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RESEARCH ARTICLE

Synthesis and Biological Activities of Some Novel Aminomethyl Derivatives of 5,5′-Butane-1,4-diyl-bis[4-allyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones

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Abstract: A series of aminomethyl containing 5,5′-Butane-1,4-diyl-bis[4-allyl-2,4-dihydro-3H-1,2,4-triazole-3-thiones were prepared under conventional cyclic condensation. The structures of newly synthesized compounds were established based on analytical and spectral studies. Further these compounds were evaluated for their antioxidant, antifungal and antibacterial activities. Most of the compounds showed good activity when compared with standard.

Keywords: 5,5′-Butane-1,4-diyl bis [4-allyl-2,4-dihydro-3*H*-1,2,4-triazole-3- thiones, Mannich bases, Biological activity

Introduction

In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. 1,2,4-Triazole moieties have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatories, CNS stimulants, sedatives, antianxiety compounds, antimicrobial agents[1-3] and antimycotic ones such as fluconazole, intraconazole, voriconazole[4,5]. There are marketed drugs containing the 1,2,4-triazole group, *e.g*., triazolam[6], alprazolam[7], etizolam[8] and furacylin[9].

Many studies have shown that Mannich bases have possess potent biological characteristics such as antibacterial, antifungal, anti-inflammatory, antimalarial and pesticide properties[10–13]. Few Mannich bases derived from 1,2,4-triazoles carrying *N*-methylpiperazine substituent were biologically active[14,15]. 4,5-Substituted products containing 1,2,4-triazole in their molecules seem to be suitable candidates for further chemical modifications and might be of interest as pharmacologically active compounds and ligands useful in coordination chemistry[16]. Derivatives of 4,5-substituted 1,2,4-triazole were synthesized by intramolecular cyclization of 1,4-disubstituted thiosemicarbazides[17]. In addition there are some studies on electronic structures and thiol-thione tautomeric equilibrium of heterocyclic thione derivatives[18–21].

In view of these findings, we report on the synthesis, characterization and antibacterial, antifungal and antioxidant activities some of 5,5'-butane-1,4-diyl-bis(4-allyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione and their Mannich bases.

Experimental

Melting points were determined on a Thomas Hoover melting point apparatus and uncorrected, but checked by differential scanning calorimeter (DSC). The IR spectra were measured with Perkin–Elmer Spectrum one FT–IR spectrophotometer. Electronic spectral studies were conducted on a Shimadzu model UV-1700 spectrophotometer in the wavelength 1100–200 nm. The ^{1}H and ^{13}C spectra were taken on Bruker AC-300 and Bruker AC-400 NMR spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C NMR. Compounds were dissolved in $CDCl_3$, DMSO and chemical shifts were referenced to TMS (^{1}H and ^{13}C NMR). Starting chemicals were obtained from Merck or Aldrich.

*General procedure for the synthesis of **4***

A mixture of bis-thiosemicarbazides (**3**) (0.01 mol) and 10% potassium hydroxide solution (10 mL) was refluxed for 3 h. The mixture was then cooled to room temperature and filtered. The filtrate was neutralized by the gradual addition of glacial acetic acid. The resulting solid was collected by filtration, dried and recrystallized from ethyl alcohol.

*5,5'-Butane-1,4-diylbis(4-allyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione **(4)***

This compound was obtained as light white solid yield: 76%; mp: 278-279 ^{o}C; IR (KBr, cm^{-1}) υ: 3105, 3057, 2952, 2767, 1645, 1277, 996, 916, 781. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.68 (s, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.61 (s, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 4.58 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 4.96 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$, J_{trans} = 17.2 Hz), 5.15 (d, 2H, -N-CH_2-CH-$\underline{CH_2}$, J_{cis} = 10.0 Hz), 5.79-5.86 (ddt, 2H, N-CH_2-$\underline{CH}$=CH_2), 14.08 (s, 2H, -SH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.4, 24.7, 45.0, 117.5, 131.9, 152.3, 166.7 (Figure 1). MW: $C_{14}H_{20}N_6S_2$ (336). Elemental analysis: calcd C, 49.97; H, 5.99; N, 24.98; S, 19.06; found, C, 49.95; H, 5.96; N, 24.96; S, 19.09.

