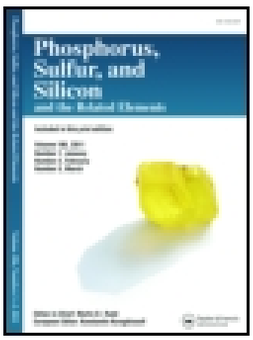

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# Synthesis and Fungicidal Activities of Novel 1,2,4-Triazole Thione Derivatives Containing 1,2,3-Triazole and Substituted Piperazine Moieties

**Bao-Lei Wang*, Yi-Zhou Zhan, Li-Yuan Zhang, Yan Zhang, Xiao Zhang, Zheng-Ming Li***

State-Key Laboratory of Elemento-Organic Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin, China

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**Running head**: Novel 1,2,4-Triazole Thione Derivatives

Address correspondence to Bao-Lei Wang and Zheng-Ming Li, State-Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China

E-mails: wangbaolei@126.com, nkzml@vip.163.com

**Abstract** *A series of novel 1,2,4-triazole thione derivatives containing 1,2,3-triazole and substituted piperazine moieties were synthesized via the Mannich reaction of 1,2,3-triazole-containing 1,2,4-triazole thiol intermediates with various substituted piperazines and formaldehyde in high yields. The structures of 14 title compounds were confirmed by melting points, IR, $^{1}H$ NMR, $^{13}C$ NMR and elemental analysis. The bioassay results showed that some of the title compounds exhibit significant fungicidal activities against several plant fungi at 50 μg/mL, especially trifluoromethyl-containing triazole thione derivative* ***9g*** *showed broad activities and could be made further structural optimization for novel fungicides innovation research.*

**Keywords**: triazole, thione derivatives, piperazine, Mannich reaction, synthesis, fungicidal activities

## INTRODUCTION

Sulfur- and nitrogen-containing organic compounds play very important roles in the research areas of organic chemistry, medicinal chemistry, and pesticidal chemistry [1-7]. In recent times, the chemistry of heterocyclic thione derivatives with substituted aminomethyl at α-carbon of thiocarbonyl group, that is the sulfur-containing heterocyclic Mannich base, has attracted much attention owing to their versatile pharmacological properties. Different heterocyclic Mannich bases have been regarded as antifungal[8], antitumor[9], antimicrobial[10], anticancer[11] agents. Furthermore, 1,2,3-triazoles possess various bioactivities, such as antibacterial[12], fungicidal[13], and insect GABA receptors antagonists[14]. As a result, 1,2,3-triazole is often used as an important pharmacophore to design novel bioactive molecules. In addition, piperazine is one of important nitrogen-containing five-membered heterocycles. It has been found that *N*-substituted piperazine compounds have a wide range of pharmaceutical activities[15,16], but only a few reports about their herbicidal and insecticidal properties are available in the literature[17].

It is known that many reports have appeared in the literature for the synthesis and medicinal activities of azole Mannich bases[10,11], however there is no research reported about the synthesis and pesticidal activities of 1,2,4-trizole Mannich bases containing 1,2,3-triazole and piperazine groups. In view of the above reasones mentioned and with our pursuit for the synthesis of novel heterocyclic compounds with pesticidal potential in mind, in this paper series of novel

1,2,4-triazole thione derivatives containing methyl/trifluoromethyl, 1,2,3-triazole and substitutedbenzylpiperazine or arylpiperazine moieties were synthesized *via* Mannich reaction of the intermediate Schiff bases based on our previous work[18]. The research ideas for this new work were to replace the (substituted)benzylideneamino group on triazole ring of the lead structure, (substituted)benzylpiperazine-containing 1,2,4-triazole Mannich bases[18], with 1,2,3-triazolylmethyleneamino group, introduce various substituted piperazine moieties ---- phenyl piperazine, (pyridin-2-yl)piperazine, and 4,6-disubstituted pyrimidyl piperazine in addition to the substituted benzyl piperazine (involved in the lead structure) into the triazole ring, maintain the trifluoromethyl group or change it to methyl group, and study the synthesis and the pesticidal activities of the new triazole thione derivertives. Meanwhile, considering there are two secondary amine structures (-NH-) in piperazine ring that can be both used for undergoing Mannich reaction, a series of novel bis(1,2,4-triazole thione) derivatives containing methyl/trifluoromethyl, 1,2,3-triazole and piperazine moieties were also successfully synthesized. The fungicidal activities of these novel herterocyclic Mannich bases against six plant fungi were also investigated.

( insert **SCHEME 1** )

( insert **SCHEME 2** )

( insert **SCHEME 3** )

## RESULTS AND DISCUSSION

### Synthesis

The intermediates and the title compounds were synthesized as shown in **SCHEME 1**, **SCHEME 2** and **SCHEME 3**. According to the method reported [19], the intermediate triazole carbaldehyde **5** was successfully prepared by the oxidation of phenyl-*D*-glucosotriazole **4** at room temperature using sodium periodate as an oxidant. In our experiment the crude product could be recrystallized from light petroleum to give pure carbaldehyde **5**. It was further condensed with 4-amino-5-methyl/trifluoromethyl-4*H*-1,2,4-triazole-3-thiol **6** in ethanol with a small amount acetic acid as catalyst at refluxing temperature to afford Schiff base **7**. The triazole thione derivatives **8** and **9** that containing different substituted piperazine moiety were conveniently synthesized *via* Mannich reaction of Schiff base **7**, formalin, and various 4-substituted piperazine intermediates. It was found that the yields for **8a-d** and **9a-h** were 58-67% and 70-88% respectively. As the amine reactant of Mannich reaction, the arylpiperazines (4-phenylpiperazine, 4-(pyridyl-2-yl)piperazine, 4-(4,6-disubstituted)piperazine) seemed have higher reactivity than those of substituted benzylpiperazines. In a 2:1 molar ratio of triazole thiol **1** and anhydrous piperazine, novel bis(1,2,4-triazole thione) containing piperazine and 1,2,3-triazole moieties **10** was also conveniently prepared in about 90% yield using the same procedure, because there are two secondary amine group (>NH) available in piperazine structure that can both occur such Mannich reaction. It's known that the intermediate Schiff base **7** can

exist either as a thiol or the thione tautomeric forms or as an equilibrium mixture of both forms owing to the thioamide structure (-NH-C(=S)). In some cases the mercapto group (-SH) took part in nucleophilic reaction and the sulfoether products obtained[20,21]. The results in our experiment indicate the thione isomer undertakes the Mannich reaction *via* the N-H at *α*-position of thiocarbonyl (C=S), though Schiff bases **7a** and **7b** exist in thiol isomer themselves which was proven by their –SH proton signals at very downfield *δ* 13.89 and 15.02, respectively in $^{1}$H NMR spectra.

