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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Pharmacological screening of some newly synthesized triazoles for H_1 receptor antagonist activity

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Abstract The present work deals with the pharmacological screening of some newly synthesized triazoles. A series of 1,2,4-triazoles have been synthesized using benzoic acid or 4-chloro benzoic acid as the starting materials. The synthesized compounds were characterized by physical and spectral analysis viz., Fourier transform infrared spectroscopy, ^{1}H nuclear magnetic resonance, ^{13}C nuclear magnetic resonance, Gas Chromatography-Mass Spectrometry and elemental analysis Carbon, Hydrogen and Nitrogen analysis in order to confirm the structure. Acute toxicity studies were carried out in accordance with the Organization for Economic Co-operation and Development guideline 425. The compounds were not found to be lethal even at a dose level of 2000 mg/kg. Pharmacological evaluation was done following three intact animal experiments and one experiment on the isolated tissue. Results of the study indicated that the compound **7bi** and **7bj** protected up to 60% against histamine-induced dyspnea. Antihistaminic nature of the test compounds **7bi**, **7bj**, **7ai**, and **7bk** were also confirmed by the loss of catalepsy after the administration of clonidine (1%, s.c.). During experiments on isolated tissue, suppression of dose-response curve of histamine indicates a noteworthy denouement in favor of the said effect.

Keywords Triazoles · Amines · Antihistaminic · H_1 antagonist

Introduction

The interests in heterocyclic compounds are growing day-by-day, as they are occupying a significant role in our biological system. Naturally occurring heterocyclic compounds are extremely common as, for example, DNA, RNA, enzyme cofactors, plant pigments, hemoglobin, vitamins, and alkaloids etc. (Saini et al. 2013). Especially the chemistry of heterocyclic compounds containing three nitrogen atoms continue to be of interest on account of their different biological activities (Martins et al. 2015). Among heterocyclic compounds, triazoles are five membered compounds having three nitrogen atoms at different positions. Most of the triazole containing compounds are biologically active which attribute to the sense that they are deep seated part of the biomolecular diversity (Al-Masoudi et al. 2006; Asif 2014). In the last few decades, the pharmacology of 1,2,4-triazoles and their analogues achieved considerable relevance because of their wide range of biological importance (Godhani et al. 2015). The synthesis of triazoles and their derivatives have acquired considerable attention attributing to their medicinal and biological importance. Compounds bearing 1,2,4-triazole possess a variety of biological applications such as anti-inflammatory (Pattan et al. 2012), hypoglycemic (Blank et al. 1972), anticonvulsant (Kamboj et al. 2015), antitubercular (Ozdemir et al. 2007), anxiolytic (Cetin and Gecibesler 2015), antidepressant (Kane et al. 1988), antimicrobial (Mange et al. 2013; Patil et al. 2013; Bhat et al. 2001; Gupta and Jain 2015), antitumor (Romagnoli et al. 2010; Li et al. 2012;

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Hou et al. 2011), anticancer (Bekircan et al. 2006), and antihistaminic activities (Alagarsamy et al. 2006). A number of triazoles are used with significant importance and clinical applications e.g., fluconazole, itraconazole, voriconazole, triazolam, alprazolam, etizolam, furacylin and ribavirin (Uygun et al. 2013). The incorporation of various substituent into 1,2,4-triazole ring and its fusion with various heterocyclic systems have resulted in compounds with enhanced activities. Amongst these, the mercapto and amino substituted 1,2,4-triazoles occupy broad spectrum as well as are also of great utility in preparative organic chemistry (Shaker 2006). In continuation of our ongoing studies of the chemistry of 1,2,4-triazole, herein we report the synthesis of several amine linked triazoles. Terminal amine functional groups are present in many antihistaminic compounds (DeRuiter 2001). However, the primary structural differences involve the nature of the para aromatic ring substituent and, more significantly, the nature of the terminal amine nitrogen substituent. Cyclizine and chlorcyclizine are the simplest examples of the N-methylpiperazines having antihistaminic (H_1 antagonist) property. Nitrogen-containing heterocyclic compounds, viz.: pemirolast and azelastine, are also known for their similar action. In view of the facts stated above, we synthesized a series of triazoles for their significant biological activities.

Materials and methods

All the chemicals and solvents used are of L.R. grade procured from Merck, Sigma-Aldrich or Spectrochem. The method for synthesis of the title compounds involves the conversion of carboxylic acids to their corresponding esters, then to hydrazides, and finally into triazoles via dithiocarbazate salts (Upmanyu et al. 2011b, c). The triazoles then reacted with chloroacetyl chloride and further treated with the secondary amines in order to get the final product. The route of synthesis for the triazoles was outlined in scheme of synthesis (Figs. 1 and 2).

Steps of synthesis

*Synthesis of methyl ester (**2a–b**)*

The aromatic carboxylic acid (0.1 mol) was taken in a round-bottom flask and dissolved in methanol (100 mL). Concentrated sulphuric acid (2 mL) was added to it. The mixture was refluxed for 6 h. Excess of methanol was then distilled off at reduced pressure. After cooling, water was added to the flask and extracted several times with carbon tetrachloride. The organic layer was washed with 5% sodium bicarbonate to remove unreacted acid. The solvent was then distilled off after drying with exsiccated sodium sulfate (Chernyshev et al. 2006). The precipitate so obtained was weighed and melting/boiling point determined.

Methyl benzoate (**2a**) Colorless liquid. This compound (**2a**) was prepared from benzoic acid (**1a**) (0.1 mol, 12.2 g) and methanol (100 mL) according to the general procedure. The product obtained as colorless liquid. 9.5 g (70%); b.p. 198–201 °C; Fourier transform infra red (FTIR) (KBr) ν_{max}: 3080, 2969, 1720, 1602, 1375, 1113, 745 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 7.88$ (2H, m, H-2′, H-6′), 7.54 (3H, m, H-3′, H-4′, H-5′), 3.83 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 165.2$ (C, C=O), 131.7 (CH, C-4′), 130.4 (C, C-1′), 128.0 (CH, C-3′, C-5′), 124.5 (CH, C-2′, C-6′), 48.2 (CH_3, O–CH_3); Gas Chromatography-Mass Spectrometry (GC-MS) *m/z* 136 [M^+]; anal. calcd. for $C_8H_8O_2$: C, 70.57; H, 5.92; found: C, 70.26; H, 5.89.

Methyl 4-chloro benzoate (**2b**) White solid (MeOH). This compound (**2b**) was prepared from 4-chloro benzoic acid (**1b**) (0.1 mol, 15.6 g) and methanol (100 mL) according to the general procedure. The product obtained as white solid was purified from methanol. 13.4 g (79%); m.p. 42–45 °C; FTIR (KBr) ν_{max}: 3056, 2955, 1717, 1589, 1386, 1120, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 7.96$ (2H, d, $J = 8.08$, H-2′, H-6′), 7.41 (2H, d, $J = 8.04$, H-3′, H-5′), 3.75 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 165.0$ (C, C=O), 137.2 (C, C-4′), 128.6 (C, C-1′), 128.4 (CH, C-3′, C-5′), 126.3 (CH, C-2′, C-6′), 49.8 (CH_3, O–CH_3); GC-MS *m/z* 170 [M^+]; anal. calcd. for $C_8H_7ClO_2$: C, 56.32; H, 4.14; found: C, 56.52; H, 4.16.

