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Original article

Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety

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Abstract

Two new series of Mannich bases, namely, 1-(morpholino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones **3** and 1-(*N*-methylpiperazino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones **4** have been synthesized by a three-component Mannich reaction (MCR) involving 3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-5-mercapto-1,2,4-triazoles **2**, formaldehyde and morpholine/*N*-methylpiperazine. The newly synthesized compounds were well characterized by elemental analysis, IR, ^{1}H NMR and mass spectral studies. They were also screened for their antibacterial and antifungal activities against a variety of microorganisms and the results of such studies have been discussed in this article.

Keywords: Aminomethylation; Mannich bases; 4-Methylthiobenzyl; Antibacterial; Antifungal

1. Introduction

Multicomponent reactions (MCRs) constitute a major part in the present day organic synthesis with advantages ranging from lower reaction times, increased reaction rates to higher yields and reproducibility. The diversity, efficiency and rapid access to small and highly functionalized organic molecules make this approach of central current interest in the construction of combinatorial libraries and optimization in drug discovery process [1]. Mannich reaction is a three-component condensation reaction involving active hydrogen containing compound, formaldehyde and a secondary amine [2]. The amino alkylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [3].

Mannich bases have been reported as potential biological agents. They find application as antitubercular [4], antimalarial [5], vasorelaxing [6], anticancer [7], and analgesic drugs [8]. They are also used in polymer industry as paints and surface active agents [9]. Mannich bases of 1,2,4-triazoles carrying *N*-methylpiperazine moiety are reported to possess protozocidal and antibacterial activity. The drugs such as Prazosin, Lidoflazine and Urapidil carrying piperazine nucleus are good cardiovascular agents [10]. Some Mannich bases are reported to exhibit activity in vitro against murine P388 lymphocytic leukemia cells [11]. Various 1,2,4-triazole derivatives have been reported to possess antibacterial, antifungal, anticancer [12], antitubercular [13], analgesic, anti-inflammatory [14], antiviral [15], anticonvulsant [16], and anti-depressant properties [17]. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs. Some of the present day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are the best examples for potent molecules possessing triazole nucleus.

The incorporation of 4-methylthiophenyl moiety into various heterocyclic systems has found to increase their biological

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activities. We have recently reported a few heterocyclic analogues carrying 4-methylthiophenyl moiety as potent antimicrobial agents [18,19]. As a continuation of our studies on Mannich bases of 1,2,4-triazoles where the tested compounds had shown significant antibacterial and antifungal activities [20], we have herein synthesized some new Mannich bases possessing both the triazole nucleus as well as 4-methylthiophenyl moiety and studied their biological properties. The results of such studies are discussed in this article.

2. Chemistry

4-Amino-3-(4-methylthiobenzyl)-5-mercapto-1,2,4-triazole **1** was synthesized and reported by us recently [21]. The triazole **1** was condensed with various substituted benzaldehydes in the presence of catalytic amount of concentrated sulphuric acid in refluxing ethanol medium to afford a series of 3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-5-mercapto-1,2,4-triazoles **2**. The title compounds i.e. 1-(morpholino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones **3** and 1-(*N*-methylpiperazino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones **4** were synthesized in a one pot multi-component Mannich reaction involving **2**, formaldehyde and morpholine/*N*-methylpiperazine in ethanol medium (Scheme 1). The structures of some of the newly synthesized compounds have been established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral studies.

3. Biological activity

3.1. Antibacterial studies

The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains, namely *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) by disc diffusion method [22,23]. The discs measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed in each screw-capped bottles and sterilized by dry heat at 140 °C for 1 h. The test compounds were prepared in different concentrations using dimethylformamide. Exactly 1 ml containing 100 times the amount of chemical in each disc was added to each bottle, containing 100 discs. The discs of each concentration were placed in nutrient agar medium inoculated with fresh bacterial strains separately. The plates were incubated at 37 °C for 24 h. Ciprofloxacin was used as a standard drug. It has an inhibition length of 19–29 mm for *S. aureus*, 25–33 mm for *P. aeruginosa*, 20–25 mm for *K. pneumoniae* and 18–26 mm for *E. coli* at 10 μg/mL concentration. Solvent and growth controls were kept separately and the zone of inhibition and minimum inhibitory concentrations (MICs) were measured. The antibacterial activity data are given in Table 1.