**Spectroscopic Characterization**

The title compounds 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 ones. In $^{1}$H NMR, the –N=CH- proton and the proton in 1,2,3-triazole ring appeared at *δ* 10.33-10.95 and *δ* 8.29-8.74 as a singlet, respectively. The resonance signals of $CH_2$ protons neighboring to the 1,2,4-triazole ring were observed at *δ* 5.12-5.23 in Mannich bases **8**, while at *δ* 5.17-5.29 in Mannich bases **9** and *δ* 5.08-5.19 in Mannich bases **10** as a singlet. The chemical shift of these $CH_2$ proton in trifluoromethyl-containing compounds appeared at downfield position compared with those in trimethyl-containing ones, e.g. **8c** (*δ* 5.23) vs. **8a** (*δ* 5.12); **9h** (*δ* 5.29) vs. **9d** (*δ* 5.17); **10b** (*δ* 5.19) vs. **10a** (*δ* 5.08), which could be owing to the strong deshielding effect of trifluoromethyl group. The chemical shifts at *δ* 2.81-3.96 and *δ* 2.40-3.20 can be ascribed to piperazine ring protons, which appeared as two multiplet or broad signals, respectively. However

the signals of these piperazine protons were observed at $\delta$ ~ 2.88 as a singlet in compounds **10** because of the symmetric structure of bis (1,2,4-triazole thione). In the $^{13}C$ NMR spectra of compounds **8** and **9**, the typical carbon resonance at $\delta$ 162.9–167.7 was indicative of a thiocarbonyl group (C=S). The piperazine carbons appeared at $\delta$ 50.3–53.0 and $\delta$ 43.5–50.5 as two singlets. The signals of $C_5$ in 1,2,4-triazole ring and carbon adjacent to it were observed at $\delta$ 139.4-151.3 and $\delta$ 11.1 ($CH_3$) as singlet respectively in **8a**, **8b** and **9a-9d**, while at $\delta$ ~138.7 and $\delta$ ~116.8 ($CF_3$) as quartet respectively in **8c**, **8d** and **9e-9h**, which is due to the "F" splitting of the latter. The chemical shifts of all carbons for compounds **8d** and **9h** were shown in **SCHEME 4** corresponding to their structures.

In IR spectra, the characteristic stretching vibration $\nu$(C=S) appeared at 1153-1167 $cm^{-1}$. The strong absorption peak at 1575-1600 $cm^{-1}$ can be ascribed to the C=N stretching. In the spectra of trifluoromethyl-containing compounds, it showed bands at 1316-1321 $cm^{-1}$ and 1194-1207 $cm^{-1}$ respectively for C-F stretching.

( insert **SCHEME 4** )

**Fungicidal Activities**

As shown in **Table S 1** (Supplemental Materials), most of the compounds synthesized showed obvious fungicidal activities against six test fungi at 50 μg/mL. For *Gibberella sanbinetti*, compounds **9e**, **9f** and **9g** possessed an inhibitory rate 68.4%, 63.2% and 68.4%, respectively, and were more effective than the control Triadimefon (52.9%). In particular, the activities of **9e**

and **9g** were closed with that of the control Chlorothalonil. For *Cercospora arachidicola*, compounds **9g** and **9h** whose inhibitory activity were 70.0% and 65.0%, were comparable with Triadimefon (66.7%). Compounds **8c**, **9d**, **9g**, **10a** and **10b** exhibited 65.1%-88.4% fungicidal activities against *Physalospora piricola*, especially **9d** (72.1%), **10a** (72.1%) and **10b** (88.4%) were more effective than Triadimefon (71.4%). Some compounds also displayed good fungicidal activities against *Alternaria solani Sorauer* and *Fusarium omysporum* at 50 μg/mL. It was worthy to note that most of compounds showed favorable fungicidal activities against *Rhizoctonia cerealis*, such as **8c**, **9e**, **9f** and **9g** had inhibitory rate 85.7%-89.8%. Although these compounds were less effective than the control Triadimefon, their structural information could still be useful for further optimization to find novel fungicides against *Rhizoctonia cerealis*.

In analyzing the structure-activity relationship (SAR) of these compounds, it was found that trifluoromethyl-containing triazole thione derivatives showed favorable fungicidal activities in most cases, which may be due to the excellent properties (such as hydrophobicity and permeability) of the trifluoromethyl group introduced into the parent structure. The activity of most compounds, when $R^4$ was fixed as H, indicated the trend Ph > Py, and 4-methylpyrimidyl > 4,6-dimethylpyrimidyl in the aryl group of piperazine 4-position; when $R^4$ was fixed as F, indicated the trend benzyl > 2,4-dichlorobenzyl, Ph ≈ Py, and 4-methylpyrimidyl > 4,6-dimethylpyrimidyl in the group of piperazine 4-position.

## EXPERIMENTAL

The melting points were determined on a Taike X-4 apparatus and were 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 (400MHz) using TMS as an internal standard and DMSO-$d_6$ or $CDCl_3$ as solvent. Elemental analysis was performed on a Vario EL CUBE element 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. The Supplemental Materials contains sample $^{1}$H and $^{13}$C NMR spectra for 8c and 9d (Figures S 1 – S 4).

4-Amino-5-methyl/trifluoromethyl-4*H*-1,2,4-triazole-3-thiol **6a** and **6b** were prepared according to the literature[22].

### Synthesis of the Intermediates 4-(Substituted)benzylpiperazine 1

Referring to the literature [23] method, to a solution of anhydrous piperazine (50 mmol) in 95% ethanol (20 mL) was added 36% hydrochloric acid (25 mmol). The mixture was stirred under refluxing and substituted benzyl chlorine (25 mmol) added dropwise over 5 min. The mixture was refluxed for 4–8 h with TLC monitoring, then left standing 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 saturated $K_2CO_3$ aq. (30 mL), extracted with chloroform (5 × 8 mL). 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 **1** as a colorless liquid.

**1a**: yield 58%, bp 131-134 $^oC$/10mmHg; **1b**: yield 36%, bp 147-150 $^oC$/6 mmHg.

**Synthesis of the Intermediates 4-(4,6-Disubstituted-pyrimidin-2-yl)piperazine 3**

By reacting the diazonium salts of 4,6-disubstituted pyrimidin-2-amines with concentrated HCl and $ZnCl_2$[24], 2-chloro-4,6-disubstituted pyrimidines **2** were prepared. Referring to the literature[23], compounds **3** were prepared 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 **2** (18 mmol) in small portions at 50–60 °C. The mixture was stirred for 1 h at 60-65 $^oC$ and cooled to room temperature. 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 **3**, which was used for the following reactions without further purification.