Synthesis of acid hydraz ide (3a–b)

Compound (2) (0.1 mol) was taken in a round-bottom flask with methanol (100 mL). Hydrazine hydrate (99%) (0.15 mol, 5.7 mL) was added drop wise with gentle stirring. The reaction mixture was refluxed for 4–6 h. Excess of methanol was distilled off under reduced pressure. The precipitated hydrazide was dried and re-crystallized from methanol (Zamani and Faghihi 2003).

Benzohydrazide (**3a**) Off-white solid (MeOH). This compound (**3a**) was prepared from methyl benzoate (**2a**) (0.1 mol, 13.6 g) and methanol (100 mL) according to the general procedure. The product obtained as off-white solid was purified from methanol. 11.6 g (85.3%); m.p. 112–114 °C; FTIR (KBr) ν_{max}: 3457, 3300, 3032, 1660, 1590, 685 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 9.81$ (1H, s, NH), 7.88 (2H, m, H-2′, H-6′), 7.41 (3H, m, H-3′, H-4′, H-5′), 4.46 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 166.2$ (C, C=O), 133.1 (C, C-1′), 130.9 (CH, C-4′), 128.1 (CH, C-3′, C-5′), 126.9 (CH, C-2′, C-6′); GC-MS *m/z*

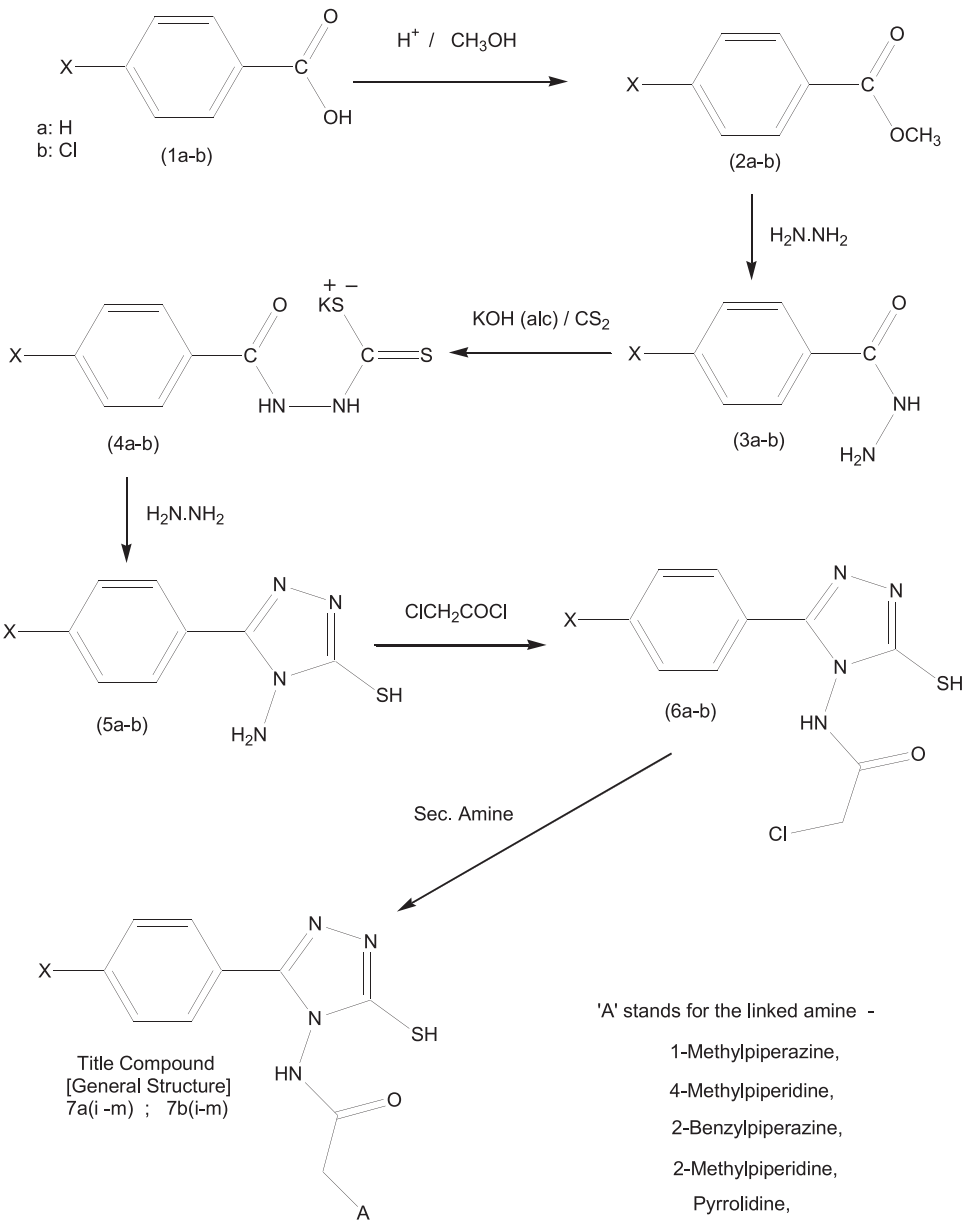


Fig. 1 Scheme of synthesis

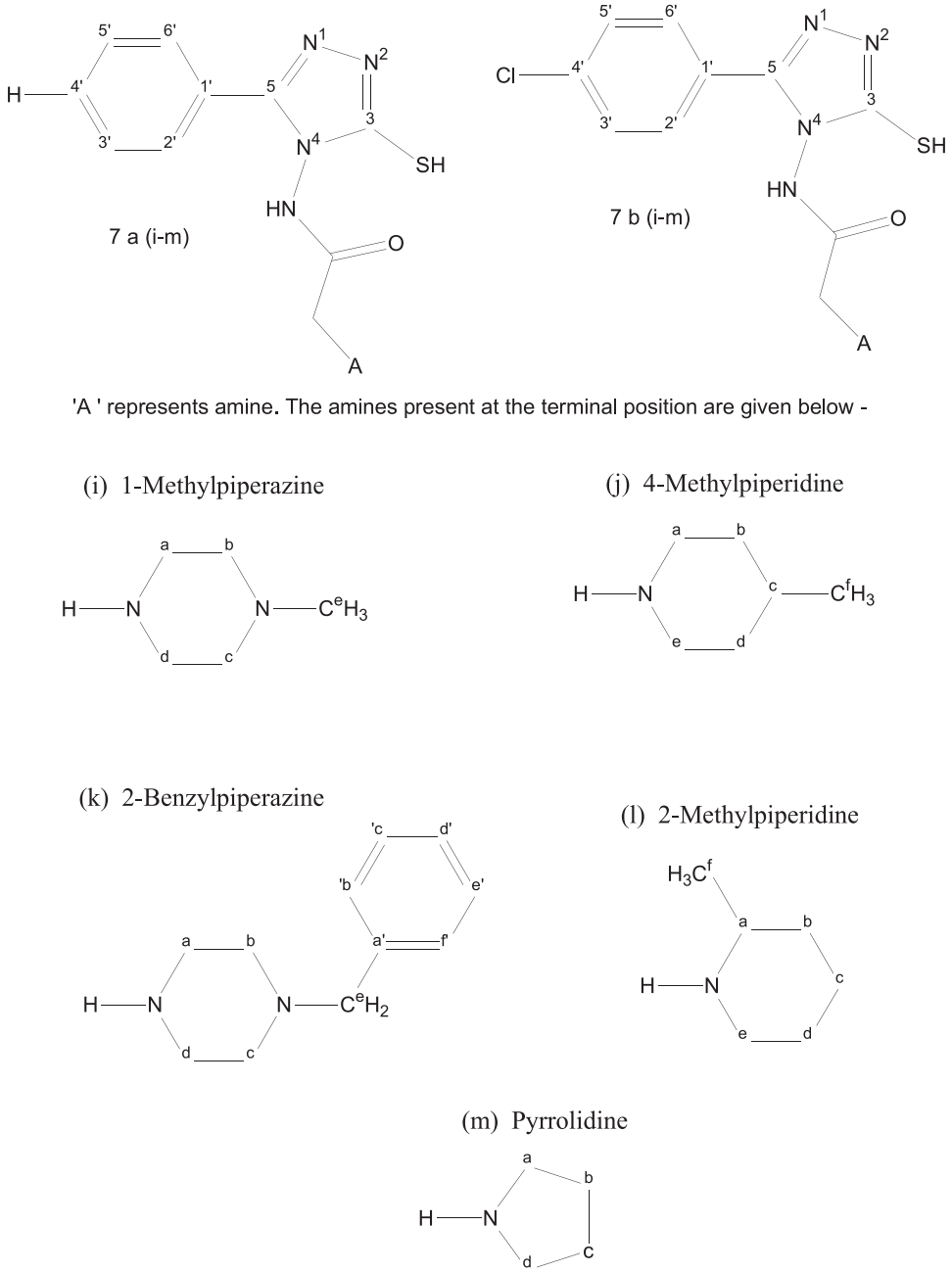
136 [M^+]; anal. calcd. for $C_7H_8N_2O$: C, 61.75; H, 5.92; N, 20.58; found: 61.68; H, 5.89; N, 20.52.

4-Chloro benzohydrazide (**3b**) Off-white solid (MeOH). This compound (**3b**) was prepared from methyl 4-chloro benzoate (**2b**) (0.1 mol, 17 g) and methanol (100 mL) according to the general procedure. The product obtained as off-white solid was purified from methanol. 15.6 g (92%); m.p. 161–163 °C; FTIR (KBr) ν_{max}: 3461, 3309, 3019, 1654, 1597, 678 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 9.87$ (1H, s, NH), 7.87 (2H, d, $J = 8.04$, H-2′, H-6′), 7.45 (2H, d, J = 8.04, H-3′, H-5′), 4.50 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 165.1$ (C, C=O), 136.1 (C, C-4′), 131.6 (CH, C-1′), 128.6 (CH, C-3′, C-5′), 128.1 (CH, C-2′, C-6′); GC-MS *m/z* 170 [M^+]; anal. calcd. for $C_7H_7ClN_2O$: C, 49.28; H, 4.14; N, 16.42; found: C, 49.08; H, 4.12; N, 16.35.

*Synthesis of potassium 3-aroyl dithiocarbazate (**4a–b**)*

Potassium hydroxide (0.15 mol, 8.4 g) was mixed with ethanol (100 mL) in a flat bottom flask. The synthesized hydrazide (0.1 mol) was transferred to the flask. Carbon disulfide (0.15 mol, 9 mL) was then added to it. The reaction mixture was stirred for 16 h at room temperature. The product was filtered off through whatman paper and washed with ether to remove excess of carbon disulfide. Further the filtered product was dried at room temperature (Cansiz et al. 2004). The salt, so obtained was taken for the next step without further

Fig. 2 Title compounds