3.2. Antifungal studies

The newly synthesized compounds were also screened for their antifungal activity against four fungal strains, namely *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigatus* (NCIM No. 902), *Candida albicans* (NCIM No. 3100), and *Penicillium marneffei* (recultured) by serial dilution method [24,25]. Sabourauds agar (prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7) was used as the medium for fungal growth. Normal saline was used to make the spore suspension of the fungal strains (i.e. a loopful of particular fungal strain was transferred to 3 ml of saline in order to obtain a suspension of the corresponding species). Twenty milliliters of the prepared Sabourauds agar media was poured into each of the petri dishes. Excess of media was decanted and the plates were dried by placing in an incubator for 1 h. Wells were made on these seeded agar plates using an agar punch and labeled. A 10 μg/ml solution of the test compounds in DMSO was then added into each of these labeled wells. A control was also prepared in the same way using DMSO. The petri dishes were then incubated at 37 °C for 3–4 days. The zone of inhibition and minimum inhibitory concentrations (MICs) were determined in comparison with the standard drug Ciclopiroxolamine. Ciclopiroxolamine has an inhibition length of 22–30 mm for *A. fumigatus*, 18–26 mm for *A. flavus*, 20–25 mm each for *C. albicans* and *P. marneffei* at 10 μg/mL concentration. The antifungal activity data are given in Table 2.

