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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of 4-(substituted)-acetylamino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole derivatives as antimicrobial agents

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Abstract Triazoles and its derivatives are found to possess diverse applications in the field of medicine and industry. A series of novel 1,2,4-triazole derivatives have been synthesized as novel antimicrobial agents starting from different 4-substituted benzoic acids. The chemical structures of these newly synthesized compounds were elucidated by Fourier transform infrared spectroscopy (FTIR), ^{1}H NMR, ^{13}C NMR, FAB^{+}-MS spectral data, and elemental analysis. Their antimicrobial activities were investigated against four bacterial strains *S. aureus* (ATCC-25923), *P. aeruginosa* (ATCC-27853), *E. coli* (ATCC-8739), *B. subtilis* (ATCC-6633) and three fungal strains *C. albicans* (MTCC-227), *A. niger* (MTCC-3323), and *F. oxysporum* (MTCC-2087). Preliminary results indicate that some of them exhibit promising activities and deserve further consideration.

Keywords 1,2,4-Triazoles · Cyclization · Zone of inhibition · Antibacterial · Antifungal

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Introduction

The treatment of infectious diseases still remain an important and challenging problem because of a combination of factors including emerging infectious disease and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents (Kaplancikli *et al.*, 2008). There is real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanism of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant.

Through the various molecules designed and synthesized for this aim, in recent years active research has been initiated on heterocycles and the chemistry of 1,2,4-triazoles 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-inflammatory, analgesic antimicrobial agents (Mathew *et al.*, 2006), and antimycotic ones such as fluconazole, itraconazole, voriconazole (Mishra *et al.*, 2010). During the last few decades, considerable attention has been devoted to synthesis of 1,2,4-triazole derivatives possessing such comprehensive biological activities such as antibacterial, antifungal (Holla *et al.*, 1994; Ulusoy *et al.*, 2001; Muhi-Eldeen *et al.*, 1991), antitubercular (Shiradkar *et al.*, 2007 and Mir and Siddiqui, 1970), anticancer, anti-tumor (Shivarama Holla *et al.*, 2003; Al-Soud *et al.*, 2003), anti inflammatory (Wade *et al.*, 1982; Navidpour *et al.*, 2006), anticonvulsant (Ainsworth *et al.*,

1962; Parmar *et al.*, 1977), antidepressant (Kane *et al.*, 1988), hypoglycemic properties (Mhasalkar *et al.*, 1970), openers of Ca^{++}-activated potassium (Maxi-K) channels (Romine *et al.*, 2002), anxiolytic (Gall *et al.*, 1978), and antiviral activity (Witkowski *et al.*, 1972).

Regarding antimicrobial activity, triazole is structurally similar to imidazole molecule. Although triazole and imidazole act by the same mechanism of action, triazole possess advantages over imidazole, which have slow metabolic rate, oral bioavailability and less effect on human sterol synthesis (Kharb *et al.,* 2010). For these reasons imidazoles are slowly being replaced by triazole molecules. It was reported that incorporation of various substituent into heterocyclic ring systems augments biological activities considerably (Wang and Shi, 2001). As secondary amine incorporated heterocycles like thiazole, oxadiazole, and 1,2,4-triazole displayed varied Pharmacological properties. In our previous work, *p*-chloro benzoic acid and *p*-nitro benzoic acid were used for synthesizing 1,2,4-triazole (Mishra *et al.,* 2006 and upmanyu *et al.,* 2011) evaluated for in vitro antimicrobial evaluation. Prompted by these investigations, it was thought to be interesting to synthesize compounds containing 1,2,4-triazole on which secondary amine group was attached and study their antimicrobial activities.

Materials and methods

Chemistry

All the solvents were of LR grade and were obtained from Merck, Rankem, Fluka, and S.D. fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. Reaction sequence employed for the synthesis of title compounds is shown in Scheme 1.

Step 1 general procedure for the synthesis of methyl esters

Required carboxylic acid viz. 4-methyl benzoic acid (a) and 4-methoxy benzoic acid (b) (0.1 mol) was taken in methanol (100 ml) in a round-bottom flask and concentrated sulphuric acid (5.7 ml) was added to that. The mixture was refluxed for 4–6 h. Excess of methanol was then distilled off. After cooling the contents were transferred to separating funnel containing 100 ml of distilled water. The synthesized ester was repeatedly extracted several times with carbon tetrachloride (30 ml). The combined organic layer was washed with solution of sodium-bi-carbonate (20%) to remove any unreacted acid. After washing with distilled water the organic layer was dried over anhydrous $MgSO_4$. Carbon-tetrachloride was then distilled off under reduced pressure to get the ester **1(a, b)**, which was recrystallized from absolute alcohol and melting point determined (Furniss *et al.*, 1989).

Step 2 general procedure for the synthesis of hydrazides

Hydrazine hydrate (99%) (5.7 ml, 0.15 mol) was taken in a flat-bottom flask and solution of methyl ester (0.1 mol) in ethanol was added drop wise with gentle stirring. After complete addition, the mixture was transferred into a round-bottomed flask and refluxed for 4–6 h. Ethanol was distilled off under reduced pressure. The precipitate of acid hydrazide **2(a, b)** found was filtered and recrystallise from ethanol and melting point determined (Yale *et al.*, 1953).