purification. The formation of compounds, potassium salt of 3-benzoyl dithiocarbazate (**4a**) as well as 3-(4-chloro-benzoyl) dithiocarbazate (**4b**) was confirmed by IR spectra (characteristic peaks at 3320, 1640, 1230, and 1065 cm^{-1} due to N–H, C=O, C=S and C–N stretchings respectively).

*Synthesis of 4-amino-3-mercapto-5-phenyl-1,2,4-triazole (**5a–b**)*

Compound (**4a–b**) collected above was dissolved in little portion of water in a round-bottom flask equipped with condenser and an outlet. Hydrazine hydrate (0.2 mol, 7.6 mL) in 100 mL absolute ethanol was added to it. Refluxing was done for 2–3 h. The gas coming out from out let was tested for hydrogen sulfide by lead acetate paper. The whole solution turned green. When the evolution of gas ceased, the excess of alcohol was distilled off. After cooling, water was added. With the aid of hydrochloric acid (1N), the precipitate was separated. The precipitate so formed was filtered off and crystallization affected by hydro-alcoholic solution (Jadhav et al. 2010).

4-Amino-3-mercapto-5-phenyl-1,2,4-triazole (**5a**) Off-white solid (EtOH). This compound (**5a**) was prepared

from potassium 3-aroyl dithiocarbazate (**4a**), hydrazine hydrate (0.2 mol, 7.6 mL) and ethanol (100 mL) according to the general procedure. The product obtained as off-white solid was purified from ethanol. 13.8 g (71.9%); m.p. 205–207 °C; FTIR (KBr) ν_{max}: 3488, 3062, 2555, 1542, 1485, 712 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.83 (1H, s, SH), 7.88 (2H, m, H-2′, H-6′), 7.54 (3H, m, H-3′, H-4′, H-5′), 5.70 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 166.7 (C, C-5), 149.1 (C, C-3), 130.1 (CH, C-4′), 128.2 (CH, C-3′, C-5′), 127.8 (CH, C-2′, C-6′), 125.7 (C, C-1′); GC-MS *m/z* 192 [M^+]; anal. calcd. for $C_8H_8N_4S$: C, 49.98; H, 4.19; N, 29.14; found: C, 49.87; H, 4.17; N, 29.06.

4-Amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**5b**) Off-white solid (EtOH). This compound (**5b**) was prepared from potassium 3-aroyl dithiocarbazate (**4b**), hydrazine hydrate (0.2 mol, 7.6 mL) and ethanol (100 mL) according to the general procedure. The product obtained as off-white solid was purified from ethanol. 14.5 g (64.1%); m.p. 214–216 °C; FTIR (KBr) ν_{max}: 3490, 3108, 2578, 1595, 1478, 711 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.87 (1H, s, SH), 8.10 (2H, d, J = 8.56, H-2′, H-6′), 7.87 (2H, m, H-3′, H-5′), 5.70 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 166.9 (C, C-5), 148.2 (C, C-3), 135.3 (CH, C-4′), 129.3 (CH, C-3′, C-5′), 128.3 (CH, C-2′, C-6′), 124.4 (C, C-1′); GC-MS *m/z* 226 [M^+]; Anal. Calcd. for $C_8H_7ClN_4S$: C, 49.39; H, 3.11; N, 24.72; found: C, 49.26; H, 3.10; N, 24.65.