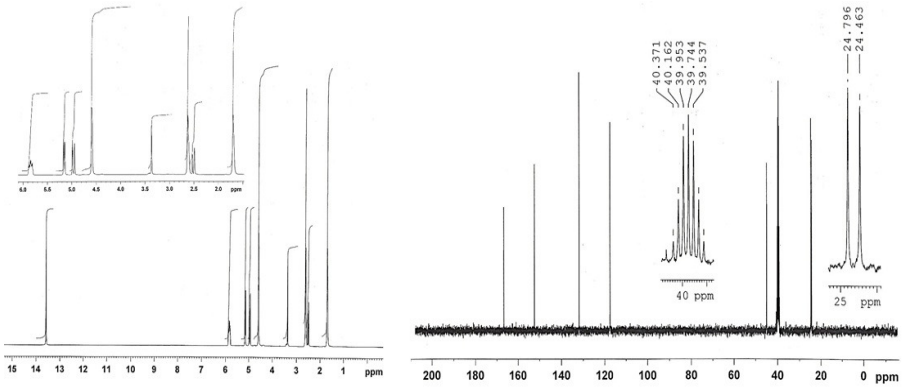
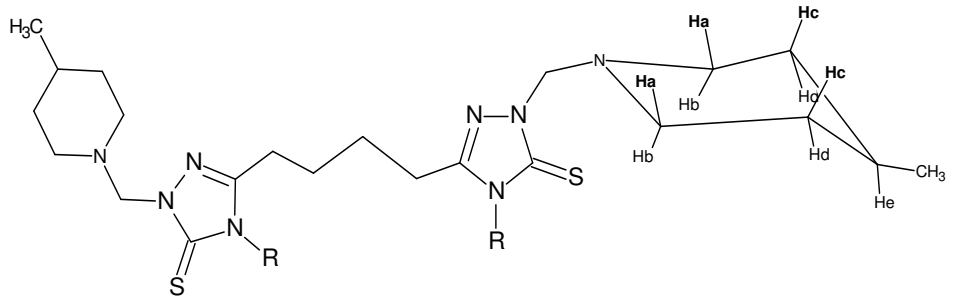


Figure 1. ^{1}H and ^{13}C NMR spectrum of **4**

*General procedure for the synthesis of **5a-h***

A solution of appropriate bis-triazoles (**4**) (0.01 mol), formaldehyde (40%, 1,5 mL) and suitably substituted secondary amines (0.01 mol) in ethanol (20 mL) was stirred for an hour and left overnight at room temperature. The solid mass thus separated was collected by filtration, dried and recrystallized from ethyl alcohol (Scheme 1).



Scheme 1. Structure of **5c**

5,5'-Butane-1,4-diylbis{4-allyl-2-[(dipropylamino)methyl]-2,4-dihydro-3H-1,2,4-triazole -3-thione} ***(5a)***

This compound was obtained as shine white solid yield: 62%; mp: 89-90 °C. IR (KBr, cm^{-1}) υ: 2958, 2870, 1647, 1251, 992, 916, 751 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.88 (t, 12H, -N-CH_2-CH_2-$\underline{CH_3}$, J = 7.2 Hz), 1.49-1.61 (m, 8H, -N-CH_2-$\underline{CH_2}$-CH_3), 1.83-1.87 (m, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.62-2.67 (m, 8H, -N-$\underline{CH_2}$-CH_2-CH_3 and 4H, -CH_2-$\underline{CH_2}$-triazole rings), 4.68 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.09 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H)), 5.13 (s, 4H, N-$\underline{CH_2}$-N), 5.24 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ J_{cis} = 10.5 Hz), 5.85-5.94 (ddt, 2H, CH_2-$\underline{CH}$=CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 11.7, 15.2, 24.9, 25.1, 46.4, 54.0, 66.6, 117.8, 130.8, 150.0, 167.9. MW: $C_{28}H_{50}N_8S_2$ (562). Elemental analysis: calcd C, 59.75; H, 8.95; N, 19.91; S, 11.39; found, C, 59.72; H, 8.97; N, 19.94; S, 11.41.

5,5'-Butane-1,4-diylbis[4-allyl-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione] ***(5b)***

This compound was obtained as shine white solid yield: 64%; mp: 166-167 °C. IR (KBr, cm^{-1}) υ: 2963, 2824, 1641, 1252, 993, 908, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.73 (s, 8H, –CH_2 (pyrrolidin rings)), 1.85 (s, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.65 (s, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 2.83 (s, 8H, –CH_2 (pyrrolidin rings)), 4.68 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.05-5.27 (m, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H) and 4H, N-$\underline{CH_2}$-N), 5.25 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ J_{cis} = 10.2 Hz), 5.82-5.95 (ddt, 2H, CH_2-$\underline{CH}$=CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 23.8, 25.0, 25.2, 46.6, 50.5, 65.7, 118.1, 130.7, 150.3, 168.3. MW: $C_{22}H_{38}N_8S_2$ (478). Elemental analysis: calcd C, 55.20; H, 8.00; N, 23.41; S, 13.40; found, C, 55.22; H, 8.02; N, 23.38; S, 13.43.