**3a**: yellow solid, yield 81%, mp 45-48 $^oC$; **3b**: yellow solid, yield 79%, mp 82-84 $^oC$.

**Synthesis of the Intermediate 2-Phenyl-2*H*-1,2,3-triazole-4-carbaldehyde 5**

To a solution of sodium periodate (9.63 g, 0.045 mol) in water (150 mL) was added phenyl-*D*-glucosotriazole **4** (4.0 g, 0.015 mol) (prepared according the reported method[19]). The

mixture was stirred at room temperature for 24 h and filtrated. The solid was washed with water, dried and recrystallized with light petroleum (30~60$^{o}$C) to give intermediate triazole carbaldehyde **5** as light yellow crystals. Yield 68.2%, mp 66-67$^{o}$C (Lit.[19] mp 68-69$^{o}$C).

**Synthesis of the Intermediates 4-(((2-Phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-5-(methyl/trifluoromethyl)-4*H*-1,2,4-triazole-3-thiol 7**

Compound **6a** or **6b** (10 mmol), triazole carbaldehyde **5** (10.5 mmol) and acetic acid (10 drop) 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 were filtered and recrystallized from ethanol to give Schiff base **7**.

**7a**: colorless crystals, yield 79.4%, mp 257-259 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO), $\delta$: 13.89 (s, 1H, SH), 10.59 (s, 1H, CH), 8.74 (s, 1H, Triazole-H), 8.10 (d, $J$ = 8.2 Hz, 2H, Ph-H), 7.63 (t, $J$ = 7.8 Hz, 2H, Ph-H), 7.51 (t, $J$ = 7.3 Hz, 1H, Ph-H), 2.40 (s, 3H, $CH_3$). Elemental anal. (%) calcd. For $C_{12}H_{11}N_7S$: C 50.51, H 3.89, N 34.36; found: C 50.14, H 4.25, N 34.03.

**7b**: yellow crystals, yield 72.3%, mp 210-212 $^{o}$C; $^{1}$H NMR (400 MHz, DMSO), $\delta$: 15.02 (s, 1H, SH), 10.43 (s, 1H, CH), 8.72 (s, 1H, Triazole-H), 8.11 (d, $J$ = 8.1 Hz, 2H, Ph-H), 7.64 (t, $J$ = 7.8 Hz, 2H, Ph-H), 7.53 (t, $J$ = 7.3 Hz, 1H, Ph-H). Elemental anal. (%) calcd. for $C_{12}H_8F_3N_7S$: C 42.48, H 2.38, N 28.90; found: C 42.22, H 2.65, N 28.58.

**Synthesis of 4-(((2-Phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2-((4-(substitutedbenzyl) piperazin-1-yl)methyl)-5-(methyl/trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione 8**

Schiff base **7** (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-(substituted)benzylpiperazine **1** (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 **8**.



**2-((4-Benzylpiperazin-1-yl)methyl)-5-methyl-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (8a):** colorless crystals, yield 61.4%, mp 154-156 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.94 (s, 1H, CH=N), 8.29 (s, 1H, Triazole-H), 8.12 (d, $J$ = 8.0 Hz, 2H, Ph-H), 7.52 (t, $J$ = 7.8 Hz, 2H, Ph-H), 7.41 (t, $J$ = 7.3 Hz, 1H, Ph-H), 7.33 – 7.20 (m, 5H, Ph-H), 5.12 (s, 2H, $NCH_2N$), 3.50 (s, 2H, $PhCH_2$), 2.87 (bs, 4H, Piperazine-H), 2.49 (bs, 4H, Piperazine-H), 2.46 (s, 3H, Triazole-$CH_3$); $^{13}$C NMR (101 MHz, $CDCl_3$), $\delta$: 162.9 (s, 1C, C=S), 150.7 (s, 1C, 1,2,4-Triazole-$C_5$), 147.7 (s, 1C, 1,2,3-Triazole-$C_4$), 144.2 (s, 1C, N=CH), 139.4 (s, 1C, Ph-C), 138.0 (s, 1C, Ph-C), 134.8 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 129.2 (s, 2C, Ph-C), 128.4 (s, 1C, Ph-C), 128.2 (s, 2C, Ph-C), 127.1 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 68.7 (s, 1C, $CH_2$), 63.1 (s, 1C, $CH_2$), 53.0 (s, 2C, Piperazine-C), 50.5 (s, 2C, Piperazine-C), 11.1 (s, 1C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2932, 2813(C-H), 1595(C=N), 1494, 1455(Ar), 1167(C=S). Elemental anal. (%) calcd. for $C_{24}H_{27}N_9S$: C 60.87, H 5.75, N 26.62; found: C 60.58, H 5.91, N 26.29.

**2-((4-(2,4-Dichlorobenzyl)piperazin-1-yl)methyl)-5-methyl-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (8b):** white solid, yield 66.9%, mp 109-110 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.95 (s, 1H, CH=N), 8.30 (s, 1H, Triazole-H), 8.13 (d, $J$ = 7.6 Hz, 2H, Ph-H), 7.53 (t, $J$ = 7.9 Hz, 2H, Ph-H), 7.48 – 7.32 (m, 3H, Ph-H), 7.19 (dd, $J$ = 8.3, 2.1 Hz, 1H, Ph-H), 5.13 (s, 2H, $NCH_2N$), 3.56 (s, 2H, $ArCH_2$), 2.87 (bs, 4H, Piperazine-H), 2.54 (bs, 4H, Piperazine-H), 2.48 (s, 3H, $CH_3$); $^{13}$C NMR (101 MHz, $CDCl_3$), $\delta$: 162.9 (s, 1C, C=S), 150.8 (s, 1C, 1,2,4-Triazole-$C_5$), 147.8 (s, 1C, 1,2,3-Triazole-$C_4$), 144.2 (s, 1C, N=CH), 139.4 (s, 1C, Ph-C), 134.8 (s, 1C, Ph-C), 134.7 (s, 1C, Ph-C), 134.6 (s, 1C, Ph-C), 133.1 (s, 1C, 1,2,3-Triazole-$C_5$), 131.4 (s, 1C, Ph-C), 129.5 (s, 2C, Ph-C), 129.2 (s, 1C, Ph-C), 128.4 (s, 1C, Ph-C), 126.9 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 68.7 (s, 1C, $CH_2$), 58.7 (s, 1C, $CH_2$), 53.0 (s, 2C, Piperazine-C), 50.5 (s, 2C, Piperazine-C), 11.1 (s, 1C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2940, 2812(C-H), 1593(C=N), 1560, 1496, 1464(Ar), 1172(C=S). Elemental anal. (%) calcd. for $C_{24}H_{25}Cl_2N_9S$: C 53.14, H 4.65, N 23.24; found: C 52.88, H 4.68, N 23.02.