*Synthesis of 4-chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a–b**)*

Compound (**5a–b**) (0.1 mol) was dissolved in dioxane (100 mL) in a two necked round-bottom flask equipped with condenser and a glass stopper. Chloroacetyl chloride (0.11 mol, 8.75 mL) was added to it. The reaction mixture was refluxed for an hour. The content was allowed to cool and poured on crushed ice. The precipitated product was filtered and repetitively washed with cold distilled water (Upmanyu et al. 2011a).

4-Chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a**) Light brown solid (dioxane). This compound (**6a**) was prepared from 4-amino-3-mercapto-5-phenyl-1,2,4-triazole (**5a**) (0.1 mol, 19.2 g), chloroacetyl chloride (0.11 mol, 8.75 mL) and dioxane (100 mL) according to the general procedure. The product obtained as light brown solid was purified from dioxane. 18.3 g (68%); m.p. 182–184 °C; FTIR (KBr) ν_{max}: 3300, 3074, 2942, 2552, 1640, 1538, 1490, 705 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.80 (1H, s, SH), 9.5373 (1H, s, NH), 7.82 (2H, m, H-2′, H-6′), 7.60 (3H, m, H-3′, H-4′, H-5′), 4.62 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 169.6 (C, NHCO), 165.8 (C, C-5), 149.5 (C, C-3), 129.5 (CH, C-3′, C-5′), 128.9 (CH, C-4′), 127.2 (CH, C-2′, C-6′), 125.3 (C, C-1′), 52.83 (CH_2); GC-MS *m/z* 268 [M^+]; anal. calcd. for $C_{10}H_9ClN_4OS$: C, 44.70; H, 3.38; N, 20.85; found: C, 44.51; H, 3.36; N, 20.73.

4-Chloroacyl amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**6b**) Light brown solid (dioxane). This compound (**6b**) was prepared from 4-amino-3-mercapto-5-(4′-chloro) phenyl-1,2,4-triazole (**5b**) (0.1 mol, 22.7 g), chloroacetyl chloride (0.11 mol, 8.75 mL) and dioxane (100 mL) according to the general procedure. The product obtained as light brown solid was purified from dioxane. 19.8 g (65.3%); m.p. 201–203 °C; FTIR (KBr) ν_{max}: 3292, 3110, 2974, 2570, 1653, 1545, 1493, 702 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.93 (1H, s, SH), 9.61 (1H, s, NH), 8.10 (2H, d, J = 8.52, H-2′, H-6′), 7.51 (3H, m, H-3′, H-5′), 4.70 (2H, s, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 170.2 (C, NHCO), 165.9 (C, C-5), 148.8 (C, C-3), 130.5 (C, C-4′), 129.8 (CH, C-3′, C-5′), 128.5 (CH, C-2′, C-6′), 124.7 (C, C-1′), 53.6 (CH_2); GC-MS *m/z* 302 [M^+]; anal. calcd. for $C_{10}H_8Cl_2N_4OS$: C, 39.62; H, 2.66; N, 18.48; found: C, 39.45; H, 2.64; N, 18.39.

*Synthesis of 4-(substituted ethanoyl)-3-mercapto-5-phenyl-1,2,4-triazole (**7a–b**)*

Compound (**6a–b**) (0.025 mol) was solubilized in very little amount of dimethyl sulfoxide (DMSO) and then the solution was diluted with benzene (75 mL approx.) in a round-bottom flask. Respective amine (0.025 mol) was added to the mixture. Triethylamine (0.025 mol) was then added. The reaction mixture was refluxed for 4–5 h. Benzene was distilled off under reduced pressure to collect the product. The precipitate so obtained was washed several times with distilled water and re-crystallized with benzene (Upmanyu et al. 2012).

Thin layer chromatography using silica gel G was used to determine the purity of synthesized compounds. Melting points were determined in open capillaries and are uncorrected. Infrared (IR) spectra in KBr phase were recorded by Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{13}C and ^{1}H NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using DMSO-d_6 as solvent. Mass spectra were recorded using direct inlet probe on Thermo Scientific TSQ 8000 Gas Chromatograph—Mass Spectrometer. Elemental estimations were carried out on Thermo Scientific (FLASH 2000) Elemental Analyzer.

4-(4′-Methyl-piperazin-1′-yl ethanoyl) amino-3-mercapto-5-phenyl-1,2,4-triazole (**7ai**) Brown solid (benzene). This compound (**7ai**) was prepared from 4-chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a**) (0.025 mol, 6.72 g), 1-methylpiperazine (0.025 mol, 2.8 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 6.39 g (77%); m.p. 153–155 °C; FTIR (KBr) ν_{max}: 3300, 3116, 2945, 2598, 1637, 1532, 1498, 1481, 1234 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.85 (1H, s, SH), 9.82 (1H, s, CONH), 7.54 (5H, m, Ar–H), 3.41 (2H, s, $COCH_2$), 2.65 (8H, m, CH_2-piperaz.), 2.42 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 173.8 (C, NHCO), 166.7 (C, C-5), 149.1 (C, C-3), 130.1 (CH, C-4′), 128.2 (CH, C-3′, C-5′), 127.8 (CH, C-2′, C-6′), 125.7 (C, C-1′), 58.0 (CH_2, $COCH_2$), 55.7 (CH_2, C-b, C-c), 52.0 (CH_2, C-a, C-d), 46.1 (CH_3, C-e); GC-MS *m/z* 332 [M^+]; anal. calcd. for $C_{15}H_{20}N_6OS$: C, 54.20; H, 6.06; N, 25.28; found: C, 54.42; H, 6.10; N, 25.19.

4-(4′-Methyl-piperidin-1′-yl ethanoyl) amino-3-mercapto-5-phenyl-1,2,4-triazole (**7aj**) Brown solid (benzene). This compound (**7aj**) was prepared from 4-chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a**) (0.025 mol, 6.72 g), 4-methylpiperidine (0.025 mol, 3 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 6.12 g (74%); m.p. 142–144 °C; FTIR (KBr) ν_{max}: 3282, 3140, 2974, 2580, 1620, 1502, 1498, 1458, 1165 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.84 (1H, s, SH), 9.81 (1H, s, CONH), 7.55 (5H, m, Ar–H), 3.40 (2H, s, $COCH_2$), 1.91 (9H, m, CH, CH_2), 1.20 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 174.6 (C, NHCO), 166.6 (C, C-5), 148.5 (C, C-3), 130.1 (CH, C-4′), 129.1 (CH, C-3′, C-5′), 127.1 (CH, C-2′, C-6′), 125.1 (C, C-1′), 59.1 (CH_2, $COCH_2$), 52.8 (CH_2, C-a, C-e), 34.4 (CH_2, C-b, C-d), 30.6 (CH, C-c), 23.1 (CH_3, C-f); GC-MS m/z 331[M^+]; anal. calcd. for $C_{16}H_{21}N_5OS$: C, 57.98; H, 6.39; N, 21.13; found: C, 58.15; H, 6.42; N, 21.22.