Step 3 synthesis of potassium 3-aroyl dithiocarbazate

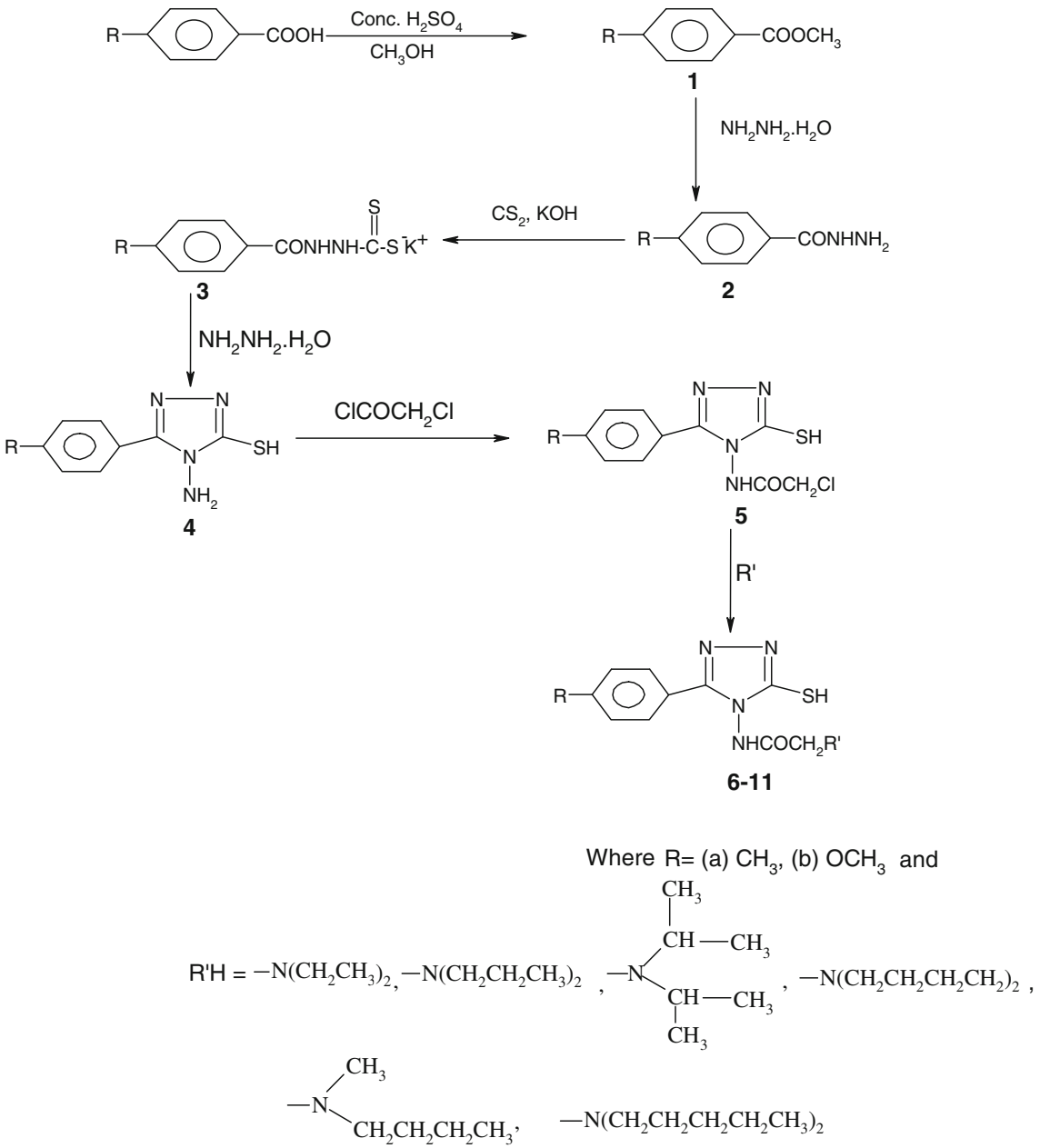
A mixture of potassium hydroxide (0.15 mol), 100 ml of absolute ethanol and (0.1 mol) of the acid hydrazide was treated with (0.15 mol) of carbon-disulfide. This mixture was diluted with 75 ml of absolute ethanol and stirred for 12–16 h. The solvent was distilled off under reduced pressure. The salts **3(a, b)**, prepared as described above, were obtained in nearly quantitative yield and were employed without further purification (Reid and Heindel, 1976).

Step 4 synthesis of 4-amino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole

A suspension of (0.1 mol) of the potassium salt in absolute alcohol (0.2 mol) of 99% hydrazine hydrate and 6 ml of water was refluxed for 2 to 3 h. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide gas and a homogenous solution resulted. Cold distilled water (100 ml) was added and the solution was acidified with concentrated hydrochloric acid. The precipitated solid **4(a, b)** was filtered, washed with two portions 30 ml each, of cold water and recrystallized from aqueous ethanol (50%) (Heindel and Reid, 1980).

Step 5 synthesis of 4-(chloro-acetyl amino)-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole

Compound 4-amino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole (0.1 mol) was taken in a 50 ml of dioxane in a two necked round-bottom flask fitted with reflux condenser and a separating funnel. Chloroacetyl chloride 8.75 ml (0.11 mol) was taken in dioxane (25 ml approx), in the separating funnel. The chloroacetyl chloride was added in small portions to the vessel. After the complete addition, the contents of the flasks were refluxed for an hour or so. After cooling the contents were poured on crushed ice. The precipitated acyl product **5(a, b)** was



Scheme 1 Synthesis of 1,2,4-triazoles derivatives

filtered and washed several times with ice cold distilled water (Eweiss *et al.*, 1986).

Step 6 synthesis of the title compound

Compound 4-(chloroacyl-amino)-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole (0.025 mol) and respective amine (0.030 mol) along with the triethylamine (0.030 mol) were taken in a round-bottomed flask in benzene (75 ml approx). The contents were refluxed for 3–4 h. The precipitated triethylamine hydrochloride was separated out. The organic layer was washed several times with distilled water to remove last traces of hydrochloride. Benzene was distilled off under vacuum and the crude product was separated. Purification of the title compounds **6–11 (a**, **b)** was done by repeated crystallization from appropriate solvents and melting point was determined.

Thin layer chromatography was used to reach the completion of the reaction and purity of the synthesized compounds. Melting points were taken in open glass capillary tubes by using melting point apparatus and were uncorrected. IR spectra in KBr were recorded on a Nicolet-6700 FTIR spectrophotometer. ^{13}C NMR spectra were recorded on Brucker aviance II 400 MHZ spectrophotometer in

DMSO-d_6/$CDCl_3$ using TMS as an internal standard (chemical shifts are expressed in δ, ppm), mass spectra were recorded on Jeol Sx 102/DA-600 mass spectrometer/Data System using fast moving bombardment (FAB) technique and nitrogen analysis were recorded using elemental analyzer Elementar Vario EL III Carlo Erba 1108. The purity of the compounds was checked on silica gel-G coated plates using a mixture of ethyl acetate and petroleum ether (1:1). Visualization was done using iodine vapors. The log *p* values were determined using Hyperchem and Chemdraw software. Substitution pattern and characterization data of the synthesized compounds are reported in Table 1. The spectral data are presented in Table 2.

Biological activities

Antibacterial activities

The newly synthesized compounds were screened for their antibacterial activity against *E. coli* (ATCC-8793), *S. aureus* (ATCC-25923), *P. aeruginosa* (ATCC-27853), and *B. subtilis* (ATCC-6633) (recultured) bacterial strains by paper disc diffusion method (Cruickshank *et al.*, 1975) at 70, 50, and 30 μg/ml concentrations, respectively. All the bacterial strains were procured from (Hi-media) ATCC, USA. A standard inoculum ($1–2 \times 10^7$ c.f.u./ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140°C for an hour. The sterile discs previously soaked in a known concentration of the test compounds (in DMSO) were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37°C. Vancomycin and Amikacin were used as a standard drug. The inhibition zones were measured and compared with the controls and the results are summarized in Table 3 (These data includes the size of filter paper 6 mm).