5,5'-Butane-1,4-diylbis{4-allyl-2-[(4-methylpiperidin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione} ***(5c)***

This compound was obtained as light white solid yield: 65%; mp: 147-148 °C. IR (KBr, cm^{-1}) υ: 2951, 2846, 1646, 1262, 975, 918, 757 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.89 (d, 6H, -CH-$\underline{CH_3}$, J = 5.4 Hz), 1.14-1.25 (m, 6H, H_d and H_e), 1.60 (d, 4H, H_c, J = 9.6 Hz), 1.84 (s, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.34 (t, 4H, Hb, J = 11.1 Hz), 2.65 (s, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 3.09 (d, 4H, H_a, J = 11.7 Hz), 4.68 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.04-5.10 (m, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H) and 4H, N-$\underline{CH_2}$-N), 5.25 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ J_{cis} = 10.5 Hz), 5.82-5.93 (ddt, 2H, CH_2-$\underline{CH}$=CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 21.9, 24.9, 25.2, 30.2, 34.2, 46.5, 51.3, 69.9, 118.0, 130.7, 150.1, 168.3 (Figure 2). MW: $C_{28}H_{46}N_8S_2$ (558). Elemental analysis: calcd C, 60.18; H, 8.30; N, 20.05; S, 11.48; found, C, 60.15; H, 8.33; N, 20.03; S, 11.45.

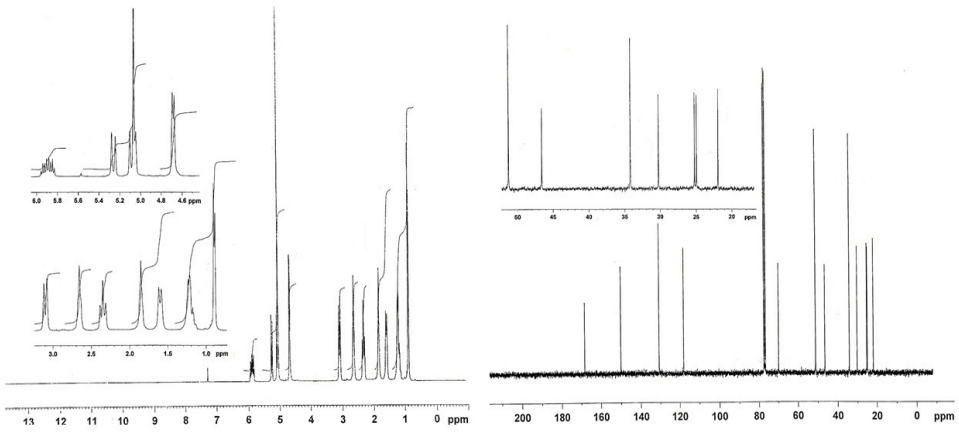


Figure 2. ^{1}H and ^{13}C NMR spectrum of **5c**

5,5'-Butane-1,4-diylbis{4-allyl-2-[(4-methylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione} ***(5d)***

This compound was obtained as light white solid yield: 60%; mp: 164-165 ^{o}C. IR (KBr, cm^{-1}) υ: 2936, 2797, 1644, 1288, 983, 917, 797 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.81-1.85 (m, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.26 (s, 6H, $\underline{CH_3}$-pyperazine ring), 2.41 (s, 8H, -CH_2 (pyperazine ring)), 2.51-2.55 (m, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 2.60 (s, 8H, -$\underline{CH_2}$ (pyperazine ring)), 4.65 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.05-5.11 (m, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H) and 4H, N-$\underline{CH_2}$-N), 5.25 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ J_{cis} = 10.5 Hz), 5.79-5.90 (ddt, 2H, CH_2-$\underline{CH}$=CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 24.9, 25.2, 46.0, 46.5, 50.1, 54.9, 68.9, 118.2, 130.6, 150.1, 168.3. MW: $C_{26}H_{44}N_{10}S_2$ (560). Elemental analysis: calcd C, 55.68; H, 7.91; N, 24.98; S, 11.44; found, C, 55.65; H, 7.94; N, 24.96; S, 11.41.