4-(4′-Benzyl-piperazin-1′-yl ethanoyl) amino-3-mercapto-5-phenyl-1,2,4-triazole (**7ak**) Brown solid (benzene). This compound (**7ak**) was prepared from 4-chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a**) (0.025 mol, 6.72 g), 1-benzylpiperazine (0.025 mol, 4.4 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 6.54 g (64%); m.p. 210–213 °C; FTIR (KBr) ν_{max}: 3301, 3124, 2957, 2591, 1612, 1512, 1469, 1491, 1236 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.83 (1H, s, SH), 9.81 (1H, s, CONH), 7.55 (2 × 5H, m, Ar–H), 3.79 (2H, s, CH_2–Ar), 3.38 (2H, s, $COCH_2$), 2.46 (8H, m, CH_2-piperaz.); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 173.8 (C, NHCO), 166.8 (C, C-5), 149.4 (C, C-3), 137.3 (C, C-a′), 130.4 (CH, C-4′), 129.2 (CH, C-b′, C-f′), 128.4 (CH, C-3′, C-5′), 128.2 (CH, C-c′, C-e′), 128.1 (CH, C-2′, C-6′), 127.1 (CH, C-d′), 125.4 (C, C-1′), 62.8 (CH_2, C-e), 58.4 (CH_2, $COCH_2$), 52.8 (CH_2, C-a, C-d), 52.7 (CH_2, C-b, C-c); GC-MS *m/z* 408 [M^+]; anal. calcd. for $C_{21}H_{24}N_6OS$: C, 61.74; H, 5.92; N, 20.57; found: C, 61.90; H, 5.95; N, 20.73.

4-(2′-Methyl-piperidin-1′-yl ethanoyl) amino-3-mercapto-5-phenyl-1,2,4-triazole (**7al**) Brown solid (benzene). This compound (**7al**) was prepared from 4-chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a**) (0.025 mol, 6.72 g), 2-methylpiperidine (0.025 mol, 3 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 5.79 g (70%); m.p. 172–174 °C; FTIR (KBr) ν_{max}: 3296, 3116, 2953, 2540, 1641, 1536, 1499, 1484, 1173 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.85 (1H, s, SH), 9.82 (1H, s, CONH), 7.62 (5H, m, Ar–H), 3.39 (2H, s, $COCH_2$), 1.94 (9H, m, CH, CH_2), 1.17 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 173.3 (C, NHCO), 166.1 (C, C-5), 148.9 (C, C-3), 131.3 (C, C-4′), 128.7 (CH, C-3′, C-5′), 127.7 (CH, C-2′, C-6′), 125.3 (C, C-1′), 59.2 (CH_2, $COCH_2$), 56.1 (CH_2, C-e), 50.8 (CH, C-a), 34.3 (CH_2, C-d), 24.9 (CH_2, C-c), 24.0 (CH_2, C-b), 18.2 (CH_3, C-f); GC-MS *m/z* 331 [M^+]; anal. calcd. for $C_{16}H_{21}N_5OS$: C, 57.98; H, 6.39; N, 21.13; found: C, 58.21; H, 6.43; N, 21.24.

4-(Pyrrolidin-1′-yl ethanoyl) amino-3-mercapto-5-phenyl-1,2,4-triazole (**7am**) Brown solid (benzene). This compound (**7am**) was prepared from 4-chloroacyl amino-3-mercapto-5-phenyl-1,2,4-triazole (**6a**) (0.025 mol, 6.72 g), pyrrolidine (0.025 mol, 2.1 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 4.99 g (66%); m.p. 135–137 °C; FTIR (KBr) ν_{max}: 3306, 3096, 2955, 2571, 1660, 1512, 1493, 1489, 1198 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.85 (1H, s, SH), 9.82 (1H, s, CONH), 7.79 (5H, m, Ar–H), 3.38 (2H, s, $COCH_2$), 2.65 (2 × 2H, t, H-a′, H-d′), 1.76 (2 × 2H, m, H-b′, H-c′); ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 174.8 (C, NHCO), 166.1 (C, C-5), 148.1 (C, C-3), 130.9 (CH, C-4′), 129.1 (CH, C-3′, C-5′), 128.1 (CH, C-2′, $C_{6'}$), 124.9 (C, C-1′), 58.4 (CH_2, $COCH_2$), 54.1 (CH_2, C-a, C-d), 23.8 (CH_2, C-b, C-c); GC-MS *m/z* 303 [M^+]; anal. calcd. for $C_{14}H_{17}N_5OS$: C, 55.42; H, 5.65; N, 23.08; found: C, 55.19; H, 5.62; N, 22.98.

4-(4′-Methyl-piperazin-1′-yl ethanoyl) amino-3-mercapto-5-(4′-chloro) phenyl-1,2,4-triazole (**7bi**) Brown solid (benzene). This compound (**7bi**) was prepared from 4-

chloroacyl amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**6b**) (0.025 mol, 7.57 g), 1-methylpiperazine (0.025 mol, 2.8 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 6.88 g (75%); m.p. 184–187 °C; FTIR (KBr) ν_{max}: 3246, 3149, 2937, 2586, 1637, 1530, 1498, 1479, 1095 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 13.90$ (1H, s, SH), 9.87 (1H, s, CONH), 8.35 (2 × 1H, d, $J = 9.1$, H-2′, H-6′), 7.74 (2 × 1H, d, $J = 9.2$, H-3′, H-5′), 3.30 (2H, s, $COCH_2$), 2.74 (8H, m, CH_2-piperaz.), 2.39 (3H, s, CH_3); ^{13}C NMR (DMSO-d6, 100 MHz): $\delta = 176.1$ (C, NHCO), 166.8 (C, C-5), 148.2 (C, C-3), 135.3 (C, C-4′), 129.5 (CH, C-3′, C-5′), 128.5 (CH, C-2′, C-6′), 124.6 (C, C-1′), 59.8 (CH_2, $COCH_2$), 55.3 (CH_2, C-b, C-c), 52.2 (CH_2, C-a, C-d), 49.4 (CH_3, C-e); GC-MS *m/z* 366 [M^+]; anal. calcd. for $C_{15}H_{19}ClN_6OS$: C, 49.11; H, 5.22; N, 22.91; found: C, 49.23; H, 5.25; N, 22.81.