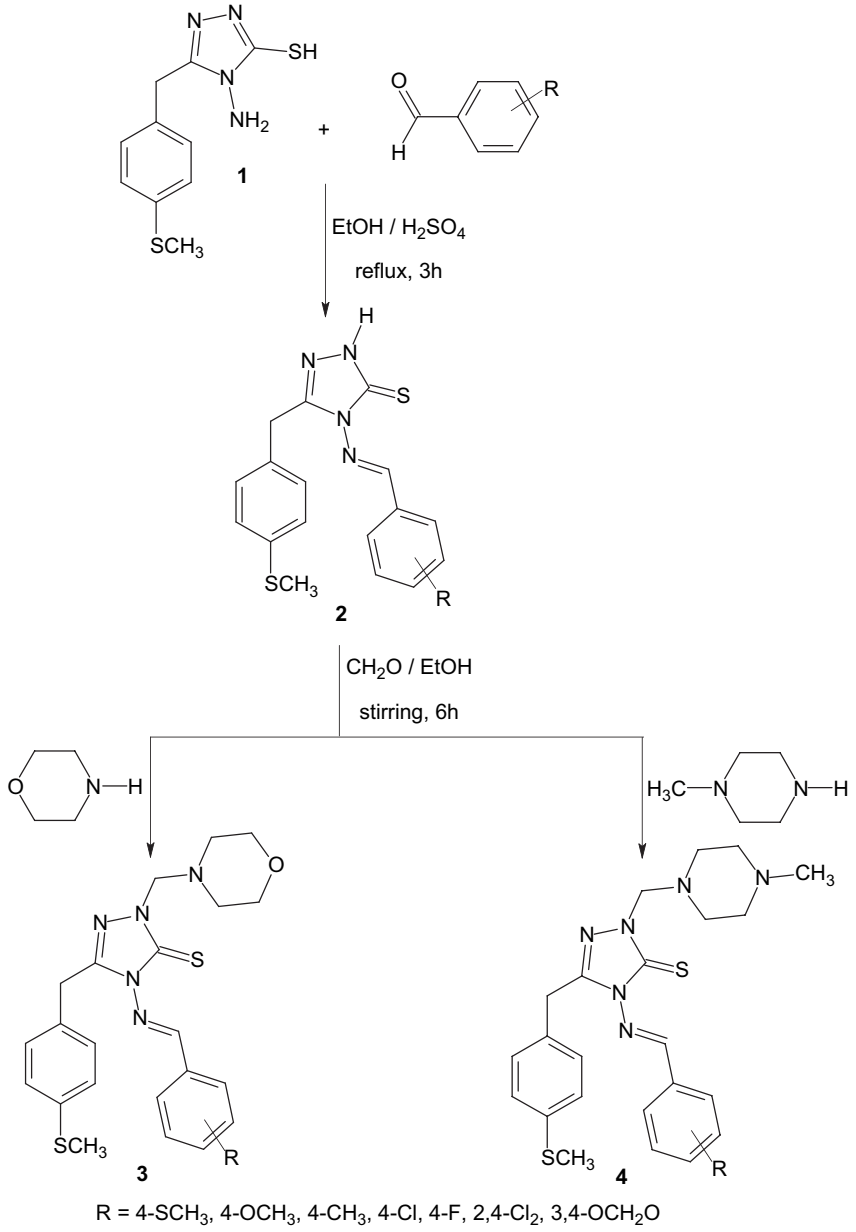
4. Results and discussion

The antibacterial and antifungal screening revealed that some of the tested compounds showed good inhibition at 10 μg/ml concentration. The antibacterial screening indicated that among the tested compounds, **3f**, **4d** and **4f** showed excellent activity against all the tested bacterial strains, namely *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli*. The remaining compounds were found to be resistant or inactive.

The antifungal screening revealed that among the tested compounds, **4d** and **4f** showed excellent activity against all the tested fungal strains, namely *A. fumigatus*, *A. flavus*, *C. albicans* and *P. marneffei*. The remaining compounds were inactive.

5. Conclusions

A series of novel Mannich bases, namely 1-(morpholino)-methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones and 1-(*N*-methylpiperazino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones have been synthesized and screened for their antibacterial and antifungal activities. The antimicrobial screening suggests that among the newly synthesized compounds, **4d** and **4f** have exhibited maximum activity against all the tested microorganisms. 1,2,4-Triazole nucleus is one of the active components present in many standard drugs



Scheme 1.

and is known to increase the pharmacological activity of the molecules. The presence of *N*-methylpiperazine moiety is also instrumental in contributing to the net biological activity of a system. Also we have already reported the increased antimicrobial activity observed in molecules possessing 4-methylthiophenyl moiety [26]. Hence herein we have combined all these three potential units, namely 1,2,4-triazole nucleus, *N*-methylpiperazine moiety and 4-methylthiophenyl moiety in one core and studied the biological behavior of the resultant system. The antimicrobial behavior in these systems was found to be moderate except for compounds **4d** and **4f**, which showed good activity against all the tested bacteria and fungi. This increased activity could also be attributed to the presence of one or more chlorine atoms in the substituted phenyl ring.

6. Experimental protocols

Melting points were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a Jeol JMS-D 300

Table 1
Antibacterial activity data of the compounds **3a–g** and **4a–g**

Compound	Diameter of the inhibition zone[a] (mm)			
	S. aureus	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
3a	10 (25)	12 (12.5)	12 (12.5)	12 (25)
3b	12 (25)	12 (12.5)	10 (12.5)	10 (25)
3c	10 (25)	12 (12.5)	10 (12.5)	10 (25)
3d	10 (25)	12 (12.5)	10 (12.5)	10 (25)
3e	12 (25)	12 (12.5)	12 (12.5)	12 (25)
3f	18 (12.5)	23 (6)	18 (6)	17 (12.5)
3g	10 (25)	12 (12.5)	10 (12.5)	10 (25)
4a	12 (25)	12 (12.5)	10 (12.5)	10 (25)
4b	10 (25)	10 (12.5)	10 (12.5)	10 (25)
4c	10 (25)	12 (12.5)	10 (12.5)	12 (25)
4d	18 (12.5)	24 (6)	20 (6)	18 (12.5)
4e	12 (25)	10 (12.5)	10 (12.5)	12 (25)
4f	19 (12.5)	25 (6)	20 (6)	18 (12.5)
4g	10 (25)	12 (12.5)	10 (12.5)	12 (25)
Standard	19 (12.5)	25 (6)	20 (6)	18 (12.5)

Standard drug – Ciprofloxacin; 12 mm or less – resistant or no inhibition; 13–17 mm – intermediate or moderate inhibition; 18 mm or more – sensitive or maximum inhibition.

[a] The values within the parentheses indicate minimum inhibitory concentration (MIC) which is defined as the lowest concentration of an antibacterial that considerably inhibits growth of the organism as visually detected.