**2-((4-Benzylpiperazin-1-yl)methyl)-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (8c):** white solid, yield 58.2%, mp 119-120 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.83 (s, 1H, CH=N), 8.32 (s, 1H, Triazole-H), 8.15 – 8.10 (m, 2H, Ph-H), 7.55-7.50 (m, 2H, Ph-H), 7.42 (t, $J$ = 7.4 Hz, 1H, Ph-H), 7.33 – 7.21 (m, 5H, Ph-H), 5.23 (s, 2H, $NCH_2N$), 3.50 (s, 2H, $PhCH_2$), 2.87 (bs, 4H, Piperazine-H), 2.48 (bs, 4H, Piperazine-H); $^{13}$C NMR (101 MHz, $CDCl_3$), $\delta$: 165.2 (s, 1C, C=S), 152.5 (s, 1C,

1,2,3-Triazole-$C_4$), 143.5 (s, 1C, N=CH), 139.4 (s, 1C, Ph-C), 138.7 (q, $J_{C-F}$ = 42.4 Hz, 1C, 1,2,4-Triazole-$C_5$), 137.9 (s, 1C, Ph-C), 135.0 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 129.2 (s, 2C, Ph-C), 128.5 (s, 1C, Ph-C), 128.3 (s, 2C, Ph-C), 127.1 (s, 1C, Ph-C), 119.3 (s, 2C, Ph-C), 116.8 (q, $J_{C-F}$ = 272.7 Hz, 1C, $CF_3$), 70.0 (s, 1C, $CH_2$), 63.1 (s, 1C, $CH_2$), 52.9 (s, 2C, Piperazine-C), 50.4 (s, 2C, Piperazine-C); IR (KBr), ν ($cm^{-1}$): 2955, 2809(C-H), 1598(C=N), 1497, 1456(Ar), 1319, 1200(C-F), 1167(C=S). Elemental anal. (%) calcd. for $C_{24}H_{24}F_3N_9S$: C 54.64, H 4.59, N 23.89; found: C 54.36, H 4.80, N 23.74.

**2-((4-(2,4-Dichlorobenzyl)piperazin-1-yl)methyl)-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (8d):** white solid, yield 62.8%, mp 127-128 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$), $\delta$: 10.83 (s, 1H, CH=N), 8.33 (s, 1H, Triazole-H), 8.12 (d, $J$ = 7.7 Hz, 2H, Ph-H), 7.53 (t, $J$ = 7.9 Hz, 2H, Ph-H), 7.46 – 7.33 (m, 3H, Ph-H), 7.19 (dd, $J$ = 8.3, 2.1 Hz, 1H, Ph-H), 5.22 (s, 2H, $NCH_2N$), 3.56 (s, 2H, $ArCH_2$), 2.88 (bs, 4H, Piperazine-H), 2.54 (bs, 4H, Piperazine-H); $^{13}C$ NMR (101 MHz, $CDCl_3$), $\delta$: 165.2 (s, 1C, C=S), 152.5 (s, 1C, 1,2,3-Triazole-$C_4$), 143.5 (s, 1C, N=CH), 139.3 (s, 1C, Ph-C), 138.7 (q, $J_{C-F}$ = 42.4 Hz, 1C, 1,2,4-Triazole-$C_5$), 135.0 (s, 1C, Ph-C), 134.8 (s, 1C, Ph-C), 134.4 (s, 1C, Ph-C), 133.2 (s, 1C, 1,2,3-Triazole-$C_5$), 131.4 (s, 1C, Ph-C), 129.5 (s, 2C, Ph-C), 129.2 (s, 1C, Ph-C), 128.5 (s, 1C, Ph-C), 127.0 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 116.8 (q, $J_{C-F}$ = 272.7 Hz, 1C, $CF_3$), 70.0 (s, 1C, $CH_2$), 58.6 (s, 1C, $CH_2$), 52.9 (s, 2C, Piperazine-C), 50.4 (s, 2C, Piperazine-C); IR (KBr), ν ($cm^{-1}$): 2950, 2816(C-H), 1597(C=N), 1498, 1462(Ar), 1320, 1201(C-F), 1170(C=S).

Elemental anal. (%) calcd. for $C_{24}H_{22}Cl_2F_3N_9S$: C 48.33, H 3.72, N 21.14; found: C 48.21, H 3.79, N 20.78.

**Synthesis of 4-(((2-Phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2-((4-arylpiperazin-1-yl) methyl)-5-(methyl/trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione 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 novel Mannich base **9**.

**5-Methyl-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2-((4-phenylpiperazin-1-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9a):** white solid, yield 70.4%, mp 151-153 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.95 (s, 1H, CH=N), 8.30 (s, 1H, Triazole-H), 8.17 – 8.09 (m, 2H, Ph-H), 7.53 (dd, $J$ = 10.7, 5.1 Hz, 2H, Ph-H), 7.42 (t, $J$ = 7.4 Hz, 1H, Ph-H), 7.29 – 7.22 (m, 2H, Ph-H), 6.92 (d, $J$ = 7.9 Hz, 2H, Ph-H), 6.86 (t, $J$ = 7.3 Hz, 1H, Ph-H), 5.19 (s, 2H, $NCH_2N$), 3.26 – 3.15 (m, 4H, Piperazine-H), 3.05 – 2.95 (m, 4H, Piperazine-H), 2.48 (s, 3H, $CH_3$); $^{13}$C NMR (101 MHz, $CDCl_3$), $\delta$: 163.0 (s, 1C, C=S), 151.3 (s, 1C, 1,2,4-Triazole-$C_5$), 150.9 (s, 1C, Ph-C), 147.9 (s, 1C, 1,2,3-Triazole-$C_4$), 144.2 (s, 1C, N=CH), 139.4 (s, 1C, 1,2,3-Triazole-$C_5$), 134.8 (s, 1C, Ph-C), 129.5 (s, 2C, Ph-C), 129.1 (s, 2C, Ph-C), 128.4 (s, 1C, Ph-C), 119.9 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 116.4 (s, 2C, Ph-C), 68.6 (s, 1C, $CH_2$), 50.5 (s, 2C, Piperazine-C), 49.4 (s, 2C, Piperazine-C), 11.1 (s, 1C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2945, 2830(C-H), 1598(C=N), 1497, 1453(Ar), 1159(C=S). Elemental anal. (%) calcd. for $C_{23}H_{25}N_9S$:

C 60.11, H 5.48, N 27.43; found: C 59.78, H 5.57, N 27.18.