4-(4′-Methyl-piperidin-1′-yl ethanoyl) amino-3-mercapto-5-(4′-chloro) phenyl-1,2,4-triazole (**7bj**) Brown solid (benzene). This compound (**7bj**) was prepared from 4-Chloroacyl amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**6b**) (0.025 mol, 7.57 g), 4-methylpiperidine (0.025 mol, 3 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 5.94 g (65%); m.p. 176–178 °C; FTIR (KBr) ν_{max}: 3278, 3134, 2974, 2578, 1620, 1502, 1477, 1451, 1165 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 13.93$ (1H, s, SH), 9.86 (1H, s, CONH), 8.28 (2 × 1H, d, $J = 9.1$, H-2′, H-6′), 7.76 (2 × 1H, d, $J = 9.2$, H-3′, H-5′), 3.30 (2H, s, $COCH_2$), 1.90 (9H, m, CH, CH_2), 1.19 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 174.2$ (C, NHCO), 166.9 (C, C-5), 148.2 (C, C-3), 135.3 (C, C-4′), 129.3 (CH, C-3′, C-5′), 127.3 (CH, C-2′, C-6′), 124.4 (C, C-1′), 59.7 (CH_2, $COCH_2$), 52.9 (CH_2, C-a, C-e), 34.2 (CH_2, C-b, C-d), 30.3 (CH, C-c), 23.8 (CH_3, C-f); GC-MS *m/z* 365 [M^+]; anal. calcd. for $C_{16}H_{20}ClN_5OS$: C, 52.52; H, 5.51; N, 19.14; found: C, 52.35; H, 5.49; N, 19.05.

4-(4′-Benzyl-piperazin-1′-yl ethanoyl) amino-3-mercapto-5-(4′-chloro) phenyl-1,2,4-triazole (**7bk**) Brown solid (benzene). This compound (**7bk**) was prepared from 4-chloroacyl amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**6b**) (0.025 mol, 7.57 g), 1-benzylpiperazine (0.025 mol, 4.4 mL), triethylamine (0.025 mol, 3.5 mL) and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 8.08 g (73%); m.p. 192–194 °C; FTIR (KBr) ν_{max}: 3286, 3142, 2965, 2582, 1620, 1516, 1489, 1472, 1236 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 13.87$ (1H, s, SH), 9.87 (1H, s, CONH), 8.19 (2H, d, $J = 8.8$, H-2′, H-6′), 7.59 (2H, d, $J = 9.2$, H-3′, H-5′), 7.48 (5H, m, Ar–H), 3.72 (2H, s, CH_2–Ar), 3.29 (2H, s, $COCH_2$), 2.45 (8H, m, CH_2-piperaz.); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 175.1$ (C, NHCO), 166.9 (C, C-5), 148.8 (C, C-3), 137.3 (C, C-a′), 135.1 (C, C-4′), 133.3 (CH, C-3′, C-5′), 129.2 (CH, C-b′, C-f′), 128.2 (CH, C-c′, C-e′), 128.1 (CH, C-2′, C-6′), 127.1 (CH, C-d′), 124.4 (C, C-1′), 62.7 (CH_2, C-e), 59.2 (CH_2, $COCH_2$), 52.8 (CH_2, C-b, C-c), 52.7 (CH_2, C-a, C-d); GC-MS *m/z* 442 [M^+]; anal. calcd. for $C_{21}H_{23}ClN_6OS$: C, 56.94; H, 5.23; N, 18.97; found: C, 57.20; H, 5.25; N, 19.05.

4-(2′-Methyl-piperidin-1′-yl ethanoyl) amino-3-mercapto-5-(4′-chloro) phenyl-1,2,4-triazole (**7bl**) Brown solid (benzene). This compound (**7bl**) was prepared from 4-chloroacyl amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**6b**) (0.025 mol, 7.57 g), 2-methylpiperidine (0.025 mol, 3 mL), triethylamine (0.025 mol, 3.5 mL), and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 5.58 g (61%); m.p. 166–169 °C; FTIR (KBr) ν_{max}: 3289, 3137, 2961, 2581, 1639, 1531, 1489, 1488, 1179 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 13.88$ (1H, s, SH), 9.85 (1H, s, CONH), 8.71 (2H, d, $J = 9.1$, H-2′, H-6′), 7.62 (2H, d, $J = 9.1$, H-3′, H-5′), 3.29 (2H, s, $COCH_2$), 1.96 (9H, m, CH, CH_2), 1.18 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 174.1$ (C, NHCO), 166.6 (C, C-5), 148.5 (C, C-3), 134.1 (C, C-4′), 130.1 (CH, C-3′, C-5′), 128.1 (CH, C-2′, C-6′), 124.4 (C, C-1′), 58.4 (CH_2, $COCH_2$), 56.2 (CH_2, C-e), 50.9 (CH, C-a), 34.4 (CH_2, C-d), 24.3 (CH_2, C-c), 24.1 (CH_2, C-b), 18.9 (CH_3, C-f); GC-MS *m/z* 365 [M^+]; anal. calcd. for $C_{16}H_{20}ClN_5OS$: C, 52.52; H, 5.51; N, 19.14; found: C, 52.80; H, 5.54; N, 19.20.

4-(Pyrrolidin-1′-yl ethanoyl) amino-3-mercapto-5-(4′-chloro) phenyl-1,2,4-triazole (**7bm**) Brown solid (benzene). This compound (**7bm**) was prepared from 4-chloroacyl amino-3-mercapto-5-(4′-chloro)phenyl-1,2,4-triazole (**6b**) (0.025 mol, 7.57 g), pyrrolidine (0.025 mol, 2.1 mL), triethylamine (0.025 mol, 3.5 mL), and benzene (75 mL) according to the general procedure. The product obtained as brown solid was purified from benzene. 6.42 g (76%); m.p. 124–127 °C; FTIR (KBr) ν_{max}: 3289, 3116, 2962, 2586, 1671, 1522, 1487, 1465, 1211 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): $\delta = 13.86$ (1H, s, SH), 9.87 (1H, s, CONH), 8.45 (2H, d, $J = 9.2$, H-2′, H-6′), 7.57 (2H, d, $J = 9.2$, 3′, H-5′), 3.2880 (2H, s, $COCH_2$), 2.59 (2 × 2H, t, H-a′, H-d′), 1.71 (2 × 2H, m, H-b′, H-c′); ^{13}C NMR (DMSO-d6, 100 MHz): $\delta = 175.1$ (C, NHCO), 166.1 (C, C-5), 148.2 (C, C-3), 135.2 (C, C-4′), 130.9 (CH, C-3′, C-5′), 128.4 (CH, C-2′, C-6′), 125.1 (C, C-1′), 59.1 (CH_2, $COCH_2$), 54.4 (CH_2, C-a, C-d), 23.6 (CH_2, C-b, C-c); GC-MS m/z 337 [M^+];

anal. calcd. for $C_{14}H_{16}$ ClN_5OS: C, 49.77; H, 4.77; N, 20.73; found: C, 49.92; H, 4.79; N, 20.81.

Pharmacological screening

Screening of the synthesized compounds was carried out on experimental animals. There are three types of screening viz.: simple screening, blind screening, and programmed screening. In this study, programmed screening has been carried out. Programmed screening generally used when a newly synthesized drug of specific type is to be screened for some pharmacological action. The screening protocol was reviewed and approved by the Institutional Animal Ethical Committee, Institute of Pharmaceutical Research, GLA University Mathura, India [Approval No: 1260/Po/Ere/S/09/CPCSEA], constituted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, New Delhi.