Table 2
Antifungal activity data of the compounds **3a–g** and **4a–g**

Compound	Diameter of the inhibition zone[a] (mm)			
	A. fumigatus	*A. flavus*	*C. albicans*	*P. marneffei*
3a	12 (12.5)	10 (25)	10 (12.5)	12 (12.5)
3b	12 (12.5)	12 (25)	10 (12.5)	12 (12.5)
3c	10 (12.5)	12 (25)	12 (12.5)	10 (12.5)
3d	10 (12.5)	10 (25)	10 (12.5)	10 (12.5)
3e	10 (12.5)	10 (25)	10 (12.5)	12 (12.5)
3f	12 (12.5)	10 (25)	10 (12.5)	10 (12.5)
3g	12 (12.5)	10 (25)	10 (12.5)	10 (12.5)
4a	12 (12.5)	12 (25)	10 (12.5)	12 (12.5)
4b	12 (12.5)	12 (25)	10 (12.5)	10 (12.5)
4c	10 (12.5)	12 (25)	12 (12.5)	11 (12.5)
4d	20 (6)	18 (12.5)	19 (6)	20 (6)
4e	12 (12.5)	10 (25)	10 (12.5)	10 (12.5)
4f	22 (6)	18 (12.5)	20 (6)	18 (6)
4g	10 (12.5)	10 (25)	10 (12.5)	10 (12.5)
Standard	22 (6)	18 (12.5)	20 (6)	20 (6)

Standard drug – Ciclopiroxolamine; 12 mm or less – resistant or no inhibition; 13–17 mm – intermediate or moderate inhibition; 18 mm or more – sensitive or maximum inhibition.

[a] The values within the parentheses indicate minimum inhibitory concentration (MIC) which is defined as the lowest concentration of an antifungal that considerably inhibits growth of the organism as visually detected.

mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using *n*-hexane and ethyl acetate (4:1, v/v).

*6.1. General procedure for the synthesis of 3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-5-mercapto-1,2,4-triazoles (**2a–g**)*

A mixture of 4-amino-3-(4-methylthiobenzyl)-5-mercapto-1,2,4-triazole (**1**) (10 mmol), substituted benzaldehydes (10 mmol) and 4–5 drops of concentrated sulphuric acid in ethanol medium was heated to reflux for 3 h. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol. The characterization data of these compounds are given in Table 3.

Compound **2a**. IR (KBr) ν/cm^{-1}: 3134 (N–H), 2917 (C–H), 1590 (C=N), 1275 (C=S); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 2.54 (s, 3H, SCH_3), 4.11 (s, 2H, CH_2), 7.17 (d, 2H, $J = 8.40$ Hz, 4-methylthiophenyl), 7.21 (d, 2H, $J = 8.40$ Hz, 4-methylthiophenyl), 7.30 (d, 2H, $J = 8.44$ Hz, 4-methylthiophenyl), 7.73 (d, 2H, $J = 8.40$ Hz, 4-methylthiophenyl), 10.23 (s, 1H, N=CH), 10.50 (bs, 1H, NH); FABMS (*m*/*z*, %): 387 ($M^+ + 1$, 100), 386 (M^+, 20), 362 (10), 359 (20), 341 (5), 307 (70), 289 (60), 273 (20), 253 (70), 237 (40), 221 (10), 171 (20), 154 (80), 137 (50), 136 (60), 120 (10), 107 (20), 105 (5).

Compound **2b**. IR (KBr) ν/cm^{-1}: 3128 (N–H), 2913 (C–H), 1607 (C=N), 1272 (C=S), 1165 (C–O); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 3.89 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2), 6.99 (d, 2H, $J = 8.84$ Hz, 4-methoxyphenyl), 7.17 (d, 2H, $J = 8.16$ Hz, 4-methylthiophenyl), 7.33 (d, 2H, $J = 8.16$ Hz, 4-methylthiophenyl), 7.78 (d, 2H, $J = 8.76$ Hz, 4-methoxyphenyl), 9.89 (s, 1H, N=CH), 10.08 (bs, 1H, NH); FABMS (*m*/*z*, %): 371 ($M^+ + 1$, 100), 370 (M^+, 50), 357 (75), 329 (2), 307 (20), 289 (15), 273 (2), 201 (2), 175 (5), 164 (10), 154 (55), 137 (50), 136 (40), 120 (5), 107 (10), 105 (10).