Antifungal activities

Newly prepared compounds were screened for antifungal activity against *C. albicans* (MTCC-227), *A. niger* (MTCC-3323), and *F. oxysporum* (MTCC-2087) by the same method as in the antibacterial screening but different mediums were used. All the fungal strains were procured from Institute of Microbial Technology, Chandigarh. For antifungal screening against *A. niger*, czapek yeast extract agar was used. Malt yeast agar ($\approx$pH 7.0) was employed as culture media against *C. albicans* and potato sucrose agar was used as culture medium against *F. oxysporum* (Catalogue of strain, 2000). Clotrimazole was used as a standard drug. The inhibition zones were measured and compared with the control and the results are summarized in Table 4 (These data includes the size of filter paper 6 mm).

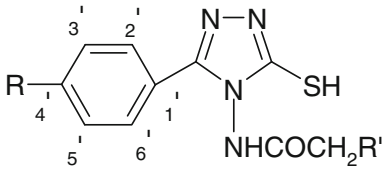
Results and discussion

The synthesis of 4-(substituted)-acetylamino-3-mercapto-5-(4-substituted) phenyl-1,2,4-triazole **6–11**(**a**, **b**) was accomplished as presented in Scheme 1. Aromatic carboxylic acid viz. 4-methyl benzoic acid (a) and 4-methoxy benzoic acid (b) were converted into their respective methyl esters **1**(**a**, **b**). Esters were converted into their hydrazides **2**(**a**, **b**) by reacting with hydrazine hydrate. Hydrazides were reacted with carob-di-sulphide and potassium hydroxide to give their potassium 3-aroyl dithiocarbazate **3**(**a**, **b**) and then these potassium salt were converted into their respective 1,2,4-triazoles **4**(**a**, **b**). These 1,2,4-triazoles were reacted with chloroacetyl chloride to give their acyl derivatives **5**(**a**, **b**). These acyl derivatives were reacted with different secondary amines to give the titled compounds **6–11**(**a**, **b**). Synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and mass spectrum. The IR spectrum exhibited characteristic bands for C–N, C=N, SH, C=O, and N–H at 1330–1370, 1503–1548, 2520–2594, 1640–1711, and 3300–3413 cm^{-1}.

In ^{1}H NMR spectra, a singlet of CONH was found in the range of δ 9.88–10.21 ppm and another singlet of thio group was observed in the range of δ 9.53–9.84 ppm. In ^{13}C NMR spectra, C3 and C5 of the 1,2,4-triazole nucleus were observed in the range of δ 139–163 ppm. Carbonyl carbon and methylene carbon of –$NHCOCH_2N<$ were found between δ 161–178 and δ 47–65 ppm, respectively. A solvent peak of DMSO-d_6 was observed at 44 ppm. Analytical and spectral data were in good agreement with the composition of synthesized compounds.

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **6–11**(**a**, **b**) showed moderate to good inhibition. The screening results indicate that some of the compounds tested exhibited significant antibacterial and antifungal activities when compared with the reference drugs. It was observed that the compound containing 4-methoxy phenyl group at position 5 of the triazole ring and *n*-butyl methyl group (at C_2 of acetamido group) at position 4 of the triazole ring (**9b**) shows maximum bactericidal as well as fungicidal activity as compared with other compounds, whereas **9a** and **7b** showed good activity. The compounds **7a** and **10b** showed moderate activity as compared with **9b**. The other compounds (**6a**, **6b**, **8a**, **8b**, **11a**, and **11b**) showed lower fungicidal effects compared with their bactericidal effects. Log *p* values demonstrated the crucial role of lipophilicity in determining the antimicrobial activity.

Table 1 Physico-chemical properties of the synthesized compounds



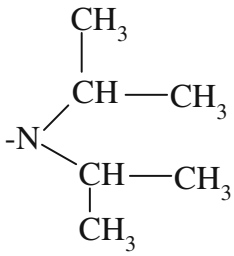
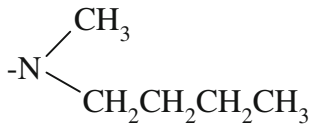
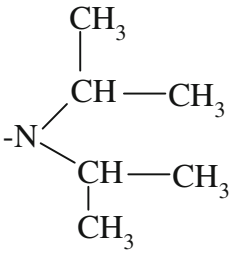
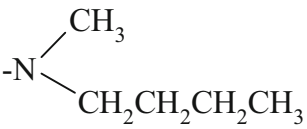
Code no.	*R*	*R′*	Yield (%)	M.P. (°C)	Molecular formula	Nitrogen estimation found (calculated) (%)	Log *P*
6a	$-CH_3$	$-N(CH_2CH_3)_2$	41.5	152–154	$C_{15}H_{21}N_5SO$	21.10 (21.92)	3.69
7a	$-CH_3$	$-N(CH_2CH_2CH_3)_2$	71.2	112–114	$C_{17}H_{25}N_5SO$	20.67 (20.15)	4.75
8a	$-CH_3$		61	142–144	$C_{17}H_{25}N_5SO$	20.01 (20.15)	4.38
9a	$-CH_3$	$-N(CH_2CH_2CH_2CH_3)_2$	74.7	120–122	$C_{19}H_{29}N_5SO$	18.58 (18.65)	5.81
10a	$-CH_3$		74.2	102–104	$C_{16}H_{23}N_5SO$	21.03 (21.00)	4.22
11a	$-CH_3$	$-N(CH_2CH_2CH_2CH_2CH_3)_2$	65.7	148–150	$C_{21}H_{33}N_5SO$	16.50 (17.35)	6.87
6b	$-OCH_3$	$-N(CH_2CH_3)_2$	44.5	192–194	$C_{15}H_{21}N_5SO2$	20.97 (20.88)	3.39
7b	$-OCH_3$	$-N(CH_2CH_2CH_3)_2$	61.7	122–124	$C_{17}H_{25}N_5SO2$	19.12 (19.27)	4.49
8b	$-OCH_3$		46.2	180–182	$C_{15}H_{25}N_5SO2$	19.23 (19.27)	4.09
9b	$-OCH_3$	$-N(CH_2CH_2CH_2CH_3)_2$	57.2	175–177	$C_{19}H_{29}N_5SO_2$	17.96 (17.89)	5.52
10b	$-OCH_3$		64	174–176	$C_{16}H_{23}N_5SO_2$	19.89 (20.04)	3.92
11b	$-OCH_3$	$-N(CH_2CH_2CH_2CH_2CH_3)_2$	62.3	159–161	$C_{21}H_{23}N_5SO_2$	16.73 (16.69)	6.58