Screening of chemical or pharmacological agent starts with an initial procedure of toxicity study, known as lethal dose (LD_{50}) study. The assessment of LD_{50} (the dose that kills 50% of the test animal population) gives an idea about the safe dose at which pharmacological experiments can be started.

LD_{50} study

It involves determination of median LD_{50} within 24 h post treatment of the test drug. Healthy albino wistar rats (150–200 g) were maintained at 25 ± 2 °C, with relative humidity 45–50%, under 12 h of light/dark cycle for 3 days in order to normalize their biorhythm. Animals were fed with standard animal diet and water ad libitum. Prior to dosing, rats were fasted overnight but allowed water ad libitum. The test compounds were administered through oral route as per guidelines set by the Organization for Economic Co-operation and Development (OECD). The referred dose (initial dose 175 mg/kg and limit dose 2000 mg/kg) of the test drug was given in accordance with OECD guideline 425 (Viswanatha et al. 2012). It is also known as Up-and-Down procedure, which is of cardinal value in reducing the number of animals required in LD_{50} study. Animals were dosed, one at a time and depending upon the outcome, the dose for the next animal was adjusted. The animals were observed for 24 h and thereafter for 14 days. The observed morbidity and mortality was analyzed through AOT425StatPgm. All the synthesized compounds were found to be non-lethal even at 2000 mg/kg dose level.

Effect of the test compound on animal behavior

This test was undertaken with a view to observe any unwanted or any substantial change in the animal behavior. The experiment involved test drug administration through intraperitoneal route in swiss albino mice (30–35 g). Mice of either sex were fasted overnight under laboratory condition and allowed for water ad libitum. The selected dose 100 mg/kg (not more than 1/10 LD_{50}) was given and the change in behavior was noticed in the open arena (Donoso and Broitman 1979). Data are presented in Table 1.

Table 1 Effect of the test compound on animal behavior

Compound	Behavior recorded in open arena for 1 h									
	Staggering gait	Rearing	Grooming	Gross locomotor activity	Palpebral closure	Visual placing reflex	Righting reflex	Thirst	Appetite	Defecation
7ai	+	0	+	− −	+	+	+++	+	+	0
7aj	+	+	0	−	0	+	+++	+	+	+
7ak	+	+	0	0	0	+	+++	+	+	+
7al	+	0	+	0	0	++	+++	0	+	+
7am	0	0	0	0	0	0	+++	+	+	0
7bi	+	0	0	− − −	++	+	+++	+	++	+
7bj	0	0	+	− −	+	+	+++	+	+	+
7bk	0	0	0	−	0	+	+++	+	++	+
7bl	+	0	0	0	0	+	+++	+	+	+
7bm	+	0	+	0	0	+	+++	0	+	+
Normal control	0	+	+	0	0	++	+++	+	+	+

− − − effect reduced strongly, − − effect reduced moderately, − effect reduced slightly, 0 no effect, + present with slight effect, ++ present with moderate effect, +++ present with strong effect

Clonidine-induced catalepsy: histamine mediated behavior

Clonidine is an alpha adrenergic agonist, but when administered intra-peritoneally in mice, it triggers histamine release and produces catalepsy, which is a condition in which the test animal has tendency to retain the imposed posture for at least 10 s. The clonidine-induced catalepsy can be attenuated by H_1 anti-histaminics (Taur and Patil 2011) and it is an indirect method to screen antihistaminic effect of test compounds.

Bar test was used to study the effect of the synthesized compounds. Swiss albino mice of average body weight (25–30 g) were divided into 12 groups, one group for vehicle control, another group for pheniramine treatment and remaining ten groups for the test compounds. Clonidine (dose: 1 mg/kg, s.c.) was administered to the mice of all groups after 30 min of the test drug administration. An elevated bar of height 3.5 cm was used, on which fore paw of each mouse was placed and duration for which the animal maintains the imposed posture was noted through stop watch. The dose of the test compound was selected on the basis of acute toxicity study. Since the optimal test dose during LD_{50} study was 2000 mg/kg body weight, hence the safe pharmacological dose should not be more than one-tenth of the LD_{50}. The selected dose was also verified through a pilot study before the main test. Effect of the test compounds on clonidine-induced catalepsy is shown in Table 2.

Histamine-induced anaphylactic shock

Anaphylactic shock induced by histamine aerosol is an allergic response mainly characterized by bronchospasm and fainting of animal. It is a well established method for screening of antihistaminic (type-H_1) drugs (Sadek et al. 2013). In this experiment, bronchospasm was induced in overnight fasted guinea pigs. Guinea pigs of either sex and average body weight were selected for this experiment. Animals were divided into 12 groups as; Group-1: Vehicle control, Group-2: Standard (Pheniramine treated, Dose: 10 mg/kg) and Group-(3 to 12): test drugs treated (dose: 100 mg/kg body weight).

Bronchospasm was induced in guinea pig with the help of 1% histamine aerosol. This study was carried out in a transparent chamber made of perplex glass (dimension—$24 \times 14 \times 24\ cm^3$). Guinea pig placed in the transparent glass chamber was exposed to histamine aerosol under constant pressure ($1\ kg/cm^2$). End point time for pre-convulsive dyspnea (PCD) was determined from the time of aerosol exposure. As soon as the PCD commenced, the guinea pig was removed from the aerosol chamber and exposed to fresh air for recovery. Effect of the test compounds on pre-convulsive dyspnea end point time is presented in Table 3.

Table 2 Effect of the test drug on clonidine-induced catalepsy

Treatment	Duration of catalepsy (mean ± SEM) in seconds at	
	15 min	30 min
Vehicle (i.p.)	89.17 ± 1.88	75.00 ± 1.59
Pheniramine maleate (10 mg/kg, i.p.)	28.67 ± 1.92**	16.00 ± 1.80**
7ai	48.67 ± 1.37**	30.5 ± 1.48**
7aj	53.17 ± 1.37*	68.67 ± 2.23*
7ak	53.83 ± 0.87	69.67 ± 1.54
7al	54.33 ± 1.17	69.83 ± 1.19
7am	54.00 ± 1.39	72.00 ± 1.12
7bi	31.17 ± 1.35**	18.17 ± 1.19**
7bj	46.33 ± 1.20**	21.00 ± 1.18**
7bk	53.33 ± 0.88*	68.00 ± 1.44*
7bl	55.00 ± 1.92	70.67 ± 0.88
7bm	54.17 ± 1.25	70.83 ± 1.07

All values are expressed as mean ± SEM, where $n = 6$ per group. Data analyzed by one way ANOVA followed by Dunnett's test. Significant when compared with control

*$p < 0.05$; **$p < 0.01$

Table 3 Effect of the test drugs on histamine-induced PCD end point time

Treatment	Pre-convulsive dyspnea end point time (in seconds)	% Protection
Vehicle control	102.83 ± 2.34	–
7ai	240.33 ± 3.79**	57.21
7aj	112.00 ± 2.00	8.18
7ak	108.33 ± 1.72	5.07
7al	111.50 ± 2.32	7.77
7am	105.17 ± 2.12	2.22
7bi	351.83 ± 2.81**	70.77
7bj	261.50 ± 2.68**	60.07
7bk	112.83 ± 3.14*	8.86
7bl	112.16 ± 1.51	8.32
7bm	105.67 ± 1.99	2.68
Pheniramine	464.50 ± 2.68**	77.86