**5-Methyl-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2-((4-(pyridin-2-yl)piperazin-1-yl)methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9b):** colorless crystals, yield 73.8%, mp 155-156 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.94 (s, 1H, CH=N), 8.29 (s, 1H, Triazole-H), 8.19 – 8.03 (m, 3H, Pyridine-H + Ph-H), 7.59 – 7.35 (m, 4H, Pyridine-H + Ph-H), 6.76 – 6.45 (m, 2H, Pyridine-H), 5.18 (s, 2H, $NCH_2N$), 3.64 – 3.49 (m, 4H, Piperazine-H), 3.00 – 2.88 (m, 4H, Piperazine-H), 2.47 (s, 3H, $CH_3$); $^{13}$C NMR (101 MHz, $CDCl_3$), $\delta$: 163.0 (s, 1C, C=S), 159.3 (s, 1C, Py-C), 150.8 (s, 1C, 1,2,4-Triazole-$C_5$), 148.0 (s, 1C, Py-C), 147.9 (s, 1C, 1,2,3-Triazole-$C_4$), 144.1 (s, 1C, N=CH), 139.4 (s, 1C, Ph-C), 137.5 (s, 1C, Py-C), 134.7 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 128.4 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 113.3 (s, 1C, Py-C), 107.1 (s, 1C, Py-C), 68.7 (s, 1C, $CH_2$), 50.4 (s, 2C, Piperazine-C), 45.2 (s, 2C, Piperazine-C), 11.1 (s, 1C, $CH_3$); IR (KBr), $\nu$ ($cm^{-1}$): 2949, 2831(C-H), 1594(C=N), 1566, 1483, 1440(Ar), 1161(C=S). Elemental anal. (%) calcd. for $C_{22}H_{24}N_{10}S$: C 57.37, H 5.25, N 30.41; found: C 59.29, H 5.36, N 30.09.

**5-Methyl-2-((4-(4-methylpyrimidin-2-yl)piperazin-1-yl)methyl)-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9c):** colorless crystals, yield 78.5%, mp 179-180 $^{o}$C; $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.94 (s, 1H, CH=N), 8.28 (s, 1H, Triazole-H), 8.14-8.11 (m, 3H, Pyrimidine-H + Ph-H), 7.52 (t, $J$ = 7.9 Hz, 2H, Ph-H), 7.41 (t, $J$ = 7.4 Hz, 1H, Ph-H), 6.34 (d, $J$ = 5.0 Hz, 1H, Pyrimidine-H), 5.17 (s, 2H, $CH_2$), 3.94 – 3.77 (m, 4H,

Piperazine-H), 2.94 – 2.82 (m, 4H, Piperazine-H), 2.45 (s, 3H, Triazole-H), 2.30 (s, 3H, Pyrimidine-$CH_3$); $^{13}C$ NMR (101 MHz, $CDCl_3$), $\delta$: 167.6 (s, 1C, C=S), 162.9 (s, 1C, Pyrimidine-$C_4$), 161.5 (s, 1C, Pyrimidine-$C_2$), 157.2 (s, 1C, Pyrimidine-$C_6$), 150.7 (s, 1C, 1,2,3-Triazole-$C_4$), 147.8 (s, 1C, N=CH), 144.2 (s, 1C, Ph-C), 139.4 (s, 1C, 1,2,4-Triazole-$C_5$), 134.7 (s, 1C, 1,2,3-Triazole-$C_5$), 129.4 (s, 2C, Ph-C), 128.4 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 109.4 (s, 1C, Pyrimidine-$C_5$), 68.9 (s, 1C, $CH_2$), 50.6 (s, 2C, Piperazine-C), 43.6 (s, 2C, Piperazine-C), 24.3 (s, C, $CH_3$), 11.1 (s, 1C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2936, 2830(C-H), 1580(C=N), 1558, 1496, 1443(Ar), 1166(C=S). Elemental anal. (%) calcd. for $C_{22}H_{25}N_{11}S$: C 55.56, H 5.30, N 32.40; found: C 55.43, H 5.36, N 32.26.

**2-((4-(4,6-Dimethylpyrimidin-2-yl)piperazin-1-yl)methyl)-5-methyl-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9d):** white solid, yield 83.3%, mp 178-179 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$), $\delta$: 10.94 (s, 1H, CH=N), 8.28 (s, 1H, Triazole-H), 8.20 – 8.04 (m, 2H, Ph-H), 7.52 (dd, $J$ = 10.7, 5.1 Hz, 2H, Ph-H), 7.41 (t, $J$ = 7.4 Hz, 1H, Ph-H), 6.23 (s, 1H, Pyrimidine-H), 5.17 (s, 2H, $NCH_2N$), 3.92 – 3.82 (m, 4H, Piperazine-H), 2.91 – 2.81 (m, 4H, Piperazine-H), 2.45 (s, 3H, Triazole-$CH_3$), 2.26 (s, 6H, Pyrimidine-$CH_3$); $^{13}C$ NMR (101 MHz, $CDCl_3$), $\delta$: 167.0 (s, 1C, C=S), 162.9 (s, 2C, Pyrimidine-$C_{4,6}$), 161.6 (s, 1C, Pyrimidine-$C_2$), 150.7 (s, 1C, 1,2,3-Triazole-$C_4$), 147.8 (s, 1C, N=CH), 144.2 (s, 1C, Ph-C), 139.4 (s, 1C, 1,2,4-Triazole-$C_5$), 134.7 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 128.4 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 108.9 (s, 1C, Pyrimidine-$C_5$), 68.9 (s, 1C, $CH_2$), 50.7 (s, 2C,

Piperazine-C), 43.5 (s, 2C, Piperazine-C), 24.1 (s, 2C, $CH_3$), 11.1 (s, 1C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2935, 2822(C-H), 1575(C=N), 1497, 1444(Ar), 1167(C=S). Elemental anal. (%) calcd. for $C_{23}H_{27}N_{11}S$: C 56.42, H 5.56, N 31.47; found: C 56.34, H 5.56, N 31.36.