Compound **2c**. IR (KBr) ν/cm^{-1}: 3138 (N–H), 3058 (Ar–H), 2919 (C–H), 1603 (C=N), 1276 (C=S); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 2.47 (s, 3H, CH_3), 4.12 (s, 2H, CH_2), 7.18 (d, 2H, $J = 8.40$ Hz, 4-methylthiophenyl), 7.22 (d, 2H, $J = 8.24$ Hz, 4-methylthiophenyl), 7.29 (d, 2H, $J = 7.88$ Hz, 4-methylphenyl), 7.72 (d, 2H, $J = 8.04$ Hz, 4-methylphenyl), 10.23 (s, 1H, N=CH), 10.34 (bs, 1H, NH); FABMS (*m*/*z*, %): 355 ($M^+ + 1$, 100), 354 (M^+, 25), 307 (40), 289 (25), 253 (20), 237 (40), 209 (2), 190 (2), 165 (5), 154 (80), 137 (50), 136 (55), 120 (10), 107 (20), 105 (5).

Compound **2f**. IR (KBr) ν/cm^{-1}: 3126 (N–H), 3053 (Ar–H), 2930 (C–H), 1582 (C=N), 1279 (C=S), 874 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 2.46 (s, 3H, SCH_3), 4.15 (s, 2H, CH_2), 7.21 (d, 2H, 4-methylthiophenyl), 7.27 (d, 2H, 4-methylthiophenyl), 7.36 (d, 1H, 2,4-dichlorophenyl), 7.50 (d, 1H, 2,4-dichlorophenyl), 7.79 (d, 1H, 2,4-dichlorophenyl), 10.53 (bs, 1H, NH), 10.98 (s, 1H, N=CH); FABMS (*m*/*z*, %): 411 ($M^+ + 2$, 70), 409 (M^+, 100), 377 (10), 307 (5), 289 (2), 253 (2), 237 (20), 190 (10), 172 (10), 154 (50), 137 (50), 136 (40), 120 (5), 107 (10), 102 (5), 91 (5), 89 (5).

Compound **2g**. IR (KBr) ν/cm^{-1}: 3132 (N–H), 2920 (C–H), 1604 (C=N), 1268 (C=S), 1158 (C–O); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 4.10 (s, 2H, CH_2), 6.07 (s, 2H, OCH_2O), 6.87–7.41 (m, 7H, Ar-H), 10.10 (s, 1H, N=CH), 10.72 (bs, 1H, NH); FABMS (*m*/*z*, %): 385 ($M^+ + 1$, 100), 384 (M^+, 10), 353 (10), 329 (5), 307 (5), 289 (5), 253 (50), 238 (40), 237 (40), 206 (10), 189 (10), 178 (5), 154 (40), 148 (30), 137 (40), 136 (35), 121 (10), 107 (10).

Table 3
Characterization data of compounds **2a–g**

Compound	R	Molecular formula	M.P (°C)	Yield (%)	Elemental analysis found (calc.)		
					C	H	N
2a	4-SCH_3	$C_{18}H_{18}N_4S_3$	140–142	76	55.98 (55.93)	4.64 (4.69)	14.58 (14.49)
2b	4-OCH_3	$C_{18}H_{18}N_4OS_2$	158–160	73	58.31 (58.35)	4.95 (4.90)	15.18 (15.12)
2c	4-CH_3	$C_{18}H_{18}N_4S_2$	166–168	78	60.90 (60.99)	5.17 (5.12)	15.86 (15.80)
2d	4-Cl	$C_{17}H_{15}ClN_4S_2$	184–186	84	49.80 (49.88)	3.42 (3.45)	13.68 (13.69)
2e	4-F	$C_{17}H_{15}FN_4S_2$	180–182	80	54.42 (54.46)	4.08 (4.03)	14.98 (14.94)
2f	2,4-Cl_2	$C_{17}H_{14}Cl_2N_4S_2$	164–166	78	56.97 (56.96)	4.22 (4.22)	15.69 (15.63)
2g	3,4-OCH_2O	$C_{18}H_{16}ClN_4O_2S_2$	166–168	76	56.28 (56.23)	4.11 (4.19)	14.56 (14.57)

*6.2. General procedure for the synthesis of 1-(morpholino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones (**3a–g**)*

A mixture of Schiff base (**2**) (10 mmol), formaldehyde (40%, 1.5 mL) and morpholine (10 mmol) in ethanol medium was stirred at room temperature for 6 h. The precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol. The characterization data of these compounds are given in Table 4.