% Protection $= [(T_T – T_C)/T_T] \times 100$; where, T_T: PCD end point time for the test drug; T_C: PCD end point time for vehicle control. All values are expressed as mean ± SEM, where $n = 6$ per group. Data analyzed by one way ANOVA followed by Dunnett's test. Significant when compared with control

*$p < 0.05$; **$p < 0.01$

Study on isolated tissue: isolated guinea pig ileum preparation

Overnight fasted guinea pig was sacrificed and abdomen was opened. Pieces of ileum (2–3 cm long) were isolated. The isolated pieces of ileum were quickly transferred to

Table 4 Effect of the test drug on isolated tissue preparation

S.N	Conc. of the test drug [A] in μM	Log [A]	% Inhibition in DRC of histamine after the exposure of test drug [histamine used (1.6 mL, strength 1 mg/mL)]										
			7ai	**7aj**	**7ak**	**7al**	**7am**	**7bi**	**7bj**	**7bk**	**7bl**	**7bm**	Pheniramine
1	10	1.00	29.76	18.31	12.98	4.57	5.35	41.99	32.83	41.17	16.02	9.15	43.50
2	20	1.30	30.55	19.09	14.51	5.35	8.40	43.50	34.34	42.75	18.31	12.22	60.31
3	40	1.60	32.05	22.89	16.80	13.73	24.29	55.73	45.04	58.78	39.70	24.43	70.98
4	80	1.90	41.99	41.21	35.88	37.41	34.34	81.68	61.07	72.52	58.02	37.41	85.50
5	160	2.20	54.95	51.15	50.37	48.08	48.86	100	72.52	85.50	79.39	55.73	100.00
6	320	2.25	66.40	63.36	61.07	61.07	57.24	100	87.79	100.00	100.00	80.14	100.00
IC_{50} value			84.80	113.79	131.86	138.29	151.32	21.80	38.64	23.12	47.98	86.60	13.16

Petri dish containing tyrode (physiological salt solution) under suitable aeration. The tissue was then mounted in organ bath containing the same physiological salt solution at 37 °C. A basal tension of 0.5 g was applied and the tissue was allowed to stabilize for 30 min. The tissue was then exposed to the test drugs and dose-response contractions were recorded (Lin et al. 2012). Effect of the test compounds on dose-response curve of histamine is given in Table 4.

Results and discussion

The synthesis was accomplished as per the given scheme. Aromatic carboxylic acids were converted into their respective methyl esters and further into hydrazides. Hydrazides were treated with carbon disulfide and alcoholic potassium hydroxide in order to form their dithiocarbazate salts. The salts were reacted with hydrazine and converted into their respective 1,2,4-triazoles. The triazoles, so obtained were reacted with chloracetyl chloride to give acyl derivatives. These acyl derivatives were treated with the amines (2°) to give the title compounds. Synthesized compounds were characterized by various spectral and physical data i.e., ^{13}C NMR, ^{1}H NMR, FTIR spectroscopy, GC-MS and elemental analysis. The ^{13}C NMR spectra for C_3 and C_5 of the 1,2,4-triazole ring were observed in the range of δ 148–149 and 166–176 ppm, while as per reported literature (Zamani and Faghihi 2003), they fall in the range of δ 148–152 and 168–169 ppm. In ^{1}H NMR spectra, singlet of CONH and SH were seen in the range of δ 9.83–9.88 and 13.85–14.03 ppm respectively. As per reported literature (Hussain et al. 2008; Kochikyan et al. 2010), the singlet of CONH and SH lie in the range of δ 6.58–7.53 and 13.25–13.95 respectively. FTIR spectra exhibited characteristic bands for S–H, C=O and C=N in the range of 2540–2596, 1612–1671, and 1469–1519 cm^{-1} respectively, however the reported literature (Kochikyan et al. 2010; Ghochikyan et al. 2016) shows the bands at 2743–2740, 1740–1743, and 1570–1586 cm^{-1}.

The compounds were tested on laboratory animals. In order to explore acute toxicity, LD_{50} determination was carried out. Effect of the test compound on animal behavior was also observed at pharmacological dose level. Preliminary screening showed that the compounds have antihistaminic (type-H_1) activity. On the basis of studies on experimental animals, the compounds were then tested on isolated tissue. Among them, compound **7bi**, **7bk**, **7bj**, and **7bl** showed potential anti-histaminic action with IC_{50} of 21.80, 23.12, 38.64, and 47.98 μM respectively. The reference drug pheniramine maleate showed an IC_{50} of 13.16 μM (Table 4). Experimental models (viz., clonidine-induced catalepsy and histamine-induced bronchospasm) confirmed the H_1 receptor antagonist activity of the compounds. Our studies showed that compounds containing 1-methyl piperazine, 4-methyl piperidine, and 1-benzyl piperazine at the terminal position of the acyl derivative of the triazole ring exhibited significant antihistaminic property. The compounds **7bk** and **7aj** showed moderate inhibition of catalepsy, while compounds **7bi**, **7bj**, and **7ai** exhibited significant anti-cataleptic effect (Table 2). In case of histamine-induced dyspnea model in guinea pig, the compound **7bi**, **7bj**, and **7ai** manifested potential protection while **7bk** showed moderate protection (Table 3). Among the ten compounds, it was observed that the electron withdrawing substituent, i.e., halogen (chloro-) substituted compounds showed comparatively better activity. The noteworthy denouement is the emergence of triazoles with secondary amines as promising antihistaminic agents.

Conclusion

The present work addresses a new series of triazoles having antihistaminic (H_1 antagonist) property. A series of novel triazoles were synthesized and their pharmacological

evaluations carried out. A few of them showed potent antihistaminic (type-H_1) activity. The recorded percentage protection by the test compounds against histamine-induced pre-convulsive dyspnoea on guinea pig was observed. When clonidine, a presynaptic alpha 2 receptor agonist was administered intra-peritoneally in mice, it triggered histamine release in the brain and produced catalepsy in mice. The bar test was used to access the effect of the synthesized drug on catalepsy. The compounds **7ai**, **7bi**, and **7bj** significantly attenuated catalepsy (Table 2). This signifies significant antihistaminic activity of the test compound. The IC_{50} values were also estimated through guinea pig isolated intestine preparation (Table 4). On the basis of the experiments performed, it is concluded that the compounds **7bi**, **7bj**, and **7ai** are most active with promising anti-histaminic activity. The persuasive outcome of this study is the emergence of 1,2,4-triazoles with secondary amines as promising anti-histaminic agent.

The field is further open for study. Preliminary findings suggest that the synthesized compounds may be considered as newer antihistaminics for future investigations. On the basis of present work, advance and detailed studies can be carried out to explore the exact mechanism of action for those compounds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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