5,5'-Butane-1,4-diylbis{4-allyl-2-[(4-benzylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione} ***(5e)***

This compound was obtained as light white solid yield: 63%; mp: 170-171 ^{o}C. IR (KBr, cm^{-1}) υ: 3076, 3027, 2939, 2769, 1642, 1264, 992, 907, 746 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.85 (s, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.47 (s, 8H, -CH_2 (pyperazine ring)), 2.63 (s, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 2.71 (s, 8H, -$\underline{CH_2}$ (pyperazine ring)), 3.49 (s, 4H, N-$\underline{CH_2}$-Ar), 4.66 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.08-5.14 (m, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H) and 4H, N-$\underline{CH_2}$-N), 5.28 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ (cisH)), 5.81-5.94 (ddt, 2H, CH_2-$\underline{CH}$=CH_2), 7.20-7.32 (m, 10H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.0, 25.2, 46.6, 50.3, 52.9, 63.1, 68.1, 118.4, 127.1, 128.2, 129.3, 130.6, 137.7, 150.1, 168.4. MW: $C_{38}H_{52}N_{10}S_2$ (713). Elemental analysis: calcd C, 64.01; H, 7.35; N, 19.64; S, 8.99; found, C, 64.04; H, 7.32; N, 19.62; S, 8.96.

5,5'-Butane-1,4-diylbis[4-allyl-2-(morpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione] ***(5f)***

This compound was obtained as shine white solid yield: 65%; mp: 181-183 ^{o}C. IR (KBr, cm^{-1}) υ: 2945, 2851, 1643, 1266, 980, 911, 775 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.85-1.88 (m, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.64-2.66 (m, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 2.77 (t, 8H, N-$\underline{CH_2}$ morpholine), 3.66 (s, 8H, O-$\underline{CH_2}$ morpholine), 4.66 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.06-5.12 (m, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H) and 4H, N-$\underline{CH_2}$-N), 5.28 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ J_{cis} = 10.5 Hz), 5.83-5.95 (ddt, 2H, CH_2-$\underline{CH}$=CH_2). ^{13}C NMR (100 MHz, $CDCl_3$,

δ, ppm): 24.9, 25.2, 46.6, 50.7, 67.0, 69.3, 118.2, 130.6, 150.3, 168.6. MW: $C_{24}H_{38}N_8O_2S_2$ (534). Elemental analysis: calcd C, 53.91; H, 7.16; N, 20.95; S, 11.99; found, C, 53.93; H, 7.19; N, 20.92; S, 12.01.

5,5'-Butane-1,4-diylbis{4-allyl-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione} ***(5g)***

This compound was obtained as light white solid yield: 66%; mp: 153-154 °C. IR (KBr, cm^{-1}) υ: 3093, 3027, 2943, 2828, 1644, 1235, 989, 916, 760 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.90 (s, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.69 (s, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 2.96 (t, 8H, -CH_2 (pyperazine ring)), 3.18 (t, 8H, -$\underline{CH_2}$ (pyperazine ring)), 4.67 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.09 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ J_{trans} = 17.2 Hz), 5.16 (s, 4H, N-$\underline{CH_2}$-N), 5.26 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$), 5.83-5.93 (ddt, 2H, CH_2-$\underline{CH}$=CH_2), 6.83-7.26 (m, 10H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.0, 25.2, 46.6, 49.3, 50.4, 69.1, 116.2, 118.3, 119.9, 129.1, 130.6, 150.3, 151.2, 168.6. MW: $C_{36}H_{48}N_{10}S_2$ (684). Elemental analysis: calcd C, 63.13; H, 7.06; N, 20.45; S, 9.36; found, C, 63.15; H, 7.09; N, 20.47; S, 9.34.