Table 2 Spectral data for the newly synthesized compounds

Compounds	FT–IR (KBr, V_{max} cm^{-1})	^{1}H NMR (DMSO-d_6) δ ppm	^{13}C NMR (DMSO-d_6) δ ppm	FAB Mass *m/z*
6a	3305 (N–H), 3000 (Ar-CH), 2550 (S–H), 1800 (C–C overtone of toluene), 1660 (C=O), 1617 (C=C Ar.), 1540 (C=N), 1350 (C–N)	0.98–1.13 (*t*, 2 × 3H, CH_3), 2.40 (s, 3H, Ar-CH_3), 2.57 (*q*, 2 × 2H, CH_2), 3.02 (s, 2H, $COCH_2$), 7.08–7.10 (d, $J = 8.9$, 2H, Ar-H), 7.89–7.91(d, $J = 8.9$, 2H, Ar-H), 9.81 (s, 1H, SH), 10.21 (s, 1H, CONH)	176.6 (NH*C*O), 143.3 (C_3 & C_5), 137 ($C_{4'}$), 132.2 ($C_{1'}$), 130.2 ($C_{3'}$ & $C_{5'}$), 126.4 ($C_{2'}$ & $C_{6'}$), 59 (*C*H_2N), 46 (N*C*H_2CH_3), 21.3 ($C^a_{4'}$), 13 (NCH_2*C*H_3)	319 $(M + H)^+$
7a	3300 (N–H), 3100 (Ar-CH), 2529 (S–H), 1800 (C–C overtone band), 1617 (C=O), 1580 (Ar-C=C), 1530 (C=N), 1374 (C–N)	0.90–1.09 (*t*, 2 × 3H, CH_3), 1.55–2.42 (m, 11H, 4 × CH_2 & Ar-CH_3), 3.06 (s, 2H, $COCH_2$), 7.04–7.06(d, $J = 9.1$, 2H, Ar-H), 7.73–7.75 (d, $J = 9.1$ 2H, Ar-H), 9.63 (s, 1H, SH), 9.98 (s, 1H, CONH)	161(NH*C*O), 139.3 (C_3 & C_5), 135($C_{4'}$),133($C_{1'}$), 129.7($C_{3'}$ & $C_{5'}$), 124.9($C_{2'}$ & $C_{6'}$), 57(CO*C*H_2N), 48.6(N*C*$H_2CH_2CH_2CH_3$), 21.1 ($C^a_{4'}$), 19.4 (NCH_2*C*H_2 CH_3), 11.1 (NCH_2 CH_2 *C*H_3)	347 $(M + H)^+$
8a	3400 (N–H), 3100 (Ar-CH), 2520 (S–H), 1800 (C–C overtone band), 1610 (C=O), 1591 (Ar-C=C), 1530 (C=N), 1406 (C–H of Gem–dimethyl group) 1350 (C–N)	1.13–1.15 (d, $J = 8.8$, 12H, 4 × CH_3), 1.67–1.86 (m, 2H, CH), 2.45 (s, 3H, Ar-CH_3), 2.92 (s, 2H, $COCH_2$), 7.09–7.11 (d, $J = 7.8$, 2H, Ar-H), 7.81–7.83 (d, $J = 8.8$, 2H, Ar-H), 9.79 (s, 1H, SH), 10.13 (s, 1H, CONH)	173(NH*C*O), 143.3(C_3 & C_5), 139.1($C_{4'}$), 130.2($C_{1'}$), 129.6($C_{3'}$ & $C_{5'}$), 126.4($C_{2'}$ & $C_{6'}$), 65.2(CO*C*H_2N), 46.5 (N*C*H), 21.3 (CH_3), 21 ($C^a_{4'}$)	347 $(M + H)^+$
9a	3300 (N–H), 3100 (Ar-CH), 2520 (S–H), 1850 (C–C overtone band), 1660 (C=O), 1604 (Ar-C=C), 1530 (C=N), 1340 (C–N)	0.92–1.11 (t, 2x3H, CH_3), 1.36–1.69 (m, 8H, 2 × CH_2), 2.36–2.59 (m, 7H, 2 × CH_2 & Ar-CH_3), 3.10 (s, 2H, $COCH_2$), 7.01–7.03 (d, $J = 9.1$, 2H, Ar-H), 7.59–7.71 (d, $J = 8.7$ 2H, Ar-H), 9.71 (s, 1H, SH), 9.88 (s, 1H, CONH)	178.6 (NH*C*O), 143(C_3 & C_5), 140.9($C_{4'}$), 130($C_{1'}$), 129.5($C_{3'}$ & $C_{5'}$), 125.9($C_{2'}$ & $C_{6'}$), 55(CO*C*H_2N) 48.6(N*C*$H_2CH_2CH_2CH_3$), 28(NCH_2*C*$H_2CH_2CH_3$), 21.6(NCH_2CH_2*C*H_2CH_3), 20.12($C^a_{4'}$), 13.5(N $CH_2CH_2CH_2$ *C*H_3)	375 $(M + H)^+$
10a	3300 (N–H), 3100 (Ar-CH), 2520 (S–H), 1850 (C–C overtone band), 1660 (C=O), 1619 (Ar-C=C), 550 (C=N), 1330 (C–N)	0.90–1.06 (t, 3H, CH_3), 1.27–1.42 (m, 4H, 2 × CH_2), 2.36–2.59 (m, 8H, 2 × CH_3 & CH_2), 3.00 (s, 2H, $COCH_2$), 7.05–7.07(d, $J = 8.6$, 2H, Ar-H), 7.88–7.90 (d, $J = 8.4$, 2H, Ar-H), 9.53 (s, 1H, SH), 10.14 (s, 1H, CONH)	178.3 (NH*C*O), 163.5 (C_3 & C_5), 140.9 ($C_{4'}$), 133 ($C_{1'}$), 129.5 ($C_{3'}$ & $C_{5'}$), 125.8 ($C_{2'}$ & $C_{6'}$), 58($COCH_2$N), 50.9 (N–*C*H_2), 49.5 (N–*C*H_3), 27.9 (N–CH_2*C*H_2), 21.5 (N–CH_2CH_2 *C*H_2), 19.98 ($C^a_{4'}$), 13.5 (N– $CH_2CH_2CH_2\underline{C}H_3$)	333 $(M + H)^+$
11a	3300(N–H), 3000 (Ar-CH), 2570 (S–H), 1850 (C–C overtone band), 1660 (C=O), 1604 (Ar-C=C), 1503 (C=N), 1344 (C–N)	0.71–1.56 (m, 16H, CH_2 & CH_3), 2.31–2.74 (m, 7H, CH_2 & Ar-CH_3), 3.21 (s, 2H, $COCH_2$), 7.33–7.35 (d, $J = 8.3$, 2H, Ar-H), 8.17–8.19 (d, $J = 8.0$, 2H, Ar-H), 9.76 (s, 1H, SH), 10.07 (s, 1H, CONH)	171(NH*C*O), 142 (C_3&C_5), 137($C_{4'}$), 133($C_{1'}$), 129.7($C_{3'}$& $C_{5'}$), 126.5($C_{2'}$&$C_{6'}$), 51 (CO*C*H_2N), 47.7(N*C*$H_2CH_2CH_2CH_2CH_3$), 28.9($NCH_2\underline{C}H_2CH_2CH_2CH_3$), 25.7($NCH_2\underline{C}H_2$ *C*$H_2CH_2CH_3$),22.1($C^a_{4'}$),22($NCH_2CH_2CH_2$*C*H_2CH_3), 13.9($NCH_2CH_2CH_2CH_2$ *C*H_3)	403 $(M + H)^+$
6b	3305 (N–H), 3100 (Ar-CH), 2843 (CH–Methyl), 2594 (S–H), 1660 (C=O), 1611 (C=C Ar.), 1523 (C=N), 1350 (C–N), 1252 (C–O–C asymm. str.), 1073 (C–O–C symm. str.)	1. 0.98–1.03 (*t*, 2 × 3H, CH_3), 2.57 (*q*, 2 × 2H, CH_2), 3.02 (s, 2H, $COCH_2$), 3.89 (s, 3H, Ar-OCH_3), 6.74 (d, 2H, Ar-H), 7.85(d, 2H, Ar-H), 9.80 (s, 1H, SH), 10.18 (s, 1H, CONH)	177.5 (NH*C*O), 162.6 ($C_{4'}$), 147 (C_3&C_5), 128.4 ($C_{1'}$), 128 ($C_{2'}$& $C_{6'}$), 115.3 ($C_{3'}$&$C_{5'}$), 59 (NHCO*C*H_2), 56 ($C^a_{4'}$), 46.2 (N*C*H_2CH_3), 13 (NCH_2*C*H_3)	335 $(M + H)^+$