**4-(((2-Phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2-((4-phenylpiperazin-1-yl)methyl) -5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9e):** white solid, yield 75.2%, mp 160-162 $^oC$; $^1H$ NMR (400 MHz, DMSO), $\delta$: 10.33 (s, 1H, CH=N), 8.74 (s, 1H, Triazole-H), 8.10 (d, $J$ = 7.9 Hz, 2H, Ph-H), 7.63 (t, $J$ = 7.8 Hz, 2H, Ph-H), 7.52 (t, $J$ = 7.3 Hz, 1H, Ph-H), 7.19 (t, $J$ = 7.8 Hz, 2H, Ph-H), 6.92 (d, $J$ = 8.2 Hz, 2H, Ph-H), 6.77 (t, $J$ = 7.2 Hz, 1H, Ph-H), 5.27 (s, 2H, $NCH_2N$), 3.13 (bs, 4H, Piperazine-H), 2.92 (bs, 4H, Piperazine-H); $^{13}C$ NMR (101 MHz, $CDCl_3$), $\delta$: 165.2 (s, 1C, C=S), 152.6 (s, 1C, Ph-C), 151.2 (s, 1C, 1,2,3-Triazole-$C_4$), 143.5 (s, 1C, N=CH), 139.3 (s, 1C, Ph-C), 138.6 (q, $J_{C\text{-}F}$ = 42.4 Hz, 1C, 1,2,4-Triazole-$C_5$), 135.1 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 129.2 (s, 2C, Ph-C), 128.5 (s, 1C, Ph-C), 120.2 (s, 1C, Ph-C), 119.3 (s, 2C, Ph-C), 116.8 (q, $J_{C\text{-}F}$ = 272.7 Hz, 1C, $CF_3$), 116.5 (s, 2C, Ph-C), 69.9 (s, 1C, $CH_2$), 50.4 (s, 2C, Piperazine-C), 49.4 (s, 2C, Piperazine-C); IR (KBr), ν ($cm^{-1}$): 2942, 2833(C-H), 1600(C=N), 1579, 1497, 1455(Ar), 1318, 1196 (C-F), 1165(C=S). Elemental anal. (%) calcd. for $C_{23}H_{22}F_3N_9S$: C 53.79, H 4.32, N 24.55; found: C 53.65, H 4.38, N 24.23.

**4-(((2-Phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-2-((4-(pyridin-2-yl)piperazin-1-yl) methyl)-5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9f):** white solid, yield 80.6%, mp 134-136 $^oC$; $^1H$ NMR (400 MHz, DMSO), $\delta$: 10.33 (s, 1H, CH=N), 8.73 (s, 1H,

Triazole-H), 8.11-8.08 (m, 3H, Pyridine-H + Ph-H), 7.63 (t, *J* = 7.8 Hz, 2H, Ph-H), 7.53-7.50 (m, 2H, Pyridine-H + Ph-H), 6.82 (d, *J* = 8.7 Hz, 1H, Pyridine-H), 6.61 (dd, *J* = 6.7, 5.1 Hz, 1H, Pyridine-H), 5.26 (s, 2H, $NCH_2N$), 3.51 (bs, 4H, Piperazine-H), 2.86 (bs, 4H, Piperazine-H); $^{13}C$ NMR (101 MHz, $CDCl_3$), *δ*: 165.2 (s, 1C, C=S), 159.3 (s, 1C, Py-C), 152.5 (s, 1C, Py-C), 148.0 (s, 1C, 1,2,3-Triazole-$C_4$), 143.5 (s, 1C, N=CH), 139.3 (s, 1C, Ph-C), 138.8 (q, $J_{C\text{-}F}$ = 42.4 Hz, 1C, 1,2,4-Triazole-$C_5$), 137.5 (s, 1C, Py-C), 135.0 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 128.5 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 116.8 (q, $J_{C\text{-}F}$ = 272.7 Hz, 1C, $CF_3$), 113.5 (s, 1C, Py-C), 107.2 (s, 1C, Py-C), 70.0 (s, 1C, $CH_2$), 50.3 (s, 2C, Piperazine-C), 45.2 (s, 2C, Piperazine-C); IR (KBr), *ν* ($cm^{-1}$): 2957, 2828(C-H), 1597(C=N), 1566, 1498, 1482, 1457(Ar), 1316, 1207(C-F), 1153(C=S). Elemental anal. (%) calcd. for $C_{22}H_{21}F_3N_{10}S$: C 51.36, H 4.11, N 27.22; found: C 51.18, H 4.28, N 27.10.

**2-((4-(4-Methylpyrimidin-2-yl)piperazin-1-yl)methyl)-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl) methylene)amino)-5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9g):** white solid, yield 80.1%, mp 149-150 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$), *δ*: 10.82 (s, 1H, CH=N), 8.31 (s, 1H, Triazole-H), 8.13 (m, 3H, Ph-H + Pyrimidine-H), 7.52 (t, *J* = 7.9 Hz, 2H, Ph-H), 7.42 (t, *J* = 7.4 Hz, 1H, Ph-H), 6.35 (d, *J* = 4.9 Hz, 1H, Pyrimidine-H), 5.28 (s, 2H, $NCH_2N$), 3.94 – 3.82 (m, 4H, Piperazine-H), 2.95 – 2.82 (m, 4H, Piperazine-H), 2.31 (s, 3H, Pyrimidine-$CH_3$); $^{13}C$ NMR (101 MHz, $CDCl_3$), *δ*: 167.7 (s, 1C, C=S), 165.2 (s, 1C, Pyrimidine-$C_4$), 161.5 (s, 1C, Pyrimidine-$C_2$), 157.2 (s, 1C, Pyrimidine-$C_6$), 152.5 (s, 1C, 1,2,3-Triazole-$C_4$), 143.5 (s, 1C,

N=CH), 139.3 (s, 1C, Ph-C), 138.7 (q, $J_{C-F}$ = 42.4 Hz, 1C, 1,2,4-Triazole-$C_5$), 135.0 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 128.5 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 116.8 (q, $J_{C-F}$ = 272.7 Hz, 1C, $CF_3$), 109.6 (s, 1C, Pyrimidine-$C_5$), 70.1 (s, 1C, $CH_2$), 50.5 (s, 2C, Piperazine-C), 43.5 (s, 2C, Piperazine-C), 24.3 (s, 1C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2946, 2854(C-H), 1578(C=N), 1560, 1493, 1451(Ar), 1316, 1194(C-F), 1166(C=S). Elemental anal. (%) calcd. for $C_{22}H_{22}F_3N_{11}S$: C 49.90, H 4.19, N 29.10; found: C 49.74, H 4.17, N 28.96.