5,5'-Butane-1,4-diylbis[4-allyl-2-({4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}methyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione] ***(5h)***

This compound was obtained as light white solid yield: 63%; mp: 145-146 °C. IR (KBr, cm^{-1}) υ: 3076, 2955, 2841, 1611, 1292, 992, 917, 788 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.87 (s, 4H, -$\underline{CH_2}$-CH_2-triazole rings), 2.67 (s, 4H, -CH_2-$\underline{CH_2}$-triazole rings), 2.96 (t, 8H, -CH_2 (pyperazine ring)), 3.23 (t, 8H, -$\underline{CH_2}$ (pyperazine ring)), 4.69 (d, 4H, N-$\underline{CH_2}$-CH=CH_2), 5.08-5.18 (m, 2H, -N-CH_2-CH=$\underline{CH_2}$ (trans H) and 4H, N-$\underline{CH_2}$-N), 5.27 (d, 2H, -N-CH_2-CH=$\underline{CH_2}$ (cisH)), 5.84-5.97 (ddt, 2H, CH_2-$\underline{CH}$=CH_2), 7.02-7.34 (m, 8H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 25.0, 25.2, 46.6, 48.7, 50.2, 69.0, 112.3, 112.4, 116.0, 118.3, 118.9, 129.6, 130.6, 150.3, 151.3, 168.6. MW: $C_{38}H_{46}F_6N_{10}S_2$ (820). Elemental analysis: calcd C, 55.59; H, 5.65; N, 17.06; S, 7.81; found, C, 55.61; H, 5.68; N, 17.03; S, 7.82.

Biological assays

Antibacterial activity

The newly synthesized compound was screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923) and *Pseudomonas aeruginosa* (ATCC-27853) bacterial strains by serial plate dilution method[23,24]. Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. Standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Agar media were poured into each Petri dish. Excess of suspension was decanted and placing in incubator at 37 °C for 1 h dried the plates. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with chloramphenicol as standard. Zone of inhibition were determined for title compounds the results are summarized in Table 1.

Table 1. Antibacterial activity of compounds **4** and **5a-h**

Compound	MIC in µg/mL and (zone of inhibition, mm)		
	E.coli	*P. aeruginosa*	*S. aureus*
4	12 (11–15)	12 (11–15)	12 (11–15)
5a	12 (11–15)	12 (11–15)	6.25 (16–20)
5b	12 (11–15)	12 (11–15)	12 (11–15)
5c	3.12 (27–33)	3.12 (25–30)	1.56 (22–30)
5d	12 (11–15)	12 (11–15)	6.25 (16–20)
5e	12 (11–15)	12 (11–15)	6.25 (16–20)
5f	6.25 (16–20)	6.25 (16–20)	3.12 (25–30)
5g	12 (11–15)	12 (11–15)	6.25 (16–20)
5h	3.12 (27–33)	3.12 (25–30)	1.56 (22–30)
*Chloramphenicol	6.25 (16–20)	6.25 (16–20)	3.12 (25–30)
DMSO (Control)	0	0	0

Note: the MIC values were evaluated at concentration range, 1.56–25 µg/mL

Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* [NCIM No.524] and *C. Albicans* ATCC 10231 in DMSO by serial plate dilution method[25,26]. Agar media were 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. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media of 20 mL were poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch each labeled well was made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dish were prepared in triplicate and maintained at 37 °C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with fluconazole as standard. Zones of inhibition were determined for title compounds the results are summarized in Table 2.

Table 2. Antifungal activity of compounds **4** and **5a-h**

Compound	MIC in µg/mL and (zone of inhibition, mm)	
	C. Albicans	*A. Flavus*
4	25 (< 10)	12 (11–15)
5a	12 (11–15)	6.25 (16–20)
5b	25 (< 10)	12 (11–15)
5c	12 (11–15)	6.25 (16–20)
5d	6.25 (16–20)	3.12 (25–30)
5e	6.25 (16–20)	3.12 (25–30)
5f	3.12 (27–33)	1.56 (35–40)
5g	6.25 (16–20)	3.12 (25–30)
5h	6.25 (16–20)	3.12 (25–30)
*Fluconazole	6.25 (16–20)	3.12 (25–30)
DMSO (Control)	0	0

*The MIC values were evaluated at concentration range, 1.56–25 µg/mL. *Fluconazole was used as a standard*

DPPH free radical scavenging activity

Free radical scavenging activity of the title compound was determined by measuring the change in the absorbance of DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazylradical) at 517 nm spectrophotometrically. Stock solutions of 500 µM of tested sample and DPPH$^{\bullet}$ were prepared in DMSO. 400 µL of DPPH$^{\bullet}$ solution was added to sample solution at different concentrations (500, 1000, 1500, 2000 and 2500 µL) and appropriately diluted with DMSO to total volume of 4.0 mL. A 400 µL from DPPH$^{\bullet}$ stock solution was also diluted to 4.0 mL using DMSO solvent to make the control. For the control, only solvent was added. Ascorbic acid was used as a standard (using the reference antioxidant) for this test. For the standard, sample was replaced with the same amount of ascorbic acid. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method was chosen to determine the antioxidant potential of the target compounds in comparison with the commercially available antioxidant ascorbic acid at the same concentrations. The reaction mixtures were thoroughly mixed by shaking the test tubes vigorously and incubated at 25 °C for 60 min in a water bath in the dark. Absorbance at 517 nm was measured and the solvent was corrected throughout. The scavenging effect was calculated using the following equation[27].