Table 2 continued

Compounds	FT–IR (KBr, V_{max} cm^{-1})	^{1}H NMR (DMSO-d_6) δ ppm	^{13}C NMR (DMSO-d_6) δ ppm	FAB Mass *m/z*
7b	3413 (N–H), 3100 (Ar-CH), 2878 (CH–Methyl), 2523 (S–H), 1660 (C=O), 1612 (C=C Ar.), 1548 (C=N), 1360 (C–N), 1254 (C–O–C asymm. str.), 1027 (C–O–C symm. str.)	0.90–0.95 (*t*, 2 × 3H, CH_3), 1.55–2.36 (m, 8H, 4 × CH_2), 3.06 (s, 2H, $COCH_2$), 3.78(s, 3H, Ar-OCH_3), 7.03 (d, 2H, Ar-H), 7.70–7.72 (d, 2H, Ar-H), 9.67 (s, 1H, SH), 9.92 (s, 1H, CONH)	177.5 (NH*C*O), 162.6 ($C_{4'}$), 147 (C_3&C_5), 128.4 ($C_{1'}$), 128 ($C_{2'}$& $C_{6'}$), 115.1 ($C_{3'}$&$C_{5'}$), 57 (NHCOCH_2), 53 (N$CH_2CH_2CH_3$), 56 ($C^{a}_{4'}$), 17 ($NCH_2CH_2CH_3$), 11.7 (NCH_2 CH_2CH_3)	363 $(M + H)^+$
8b	3317 (N–H), 3000 (Ar-CH), 2839 (CH–Methyl), 2520 (S–H), 1650 (C=O), 1605 (C=C Ar.), 1523 (C=N), 1406 (CH bend.–Gem–Dimethyl) 1352 (C–N), 1253 (C–O–C asymm. str.), 1072 (C–O–C symm. str.)	1.14 (d, 12H, 4 × CH_3), 1.67–1.86 (m, 2H, CH), 2.94 (s, 2H, $COCH_2$), 3.65 (s, 3H, Ar-OCH_3), 7.08 (d, 2H, Ar-H), 7.76 (d, 2H, Ar-H), 9.84 (s, 1H, SH), 10.11 (s, 1H, CONH)	177 (NH*C*O), 162.6 ($C_{4'}$), 160.9 (C_3&C_5), 128.6 ($C_{1'}$), 128.3 ($C_{2'}$& $C_{6'}$) , 115.3 ($C_{3'}$&$C_{5'}$), 56 ($C^{a}_{4'}$), 54 (NHCOCH_2), 46.5 (N*C*H), 19.1 (NCH–CH_3)	363 $(M + H)^+$
9b	3330 (N–H), 3100 (Ar-CH), 2860 (CH–Methyl), 2540 (S–H), 1711 (C=O), 1612 (C=C Ar.), 1518 (C=N), 1350 (C–N), 1252 (C–O–C asymm. str.), 1071 (C–O–C symm. str.), 690 (C–H bend.)	0.92–0.97 (t, 2 × 3H, CH_3), 1.36–1.69 (m, 8H, 2 × CH_2), 2.36–2.59 (m, 4H, 2 × CH_2), 3.10 (s, 2H, $COCH_2$), 3.61 (s, 3H, Ar-OCH_3), 7.07 (d, 2H, Ar-H), 7.66 (d, 2H, Ar-H), 9.79 (s, 1H, SH), 9.96 (s, 1H, CONH)	177.5 (NH*C*O), 162.6 ($C_{4'}$), 160.9 (C_3&C_5), 128.4 ($C_{1'}$), 128 ($C_{2'}$& $C_{6'}$), 115.3 ($C_{3'}$&$C_{5'}$), 59 (NHCOCH_2), 56 ($C^{a}_{4'}$), 53 (NCH_2 $CH_2CH_2CH_3$), 31.9($NCH_2CH_2CH_2CH_3$),19.7($NCH_2CH_2CCH_2CH_3$), 13.9 (NCH_2 $CH_2CH_2CH_3$)	391 $(M + H)^+$
10b	3350 (N–H), 3095 (Ar-CH), 2840 (CH–Methyl), 2520 (S–H), 1710 (C=O), 1610 (C=C Ar.), 1511 (C=N), 1351 (C–N), 1254 (C–O–C asymm. str.), 1072 (C–O–C symm. str.), 692 (C–H bend.)	0.90–0.95 (t, 3H, CH_3), 1.27–1.42 (m, 4H, 2 × CH_2), 2.36–2.59 (m, 5H, CH_3 & CH_2), 3.00 (s, 2H, $COCH_2$), 3.57 (s, 3H, Ar-OCH_3), 7.02(d, 2H, Ar-H), 7.92 (d, 2H, Ar-H), 9.61 (s, 1H, SH), 10.07 (s, 1H, CONH)	177.5 (NH*C*O), 162.6 ($C_{4'}$), 161 (C_3&C_5), 128.4 ($C_{1'}$), 128 ($C_{2'}$& $C_{6'}$), 115.1 ($C_{3'}$&$C_{5'}$), 62 (NHCOCH_2), 59 ($C^{a}_{4'}$), 56 (N–CH_2 $CH_2CH_2CH_3$), 39 (N–CH_3), 31.6(N–$CH_2CH_2CH_2CH_3$), 20 (N–CH_2 $CH_2CH_2CH_3$), 13.9 (N–CH_2 $CH_2CH_2CH_3$)	349 $(M + H)^+$
11b	3316 (N–H), 3100 (Ar-CH), 2840 (CH–Methyl), 2520 (S–H), 1665 (C=O), 1615 (C=C Ar.), 1523 (C=N), 1352 (C–N), 1253 (C–O–C asymm. str.), 1079 (C–O–C symm. str.), 693 (C–H bend.)	0.70–1.54 (m, 16H, CH_2 & CH_3), 2.33–2.76 (m, 4H, CH_2), 3.21 (s, 2H, $COCH_2$), 3.63 (s, 3H, Ar-OCH_3), 7.32 (d, 2H, Ar-H), 8.17 (d, 2H, Ar-H), 9.74 (s, 1H, SH), 10.09 (s, 1H, CONH)	177.5 (NH*C*O), 162.6 ($C_{4'}$), 160.9 (C_3&C_5) , 128.4 ($C_{1'}$), 128($C_{2'}$& $C_{6'}$),115.1($C_{3'}$&$C_{5'}$), 56(NHCOCH_2), 47.1 (N$CH_2CH_2CH_2CH_2CH_3$),28.5(NCH_2 $CH_2CH_2CH_2CH3$),25.5($NCH_2CH_2CH_2CH_2CH_3$), 22.1($NCH_2CH_2CH_2$ CH_2CH_3),14.1($NCH_2CH_2CH_2CH_2CH_3$)	419 $(M + H)^+$