**2-((4-(4,6-Dimethylpyrimidin-2-yl)piperazin-1-yl)methyl)-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene)amino)-5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9h):** white solid, yield 87.6%, mp 182-183 $^oC$; $^1H$ NMR (400 MHz, $CDCl_3$), $\delta$: 10.83 (s, 1H, CH=N), 8.31 (s, 1H, Triazole-H), 8.12 (d, $J$ = 7.9 Hz, 2H, Ph-H), 7.53 (t, $J$ = 7.8 Hz, 2H, Ph-H), 7.42 (t, $J$ = 7.4 Hz, 1H, Ph-H), 6.25 (s, 1H, Pyrimidine-H), 5.29 (s, 2H, $NCH_2N$), 3.94 – 3.83 (m, 4H, Piperazine-H), 2.96 – 2.82 (m, 4H, Piperazine-H), 2.26 (s, 6H, Pyrimidine-$CH_3$); $^{13}C$ NMR (101 MHz, $CDCl_3$), $\delta$: 167.1 (s, 1C, C=S), 165.2 (s, 2C, Pyrimidine-$C_{4,6}$), 161.6 (s, 1C, Pyrimidine-$C_2$), 152.4 (s, 1C, 1,2,3-Triazole-$C_4$), 143.5 (s, 1C, N=CH), 139.3 (s, 1C, Ph-C), 138.7 (q, $J_{C-F}$ = 42.4 Hz, 1C, 1,2,4-Triazole-$C_5$), 135.0 (s, 1C, 1,2,3-Triazole-$C_5$), 129.5 (s, 2C, Ph-C), 128.5 (s, 1C, Ph-C), 119.2 (s, 2C, Ph-C), 116.8 (q, $J_{C-F}$ = 272.7 Hz, 1C, $CF_3$), 109.1 (s, 1C, Pyrimidine-$C_5$), 70.2 (s, 1C, $CH_2$), 50.5 (s, 2C, Piperazine-C), 43.5 (s, 2C, Piperazine-C), 24.1 (s, 2C, $CH_3$); IR (KBr), ν ($cm^{-1}$): 2940, 2841(C-H), 1583(C=N), 1565, 1495, 1452(Ar), 1321, 1197(C-F), 1161(C=S). Elemental anal. (%) calcd. for $C_{23}H_{24}F_3N_{11}S$: C 50.82, H 4.45, N 28.35;

found: C 50.69, H 4.53, N 28.32.

**Synthesis of 1,1'-((Piperazin-1,4-diyl)bis(methylene))bis(4- ((2-phenyl-2*H*-1,2,3-triazol-4-yl) methylene)amino-3-methyl/trifluoromethyl-1*H*-1,2,4-triazole-5(4*H*)-thione) 10**

Schiff base **7** (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 **10**.

**2,2'-(Piperazine-1,4-diylbis(methylene))bis(5-methyl-4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl) methylene)amino)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione) (10a):** white solid, yield 90.4%, mp 226-228$^{o}$C(Dec.); $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.93 (s, 2H, CH=N), 8.29 (s, 2H, Triazole-H), 8.12 (d, $J$ = 7.8 Hz, 4H, Ph-H), 7.52 (t, $J$ = 7.8 Hz, 4H, Ph-H), 7.41 (t, $J$ = 7.3 Hz, 2H, Ph-H), 5.08 (s, 4H, $NCH_2N$), 2.87 (s, 8H, Piperazine-H), 2.46 (s, 6H, Triazole-$CH_3$); IR (KBr), ν ($cm^{-1}$): 2944, 2822(C-H), 1617(C=N), 1594, 1496(Ar), 1172(C=S). Elemental anal. (%) calcd. for $C_{30}H_{32}N_{16}S_2$: C 52.93, H 4.74, N 32.92; found: C 52.80, H 4.85, N 32.78.

**2,2'-(Piperazine-1,4-diylbis(methylene))bis(4-(((2-phenyl-2*H*-1,2,3-triazol-4-yl)methylene )amino)-5-(trifluoromethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione) (10b):** white solid, yield 88.5%, mp 215-216$^{o}$C(Dec.); $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 10.83 (s, 2H, CH=N), 8.33 (s, 2H, Triazole-H), 8.13 (d, $J$ = 7.8 Hz, 4H, Ph-H), 7.65 – 7.34 (m, 6H, Ph-H), 5.19 (s, 4H, $NCH_2N$),

2.89 (s, 8H, Piperazine-H); IR (KBr), ν ($cm^{-1}$): 2952, 2832(C-H), 1616(C=N), 1598, 1520, 1498, 1457(Ar), 1320, 1200(C-F), 1153(C=S). Elemental anal. (%) calcd. for $C_{30}H_{26}F_6N_{16}S_2$: C 45.68, H 3.32, N 28.41; found: C 45.29, H 3.68, N 28.01.

**Fungicidal Activity Tests**

Fungicidal activities of compounds against six plant fungi (**Table S 1**) were evaluated using the mycelium growth rate test[4,25]. The method was performed in an isolated culture. Under a sterile condition, 1 mL of sample was added to the culture plates, followed by the addition of 9 mL of culture medium. The final mass concentration was 50 μg/mL. The blank assay was performed with 1 mL of sterile water. Circle mycelium with a diameter of 4 mm was cut using a drill. The culture plates were cultivated at 24 ± 1 $^{o}$C. The extended diameters of the circle mycelium were measured after 72 h. The relative inhibition rate of the circle mycelium compared to blank assay was calculated *via* the following equation:

Relative inhibition rate ( % ) = $[(d_{ex} - d_{ex}') / d_{ex}] \times 100\%$

Where $d_{ex}$ is the extended diameter of the circle mycelium during the blank assay; and $d_{ex}'$ is the extended diameter of the circle mycelium during testing.

The blank test was made using acetone. Three replicates were performed.

**CONCLUSION**

A series of novel 1,2,3-triazole- and piperazine-containing 1,2,4-triazole thione derivatives

have been conveniently synthesized by the Mannich reaction of 1,2,4-triazole thiol, various substituted piperazines and formaldehyde under mild conditions in high yields. The preliminary bioassays for 14 title compounds showed that some of them exhibit significant fungicidal activities against *Gibberella sanbinetti*, *Cercospora arachidicola*, *Physalospora piricola* and *Rhizoctonia cerealis* at 50 μg/mL. Among which, trifluoromethyl-containing triazole thione derivative **9g** showed broad activities and could be made further structural optimization for novel fungicides innovation research.