$$Scavengingactivity(\%) = \frac{A_0 - A_s}{A_0} x100$$

Where A_s is the absorbance of the DPPH$^{\bullet}$ in the presence of the tested compounds and standard and A_0 is the absorbance of the DPPH$^{\bullet}$ in the absence of the tested compound and standard (control). The data for antioxidation presented as means ±SD of three determinations

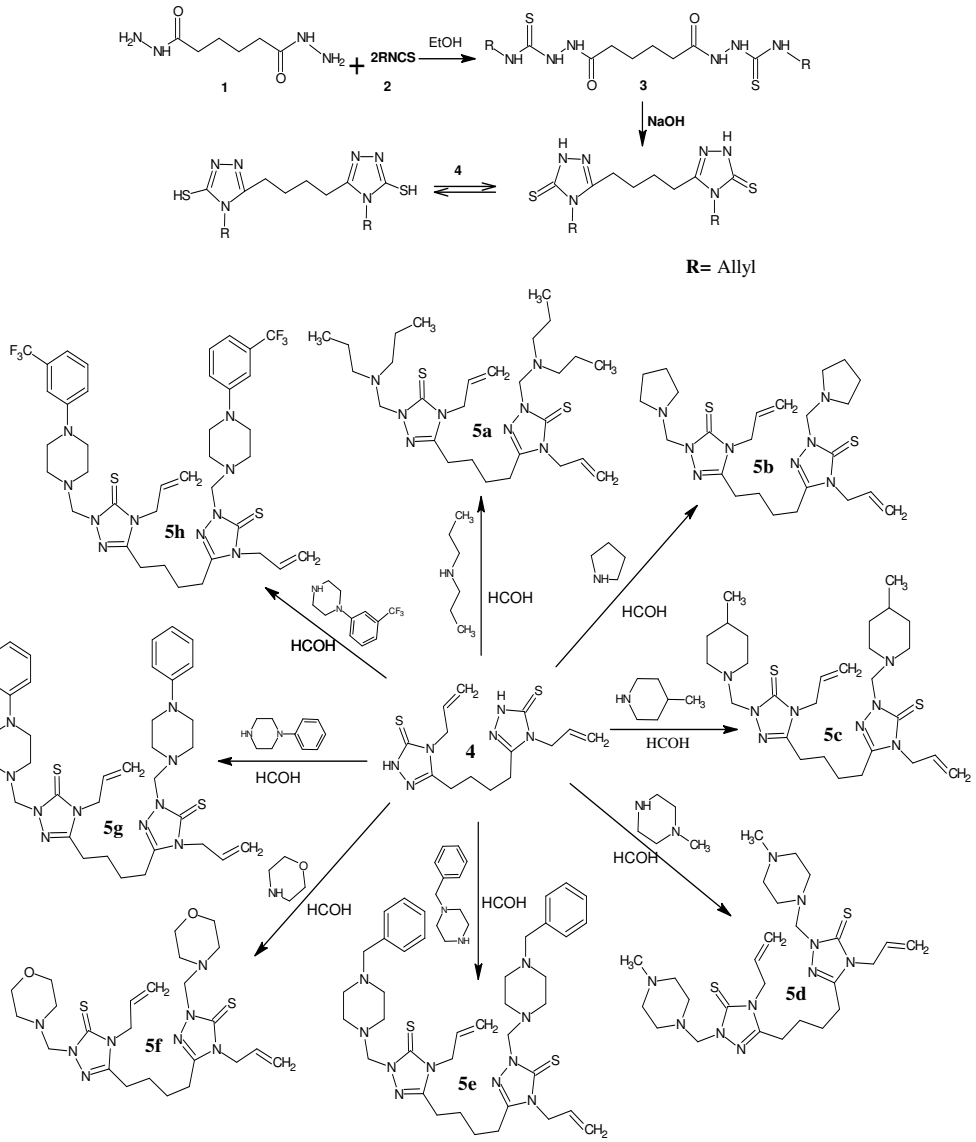
Results and Discussion

Chemistry

The reaction sequences employed for synthesis of title compounds are shown in Scheme 2. In the present work, thiosemicarbazides (**2**) were synthesized by reacting of adipic dihydrazide (**1**) with allyl isothiocyanate (**2**) in the presence of ethanol at reflux temperature by condensation method. The synthesized thiosemicarbazides were reacted with sodium hydroxide in presence of ethanol to obtain 5,5′-butane-1,4-diyl bis [4-allyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**4**). The synthesized bis-triazoles were reacted with formaldehyde and secondary amines to afford Mannich bases (**5a-h**). All compounds displayed IR, ^{1}H and ^{13}C NMR spectra and elemental analyses consistent with the assigned structures. In the ^{1}H NMR spectrum of compounds **(4)**, signal due to –SH group appeared at 13.50-14.08 ppm. Moreover, C=S group resonated at 165.6, 166.7 ppm in the ^{13}C NMR spectra of compounds (**4**). In the IR spectrum of **(4)** the most characteristic absorptions are at 3106- 3109 cm^{-1} -NH bands, 1570 and 1645 cm^{-1} (C=N), 1277 and 1279 cm^{-1}, (C=S) and (C-S-C) stretching bands at 731-781 cm^{-1}. When compounds (**4**) were converted to Mannich bases of 5,5′-butane-1,4-diyl bis [4-allyl-2,4-dihydro-3H-1,2,4-triazole-3-thiones in acidic media, -NH peaks disappeared, while new signal due to –N-CH$_2$-N- group were observed at 5.02-5.16 ppm in the ^{1}H NMR spectra of compounds (**5a-h**). ^{1}H NMR spectrum of compounds, signal due to Ar-H appeared at 6.83-7.34 ppm.