Table 3 Antibacterial activities of synthesized compounds **6–11(a, b)**

Compounds	Diameter of zone of Inhibition (mm)[a]											
	S. aureus			*B. subtilis*			*P. aeruginosa*			*E.coli*		
	70 μg/ml	50 μg/ml	30 μg/ml	70 μg/ml	50 μg/ml	30 μg/ml	70 μg/ml	50 μg/ml	30 μg/ml	70 μg/ml	50 μg/ml	30 μg/ml
6a	8.20 ± 0.92	7.40 ± 0.92	6.36 ± 0.64	8.26 ± 1.36	7.43 ± 0.60	6.40 ± 0.30	9.00 ± 0.80	8.40 ± 0.55	7.30 ± 0.62	9.00 ± 0.80	8.40 ± 0.55	7.30 ± 0.62
7a	10.03 ± 0.85	9.33 ± 0.80	8.20 ± 0.56	10.13 ± 0.56	9.86 ± 0.56	8.93 ± 0.85	11.13 ± 0.56	10.23 ± 0.51	9.56 ± 0.40	11.10 ± 0.46	10.33 ± 0.60	9.33 ± 0.55
8a	9.10 ± 0.75	8.20 ± 0.46	7.30 ± 0.44	9.23 ± 0.51	8.23 ± 0.55	7.20 ± 0.44	10.13 ± 0.56	9.26 ± 0.47	8.30 ± 0.44	10.46 ± 0.40	9.20 ± 0.53	8.20 ± 0.44
9a	13.96 ± 0.85	12.10 ± 0.60	11.10 ± 0.46	12.20 ± 0.53	11.23 ± 1.21	10.27 ± 0.65	14.13 ± 0.61	13.56 ± 0.45	12.30 ± 0.44	13.23 ± 0.70	12.10 ± 0.75	11.20 ± 0.56
10a	9.26 ± 0.38	8.23 ± 0.55	7.40 ± 0.46	9.23 ± 0.65	8.20 ± 0.53	7.43 ± 0.60	10.30 ± 0.56	9.33 ± 0.58	8.20 ± 0.66	9.20 ± 0.44	8.56 ± 0.40	7.26 ± 0.56
11a	8.16 ± 0.70	7.10 ± 0.76	6.03 ± 0.65	8.23 ± 0.56	7.20±0.66	6.46 ± 0.71	9.17 ± 0.56	8.13 ± 0.56	7.13 ± 0.65	8.13 ± 0.56	7.16 ± 0.56	6.26 ± 0.50
6b	9.36 ± 0.40	8.33 ± 1.14	7.63 ± 0.87	9.03 ± 0.56	8.53 ± 1.14	7.23 ± 0.65	10.76 ± 1.03	9.56 ± 0.99	8.26 ± 0.47	11.23 ± 1.10	10.13 ± 0.90	9.20 ± 1.25
7b	14.80 ± 0.75	13.20 ± 0.65	12.66 ± 0.45	12.83 ± 0.70	11.03 ± 0.71	10.16 ± 0.57	14.13 ± 0.56	13.16 ± 0.47	12.20 ± 0.56	14.16 ± 0.35	12.23 ± 0.56	11.10 ± 0.40
8b	10.30 ± 0.46	9.26 ± 0.41	8.20 ± 0.50	10.53 ± 0.55	9.30 ± 0.60	8.50 ± 0.41	13.33 ± 0.55	12.26 ± 0.56	11.06 ± 0.65	12.36 ± 0.38	10.26 ± 0.47	9.43 ± 0.50
9b	17.23 ± 0.35	15.16 ± 0.65	13.13 ± 0.45	15.16 ± 0.56	13.03 ± 0.75	11.20 ± 0.90	18.20 ± 0.55	16.16 ± 0.56	15.10 ± 1.05	17.30 ± 0.56	15.06 ± 0.60	13.03 ± 0.85
10b	13.46 ± 0.56	11.03 ± 0.56	10.16 ± 0.51	11.17 ± 0.65	10.20 ± 0.65	9.27 ± 0.56	12.20 ± 0.56	10.40 ± 0.56	9.20 ± 0.65	9.60 ± 0.56	8.63 ± 0.56	7.20 ± 1.15
11b	9.16 ± 0.65	8.13 ± 0.65	7.20 ± 0.75	8.03 ± 0.56	7.26 ± 0.56	6.13 ± 0.45	8.10 ± 0.56	7.13 ± 0.51	6.23 ± 0.65	10.16 ± 0.50	9.26 ± 0.56	8.97 ± 0.56
Vancomycin[b]			18.20 ± 0.30			17.43 ± 0.35						
Amikacin[b]									22.10 ± 0.36			21.27 ± 0.56