Compound **3b**. IR (KBr) ν/cm^{-1}: 2961, 2855 (C–H), 1601 (C=N), 1255 (C=S), 1167 (C–O); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 2.85 (t, 4H, CH_2NCH_2), 3.73 (t, 4H, CH_2OCH_2), 3.91 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 5.14 (s, 2H, NCH_2N), 6.99 (d, 2H, $J = 8.32$ Hz, 4-methoxyphenyl), 7.17 (d, 2H, $J = 8.32$ Hz, 4-methylthiophenyl), 7.22 (d, 2H, $J = 8.36$ Hz, 4-methylthiophenyl), 7.78 (d, 2H, $J = 8.28$ Hz, 4-methoxyphenyl), 10.06 (s, 1H, N=CH); FABMS (*m/z*, %): 470 ($M^+ + 1$, 70), 469 (M^+, 25), 383 (10), 371 (55), 370 (5), 339 (5), 307 (45), 289 (20), 237 (15), 221 (5), 166 (10), 154 (80), 137 (50), 136 (55), 107 (30), 100 (100), 98 (15), 91 (15), 89 (25).

Compound **3c**. IR (KBr) ν/cm^{-1}: 2956, 2845 (C–H), 1605 (C=N), 1258 (C=S); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 2.45 (s, 3H, CH_3), 2.84 (t, 4H, CH_2NCH_2), 3.72 (t, 4H, CH_2OCH_2), 4.13 (s, 2H, CH_2), 5.13 (s, 2H, NCH_2N), 7.18 (d, 2H, $J = 8.32$ Hz, 4-methylthiophenyl), 7.22 (d, 2H, $J = 8.28$ Hz, 4-methylthiophenyl), 7.29 (d, 2H, $J = 8.12$ Hz, 4-methylphenyl), 7.72 (d, 2H, $J = 8.04$ Hz, 4-methylphenyl), 10.22 (s, 1H, N = CH); FABMS (*m/z*, %): 454 ($M^+ + 1$, 40), 453 (M^+, 50), 383 (10), 371 (55), 355 (30), 339 (15), 307 (15), 289 (30), 237 (15), 221 (15), 166 (10), 154 (70), 137 (50), 136 (60), 107 (30), 105 (20), 100 (100), 98 (15), 91 (15), 89 (25).

Compound **3d**. IR (KBr) ν/cm^{-1}: 2957, 2919, 2854 (C–H), 1594 (C=N), 1282 (C=S), 1159 (C–O), 895 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H, SCH_3), 2.82 (t, 4H, CH_2NCH_2), 3.71 (t, 4H, CH_2OCH_2), 4.12 (s, 2H, CH_2), 5.11 (s, 2H, NCH_2N), 7.17 (d, 2H, $J = 8.28$ Hz, 4-methylthiophenyl), 7.21 (d, 2H, $J = 8.32$ Hz, 4-methylthiophenyl), 7.45 (d, 2H, $J = 8.44$ Hz, 4-chlorophenyl), 7.74 (d, 2H, $J = 8.40$ Hz, 4-chlorophenyl), 10.42 (s, 1H, N=CH); FABMS (*m/z*, %): 474 ($M^+ + 1$, 50), 473 (M^+, 15), 468 (20), 439 (25), 411 (25), 391 (20), 377 (25), 375 (55), 355 (5), 335 (5), 307 (10), 289 (10), 279 (5), 257 (15), 236 (50), 221 (25), 203 (15), 191 (20), 189 (20), 154 (45), 149 (20), 137 (45), 136 (30), 119 (20), 109 (25), 105 (20), 100 (100), 95 (40), 91 (30).

Compound **3f**. IR (KBr) ν/cm^{-1}: 2951, 2916, 2854 (C–H), 1584 (C=N), 1279 (C=S), 1159 (C–O), 859 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 2.45 (s, 3H, SCH_3), 2.83 (t, 4H, CH_2NCH_2),