The ^{1}H NMR spectrum of compounds (**5a-h**), N-<u>CH$_2$</u>-CH=CH$_2$ appeared as a doublet at 4.65-4.69 ppm, N-CH$_2$-<u>CH</u>=CH$_2$ appeared as a multiplied at 5.79-5.97 ppm and N-CH$_2$-CH=<u>CH$_2$</u> appeared as a doublet doublet triplet (ddt) trans protons at 5.04-5.09 and appeared as a doublet cis protons at 5.24-5.28. Moreover, C=S group resonated at 167.2-168.6 ppm in the ^{13}C NMR spectra of compounds (**5a-h**). In the IR spectrum of (**5a-h**) the most characteristic

absorptions are at 3021–3093 cm^{-1} aromatic bands, 1562–1678 cm^{-1} (C=N), 1235–1292 cm^{-1}, (C=S) and (C-S-C) stretching bands at 721–797 cm^{-1}. The data for all compounds are given in the experimental section.



Scheme 2

Biological evaluation

Antibacterial and antifungal activity

The antibacterial and antifungal screening revealed that some of the tested compounds showed good inhibition at 1.56–25 µg/mL in DMSO. The compound **4** showed moderate activity against all the fungal strains. Compounds **5b** showed moderate activity against all

the fungal strains. Among the screened compounds **5a** and **5c** showed comparatively good activity against all the fungal strains. Compounds **5d, 5e, 5f, 5g** and **5h** showed good antifungal activity and registered a good inhibition against all the fungal strains. Of all the synthesized derivatives, compounds **5d, 5e, 5f**, **5g** and **5h** were the most active against the investigated strains as compared to the standard drug. **5d 5e, 5g** and **5h** exhibited good antifungal activity almost equivalent to that of standard. The antifungal screening revealed that among the tested compound, **5f** showed excellent activity against all the tested fungal strains, namely C. *albicans* and A. *flavus* at 1.56-3.12 µg/mL concentration and registered a good inhibition of 25-40 mm. The remaining compounds found to be active at higher concentrations, e.g., 6.25 and 25 µg/ml and registered at lower inhibition of 11-15 mm. So, it was concluded that the presence of morpholine and piperazine moiety, besides phenyl, allyl and benzyl groups, was found to be essential for their high antifungal activity.