[a] Zone of inhibition are represented in mm at different concentration of synthesized compounds and no inhibition was showed by control.

[b] Vancomycin and Amikacin were used as standard drug at 30 μg/ml.

[c] All the values are in Mean ± SEM. Statistical analysis of data was carried out by One-way ANOVA.

Table 4 Antifungal activities of synthesized compounds **6–11(a, b)**

Compounds	Diameter of zone of Inhibition (mm)[a]								
	C. albicans			*A. niger*			*F. oxysporum*		
	70 μg/ml	50 μg/ml	30 μg/ml	70 μg/ml	50 μg/ml	30 μg/ml	70 μg/ml	50 μg/ml	30 μg/ml
6a	9.23 ± 0.56	8.70 ± 0.46	7.13 ± 0.65	10.23 ± 0.56	9.23 ± 0.56	8.20 ± 0.46	7.33 ± 0.55	6.23 ± 0.21	6.03 ± 0.60
7a	11.31 ± 0.65	10.46 ± 0.40	9.13 ± 0.70	14.43 ± 0.45	12.33 ± 0.50	11.23 ± 0.65	9.96 ± 1.25	8.40 ± 0.46	7.26 ± 0.60
8a	10.10 ± 0.75	9.16 ± 0.47	8.03 ± 1.20	12.23 ± 0.56	11.03 ± 0.65	10.13 ± 0.55	8.33 ± 0.21	7.17 ± 0.65	6.06 ± 0.65
9a	13.23 ± 0.65	12.20 ± 0.56	11.13 ± 0.65	18.03 ± 0.56	16.16 ± 0.55	15.13 ± 0.65	11.43 ± 0.56	9.20 ± 0.66	8.17 ± 1.15
10a	10.10 ± 0.70	9.23 ± 0.70	8.16 ± 0.55	9.17 ± 0.55	8.26 ± 0.65	7.00 ± 1.10	8.13 ± 0.65	7.20 ± 0.70	6.03 ± 1.35
11a	9.23 ± 0.80	8.10 ± 0.60	7.26 ± 0.65	12.13 ± 0.70	11.26 ± 0.65	9.06 ± 0.65	9.13 ± 0.65	8.23 ± 0.65	7.07 ± 0.65
6b	10.36 ± 0.75	9.13 ± 0.71	8.10 ± 0.55	11.11 ± 0.56	10.20 ± 0.66	9.13 ± 0.80	9.06 ± 1.17	8.21 ± 1.20	7.26 ± 0.81
7b	15.06 ± 0.75	13.23 ± 0.75	12.53 ± 0.40	17.03 ± 0.56	15.23 ± 0.65	14.03 ± 0.65	12.16 ± 0.65	11.10 ± 0.56	10.20 ± 0.56
8b	13.80 ± 1.00	12.13 ± 0.56	11.23 ± 0.65	13.00 ± 0.56	11.26 ± 0.60	10.13 ± 0.45	9.06 ± 1.20	8.13 ± 0.61	7.26 ± 0.81
9b	20.40 ± 0.46	18.26 ± 0.55	16.13 ± 0.65	22.43 ± 0.40	20.13 ± 0.70	18.13 ± 0.65	16.16 ± 0.70	14.26 ± 0.56	13.06 ± 0.80
10b	14.16 ± 0.65	12.20 ± 0.56	11.13 ± 1.16	13.16 ± 0.56	11.13 ± 1.16	10.56 ± 0.75	7.23 ± 0.70	6.26 ± 0.65	6.16 ± 0.55
11b	12.17 ± 0.75	11.26 ± 0.65	10.13 ± 0.65	13.03 ± 0.65	12.17 ± 0.60	11.16 ± 0.70	8.20 ± 0.76	7.21 ± 0.76	6.17 ± 0.56
Clotrimazole[b]			21.87 ± 1.07			22.56 ± 0.51			19.26 ± 0.56

[a] Zone of inhibition are represented in mm at different concentration of synthesized compounds and no inhibition was showed by control.

[b] Clotrimazole was used as standard drug at 30 μg/ml.

[c] All the values are in Mean ± SEM. Statistical analysis of data was carried out by One-way ANOVA.

Conclusion

We report successful synthesis and antimicrobial activity of a new 1,2,4-triazole moiety. The antimicrobial activity study revealed that some of the tested compounds showed moderate to good antibacterial and antifungal studies against most abundant strains. Structure and biological activity relationship of title compounds showed that *p*-methoxy group is preferable in position 5-phenyl as compare to *p*-methyl group. The antimicrobial activity was enhanced serially with increase in number of carbon atom (up to *n*-dibutyl amine) and decreased with branch chain substitution.

The field is further open for study of these compounds with respect to toxicity, chronic toxicity, pharmacokinetics, and clinical studies to establish these molecules as drugs in the market.