Table 4
Characterization data of compounds **3a–g** and **4a–g**

Compound	R	Molecular formula	M.P (°C)	Yield (%)	Elemental analysis found (Calc.)		
					C	H	N
3a	4-SCH_3	$C_{23}H_{27}N_5OS_3$	106–108	72	56.88 (56.88)	5.64 (5.60)	14.52 (14.42)
3b	4-OCH_3	$C_{23}H_{27}N_5O_2S_2$	120–122	73	58.81 (58.82)	5.85 (5.80)	14.88 (14.91)
3c	4-CH_3	$C_{23}H_{27}N_5OS_2$	102–104	76	60.96 (60.90)	6.07 (6.00)	15.46 (15.44)
3d	4-Cl	$C_{22}H_{24}ClN_5OS_2$	124–126	82	55.76 (55.74)	5.12 (5.10)	14.78 (14.77)
3e	4-F	$C_{22}H_{24}FN_5OS_2$	130–132	78	57.72 (57.75)	5.28 (5.29)	15.38 (15.30)
3f	2,4-Cl_2	$C_{22}H_{23}Cl_2N_5OS_2$	130–132	80	51.97 (51.97)	4.52 (4.56)	13.79 (13.77)
3g	3,4-OCH_2O	$C_{23}H_{25}N_5O_3S_2$	136–138	74	57.18 (57.12)	5.21 (5.21)	14.46 (14.48)
4a	4-SCH_3	$C_{24}H_{30}N_6S_3$	110–112	74	57.88 (57.80)	6.04 (6.06)	16.88 (16.85)
4b	4-OCH_3	$C_{24}H_{30}N_6OS_2$	112–114	72	59.71 (59.72)	6.25 (6.26)	17.48 (17.41)
4c	4-CH_3	$C_{24}H_{30}N_6S_2$	110–112	72	61.70 (61.77)	6.47 (6.48)	18.06 (18.01)
4d	4-Cl	$C_{23}H_{27}ClN_6S_2$	118–120	80	56.70 (56.72)	5.52 (5.59)	17.28 (17.25)
4e	4-F	$C_{23}H_{27}FN_6S_2$	140–142	79	58.72 (58.70)	5.78 (5.78)	17.81 (17.86)
4f	2,4-Cl_2	$C_{23}H_{26}Cl_2N_6S_2$	114–116	80	52.97 (52.97)	5.12 (5.02)	16.19 (16.11)
4g	3,4-OCH_2O	$C_{24}H_{28}N_6O_2S_2$	122–124	76	58.08 (58.04)	5.71 (5.68)	16.96 (16.92)

3.71 (t, 4H, CH_2OCH_2), 4.13 (s, 2H, CH_2), 5.12 (s, 2H, NCH_2N), 7.19–7.23 (m, 4H, 4-methylthiophenyl), 7.34 (dd, 1H, $J_{o/m} = 8.56$, 2.04 Hz, 2,4-dichlorophenyl), 7.48 (d, 1H, $J_m = 1.88$ Hz, 2,4-dichlorophenyl), 7.95 (d, 1H, $J_o = 8.56$ Hz, 2,4-dichlorophenyl), 11.01 (s, 1H, N=CH); FABMS (*m/z*, %): 510 ($M^+ + 2$, 40), 508 (M^+, 60), 490 (2), 421 (5), 413 (10), 411 (40), 409 (50), 391 (20), 373 (2), 335 (5), 307 (15), 289 (10), 279 (5), 239 (5), 237 (30), 221 (10), 191 (10), 167 (15), 154 (60), 149 (30), 137 (60), 136 (45), 107 (15), 100 (100), 98 (40), 91 (30).

*6.3. General procedure for the synthesis of 1-(N-methylpiperazino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones (**4a–g**)*

A mixture of Schiff base (**2**) (10 mmol), formaldehyde (40%, 1.5 mL) and *N*-methylpiperazine (10 mmol) in ethanol medium was stirred at room temperature for 6 h. The precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol. The characterization data of these compounds are given in Table 4.

Compound **4b**. IR (KBr) ν/cm^{-1}: 2938, 2836 (C–H), 1601 (C=N), 1255 (C=S), 1168 (C–O); ^{1}H NMR ($CDCl_3$) δ: 2.31 (s, 3H, NCH_3), 2.44 (s, 3H, SCH_3), 2.68 (t, 4H, CH_2NCH_2), 3.00 (t, 4H, CH_2NCH_2), 3.88 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2), 5.14 (s, 2H, NCH_2N), 6.97 (d, 2H, $J = 8.76$ Hz, 4-methoxyphenyl), 7.16 (d, 2H, $J = 8.28$ Hz, 4-methylthiophenyl), 7.20 (d, 2H, $J = 8.32$ Hz, 4-methylthiophenyl), 7.76 (d, 2H, $J = 8.72$ Hz, 4-methoxyphenyl), 10.02 (s, 1H, N=CH); FABMS (*m/z*, %): 483 ($M^+ + 1$, 60), 482 (M^+, 15), 460 (5), 391 (45), 371 (20), 348 (2), 307 (30), 289 (20), 279 (10), 264 (5), 237 (10), 221 (10), 167 (15), 154 (90), 149 (40), 137 (55), 136 (65), 113 (100), 111 (50), 97 (25), 95 (10).