The compound **4** showed moderate activity against all the bacterial strains. Among the screened compound, **5b** showed moderate activity against all the bacterial strains. All the synthesized derivatives, compounds **5a, 5g, 5d** and **5e** showed comparatively good activity against all the bacterial strains. **5f** exhibited good antibacterial activity almost equivalent to that of standard. The antibacterial screening revealed that among the tested compounds, **5c** and **5h** showed excellent activity against all the tested bacterial strains, namely, E. *coli,* P. *aeruginosa* and S. *aureus* at 3.12-1.56 µg/mL concentration and registered a good inhibition of 22-33 mm. The remaining compounds found to be active at higher concentrations, *e.g*., 6.25 and 25 µg/mL and registered at lower inhibition of 11-20 mm. Also compounds **5c** and **5h** were the most active against the investigated strains as compared to the standard drug. So, it was concluded that the presence of 4-methyl piperidine and trifluoromethyl-phenylpiperazine moiety was found to be essential for their high antibacterial activity.

Antioxidant activity

Since the antioxidants are gaining a lot of importance as panacea for a large number of lifestyle diseases like aging, cancer, diabetes, cardiovascular and other degenerative diseases, it is of immense significance to establish some new antioxidants by a convenient synthetic methodology. Although a number of methods such as ORAC, ABTS, DMPD, FRAP, TRAP, TBA, superoxide radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, xanthine oxidase, cytochrome C, reducing power method, *etc.,* available, the DPPH method is very common and proved as the best[22].

Compound **4** showed moderate antioxidant activity. Among the screened compound **5b** showed moderate antioxidant activity. Of all the synthesized derivatives, compounds **5a, 5d, 5e, 5g** and **5h** showed very good activities which were similar to reference antioxidant compound. Also compound **5c** showed good scavenging activities. From all the synthesized derivatives, compound **5f** exhibited the highest radical scavenging activities which were better than the reference antioxidant compound.

Compound **5f** with morpholine moiety was the best DPPH radical scavenging activity at all concentration, followed by compound **5d, 5e** and **5g** with piperazine moiety, **5a** with dipropylamine and **5h** having triflormethyl phenyl moiety.

As shown in Table 3, the title compounds have scavenging activity between 36.1% and 95.0% within the investigated concentration range. The antioxidant activity of the title compound is obvious that the scavenging activity increases with increasing sample concentration in the range tested.

Table 3. Antioxidant scavenging activity of compounds (**4**) and (**5a-h**) on DPPH$^{\bullet}$ free radical at different concentrations

Tested Compound	DPPH scavenging activity, %				
	62.5 µM	125 µM	187.5 µM	250 µM	312.5 µM
4	36.1±0.6	45.7±0.6	47.9±0.1	51.2±0.6	65.1±1.0
5a	51.1±0.6	61.4±0.2	70.1±0.1	80.2±0.4	88 .1±0.6
5b	40.4±0.2	43.2±0.4	44.7±0.3	55.5±0.1	66.8±0.5
5c	50.1±0.3	53.5±0.4	68.1±0.3	69.7±0.2	72.4±0.2
5d	52.1±0.1	62.9±0.2	72.4±0.2	82.3±0.3	90.1±0.1
5e	53.5±0.4	63.2±0.6	74.5±0.2	83.1±0.2	90.9±0.2
5f	62.3±0.1	71.2±0.2	83.1±0.2	89.9±0.2	95.0±0.1
5g	54.0±0.1	64.4±0.3	75.0±0.1	84.1±0.2	91.6±0.2
5h	52.0±0.1	61.1±0.4	71.3±0.3	80.2±0.2	81.6±0.2
[a]Ascorbic acid	55.1±0.2	65.0±0.2	75.2±0.2	85.8±0.4	91.7±0.2

[a]Ascorbic acid (reference antioxidant compounds) was used as a standard. The scavenging capacities were represented as percentage inhibition and values were the means of three replicates (mean ± SD,n = 3)

Conclusion

The research study reports the successful synthesis and antimicrobial activity of new 1,2,4-triazole and Mannich bases bearing allyl moiety. The antimicrobial activity study revealed that all the compounds tested showed good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of title compounds showed that presence butyl groups and biologically active groups like morpholine, 4-benzylpiperazine, 1-methyl-piperazine, *N*-methylpiperidine and trifluoromethylphenyl-piperazine groups attached to the triazole ring of the title compounds are responsible for good antimicrobial activity. The similar correlation was found to be true in the case of antioxidant activity. All compounds greatly improved their activity compared to the precursors. So, the significant antifungal, antibacterial and antioxidant activity of compounds may be due to the presence of morpholine, *N*-methyl-piperidine and piperazine moiety in addition to thieny, phenyl, methyl and allyl group. Hence it is concluded that there is ample scope for further study.

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