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References

Ainsworth C, Nelson R, Easton M (1962) The anticonvulsant activity of 1,2,4-triazoles. J Med Chem 5(2):383–389. doi:10.1021/jm01237a016

Al-Soud YA, Al-Masoudi NA, Ferwanah AS (2003) Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents. Bioorg Med Chem 11:1701–1708. doi:10.1016/S0968-0896(03)00043-9

Catalogue of strain, microbial type culture collection and gene bank (2000) published by Institute of Microbial Technology (IMTECH), 5th edn. Chandigarh, (Medium No-137), pp 130–138

Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975) Medicinal microbiology, vol II, 12th edn. Churchill Livingstone, London, pp 196–202

Eweiss NF, Bahajaj AA, Elsherbini EA (1986) Synthesis of heterocycles part VI. Synthesis and antimicrobial activity of some 4-amino-5-aryl-1,2,4-triazole-3-thiones and their derivatives. J Heterocycl Chem 23:1451–1458

Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR (1989) Vogel's textbook of practical organic chemistry, 5th edn. Longman Scientific and Technical, London, pp 1077–1078

Gall M, Lathi RA, Rudzik AD, Duchamp DJ, Chidester C, Scahill T (1978) Novel anxiolytic agents derived from alpha-amino-alpha-phenyl-o-tolyl-4H-triazoles and -imidazoles. J Med Chem 21(6):542–548. doi:10.1021/jm00204a008

Heindel ND, Reid JR (1980) 4-Amino-3-mercapto-4H-1,2,4-triazoles and propargyl aldehydes: a new route to 3-R-8-aryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazepines. J Heterocycl Chem 17:1087–1088. doi:10.1002/jhet.5570170547

Holla BS, Kallurya B, Sridhar KR, Drake E, Thomas LM, Bhandary KK, Levine MJ (1994) Synthesis, structural characterization, crystallographic analysis and antibacterial properties of some nitrofuryl triazolo [3,4-b]-1,3,4-thiadiazines. Eur J Med Chem 29:301–308. doi:10.1016/0223-5234(94)90100-7

Holla BS, Veerendra B, Shivananda MK, Poojary B (2003) Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem 38:759–767

Kane JM, Dudley MW, Sorensen SM, Miller FP (1988) 2,4-Dihydro-3H–1,2,4-triazole-3-thiones as potential antidepressant agents. J Med Chem 31:1253–1258. doi:10.1021/jm00298a021

Kaplancikli ZA, Turan-Zitouni G, Ozdemir A, Revail G (2008) New triazole and triazolothiadiazine derivatives as possible antimicrobial agents. Eur J Med Chem 43(1):155–159. doi:10.1016/j.ejmech.2007.03.019

Kharb R, Sharma PC, Yar MS (2010) Pharmacological significance of triazole scaffold. J Enz Inhib Med Chem 26(1):1–21. doi:10.3109/14756360903524304

Mathew V, Keshavayya J, Vaidya VP, Reddy BM (2006) Heterocyclic system containing bridgehead nitrogen atom: Synthesis and pharmacological activities of some substituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles. Eur J Med Chem 41:1048–1058. doi:10.1016/j.ejmech.2006.03.018

Mhasalkar MY, Shah MH, Nikam ST, Anantanarayanan KG, Deliwala CV (1970) J Med Chem 13(4):672–674. doi:10.1021/jm00298a021

Mir I, Siddiqui MT (1970) Antituberculosis agents-I:α-[5-(2-Furyl)-1,2,4-triazol-3-ylthio] acethydrazide and related compounds. Tetrahedron 26:5235–5238. doi:10.1016/S0040-4020(01)98732-0

Mishra P, Upmanyu N, Mehta A (2006) In vitro anti-microbial activity of some 5-aryl-4-(substituted amino)-3-mercapto-1,2,4-triazole. Indian J Microbiol 46(4):401–404

Mishra R, Kumar R, Kumar S, Majeed J, Rashid M, Sharma S (2010) Synthesis and in vitro antimicrobial activity of some triazole derivatives. J Chil Chem Soc 55(3):359–362

Muhi-Eldeen Z, Nadir M, Aljobory NR, Husseen F, Stohs SJ (1991) Synthesis and antimicrobial evaluation of 3-(4-tert-amino-2-butynyl)thio and alkyl/alkenylthio-4,5-disubstituted-4H–1,2,4-triazoles. Eur J Med Chem 26:237–241. doi:10.1016/0223-5234(91)90035-L

Navidpour L, Shafaroodi H, Abdi K, Amini M, Ghahremani MH, Dehpour AR, Shafiee A (2006) Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4H-1,2,4-triazoles as selective COX-2 inhibitors. Bioorg Med Chem 14:2507–2517. doi:10.1016/j.bmc.2005.11.029

Parmar SS, Chaudhary M, Chaudhary AK, Kumar S, Spiro PR (1977) Anticonvulsant activity and selective inhibition of NAD-dependent oxidations in rat brain homogenates by newer mercaptotriazoles. J Pharm Sci 66(7):971–975

Reid JR, Heindel ND (1976) Improved syntheses of 5-substituted-4-amino-3-mercapto-(4H)-1,2,4-triazoles. J Heterocycl Chem 13:925–926

Romine JL, Martin SW, Gribkoff VK, Boissard CG, Dworetzky SI, Natale J, Li Y, Gao Q, Meanwell NA, Starrett JE (2002) 4,5-Diphenyltriazol-3-ones: openers of large-conductance Ca^{2+}-activated potassium (Maxi-K) channels. J Med Chem 45(14):2942–2952. doi:10.1021/jm010569q

Shiradkar M, Suresh Kumar GV, Dasari V, Tatikonda S, Akula KC, Shah R (2007) Clubbed triazoles: a novel approach to antitubercular drugs. Eur J Med Chem 42:807–816. doi:10.1016/j.ejmech.2006.12.001

Ulusoy N, Aysel G, Gűiten Ö (2001) Synthesis and antimicrobial activity of some 1,2,4-triazole-3-mercaptoacetic acid derivatives. II Farmaco 56:947–952

Upmanyu N, Kumar S, Kharya MD, Shah K, Mishra P (2011) Synthesis and antimicrobial evaluation of some novel 1,2,4-triazole. Acta Pol Pharm Drug Res 68(2):213–221

Wade PC, Vogt BR, Kissick TP, Simpkins DM, Palmer LM, Millonig RC (1982) 1-acyltriazoles as anti-inflammatory agents. J Med Chem 25:331–333. doi:10.1021/jm00345a021

Wang Z, Shi H (2001) Novel synthesis of condensed heterocyclic systems containing 1,2,4-triazole ring. Synth Commun 31: 2841–2848. doi:10.1081/SCC-100105335

Witkowski JT, Robins RK, Sidwell RW, Simon LN (1972) Design, synthesis, and broad spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and related nucleosides. J Med Chem 15(11):1150–1154. doi:10.1021/jm00281a014

Yale HL, Losee K, Martins J, Holsing M, Perry FM, Bernstein J (1953) Chemotherapy of experimental tuberculosis VIII. The synthesis of acid hydrazides, their derivatives and related compounds. J Am Chem Soc 75:1933–1942