Compound **4c**. IR (KBr) ν/cm^{-1}: 2941, 2843 (C–H), 1603 (C=N), 1283 (C=S); ^{1}H NMR ($CDCl_3$) δ: 2.33 (s, 3H, NCH_3), 2.43 (s, 3H, CH_3), 2.44 (s, 3H, SCH_3), 2.68 (t, 4H, CH_2NCH_2), 3.09 (t, 4H, CH_2NCH_2), 4.10 (s, 2H, CH_2), 5.14 (s, 2H, NCH_2N), 7.19 (d, 2H, $J = 8.28$ Hz, 4-methylthiophenyl), 7.22 (d, 2H, $J = 8.24$ Hz, 4-methylthiophenyl), 7.28 (d, 2H, $J = 8.12$ Hz, 4-methylphenyl), 7.71 (d, 2H, $J = 7.84$ Hz, 4-methylphenyl), 10.12 (s, 1H, N=CH); FABMS (*m/z*, %): 467 ($M^+ + 1$, 50), 466 (M^+, 25), 439 (25), 411 (25), 391 (20), 377 (25), 375 (55), 307 (10), 289 (10), 257 (15), 236 (50), 221 (25), 203 (15), 191 (20), 189 (20), 154 (45), 149 (20), 137 (45), 136 (30), 119 (20), 109 (25), 105 (20), 100 (100), 95 (40), 91 (30).

Compound **4d**. IR (KBr) ν/cm^{-1}: 2945, 2840 (C–H), 1593 (C=N), 1287 (C=S), 848 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 2.35 (s, 3H, NCH_3), 2.45 (s, 3H, SCH_3), 2.66 (t, 4H, CH_2NCH_2), 3.03 (t, 4H, CH_2NCH_2), 4.12 (s, 2H, CH_2), 5.11 (s, 2H, NCH_2N), 7.18 (d, 2H, $J = 8.32$ Hz, 4-methylthiophenyl), 7.22 (d, 2H, $J = 8.28$ Hz, 4-methylthiophenyl), 7.48 (d, 2H, $J = 8.46$ Hz, 4-chlorophenyl), 7.78 (d, 2H, $J = 8.44$ Hz, 4-chlorophenyl), 10.28 (s, 1H, N=CH); FABMS (*m/z*, %): 488 ($M^+ + 1$, 60), 487 (M^+, 25), 439 (15), 411 (20), 391 (30), 377 (15), 375 (50), 335 (15), 307 (20), 289 (30), 279 (25), 257 (15), 236 (20), 221 (15), 203 (5), 191 (20), 189 (20), 154 (55), 149 (10), 137 (35), 136 (30), 109 (15), 105 (30), 100 (100), 95 (20), 91 (10).

Compound **4f**. IR (KBr) ν/cm^{-1}: 2939, 2919, 2847 (C–H), 1584 (C=N), 1279 (C=S), 860 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 2.28 (s, 3H, NCH_3), 2.44 (s, 3H, SCH_3), 2.47 (t, 4H, CH_2NCH_2), 2.88 (t, 4H, CH_2NCH_2), 4.10 (s, 2H, CH_2), 5.14 (s, 2H, NCH_2N), 7.18–7.22 (m, 4H, 4-methylthiophenyl), 7.33 (dd, 1H, $J_{o/m} = 8.52$, 1.92 Hz, 2,4-dichlorophenyl), 7.46 (d, 1H, $J_m = 1.96$ Hz, 2,4-dichlorophenyl), 7.93 (d, 1H, $J_o = 8.56$ Hz, 2,4-dichlorophenyl), 10.98 (s, 1H, N=CH); FABMS (*m/z*, %): 523 ($M^+ + 2$, 30), 521 (M^+, 45), 460 (15), 411 (15), 409 (20), 391 (70), 389 (2), 308 (10), 307 (45), 289 (25), 279 (5), 237 (2), 220 (2), 167 (10), 154 (100), 149 (25), 137 (55), 136 (70), 113 (45), 107 (20), 95 (5).

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