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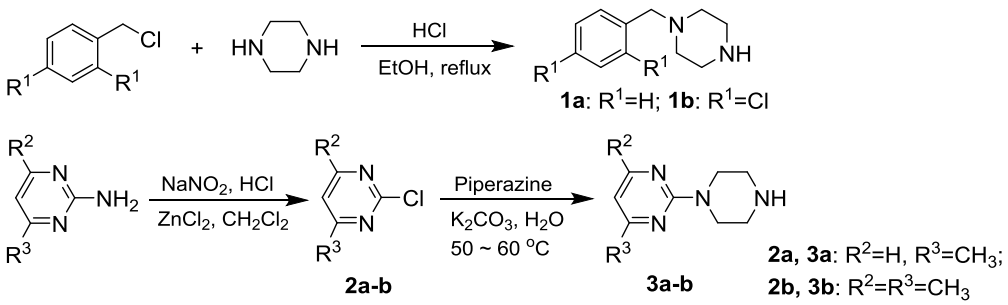
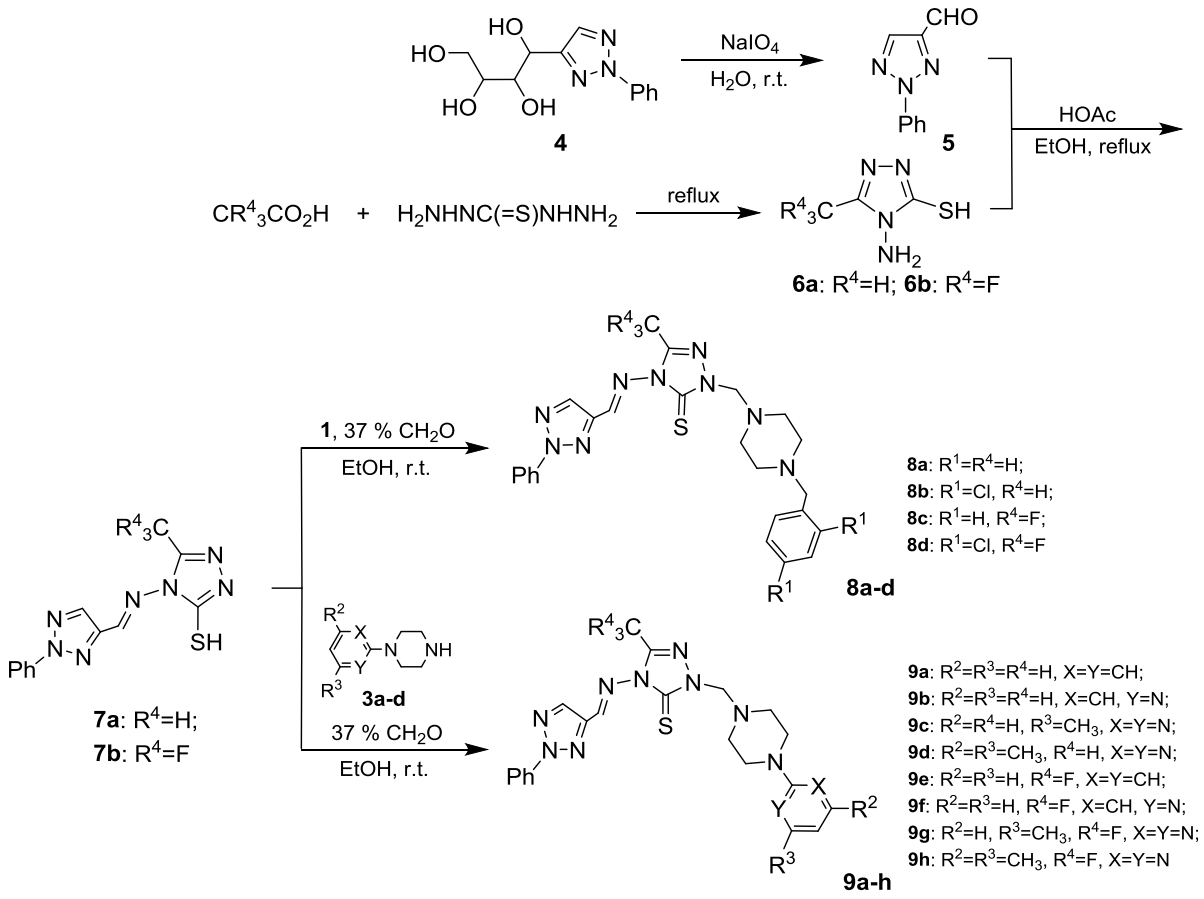
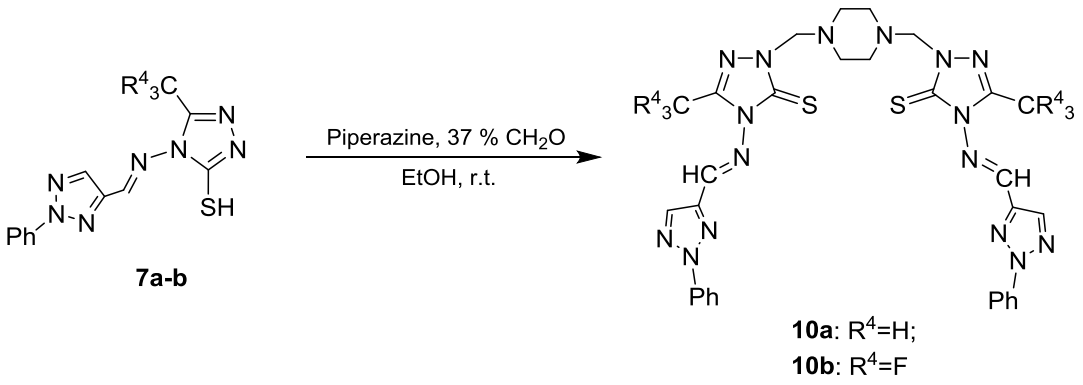


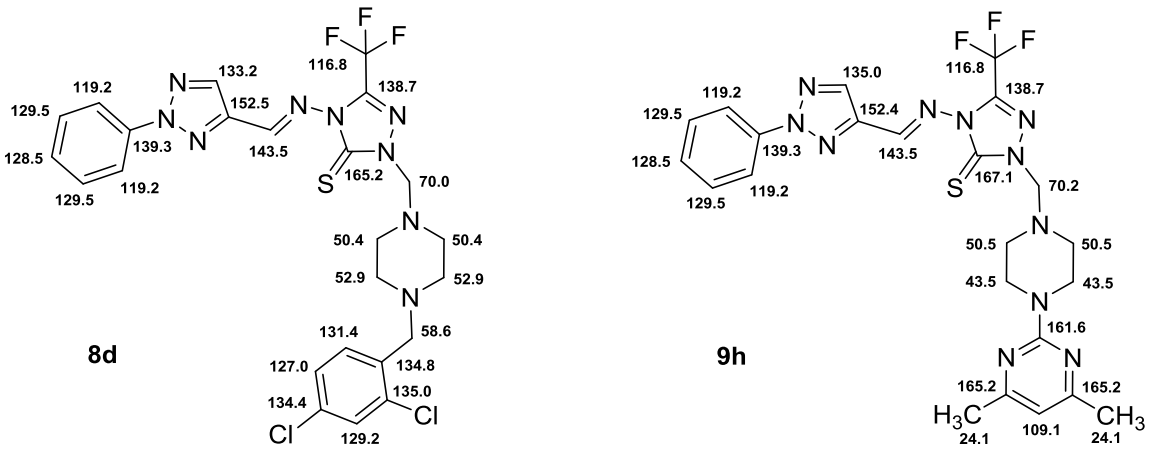

**Scheme 1:** The synthetic routes of the intermediates **1a-b** and **3a-b**.




**Scheme 2:** The synthetic routes of the title compounds **8a-d** and **9a-h**.




**Scheme 3:** The synthetic routes of the title compounds **10a-b**.




**Scheme 4:** Chemical shifts in $^{13}C$ NMR spectra of compounds **8d** and **9h**.