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Synthesis and SAR Evaluation of Mercaptotriazolo derivatives as anti-inflammatory agents.

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Abstract : A new series of benzothiazole appended triazole derivatives were synthesized. Characterization is done by FT-IR, Mass, ^{1}H and ^{13}C NMR spectroscopic techniques. The newly synthesized compounds were screened for antioxidant and anti inflammatory activities. Experimental data hypothesized compound *7b* as highly potent cytotoxicant with lengthened activity. Structure–activity relation interrelates the biological activity and chemical moiety of the molecule. Structurally, 7b is a benzothiazolyltriazole having a pyrrolidine group (five membered ring) attached to two CH_2 groups and methyl substituent at 7^{th} position of the benzothiazole ring.
Key words : Benzothiazole ;triazole; anti- inflammation.

Introduction and Experimental

In recent years benzothiazole has received an alarming response owing to its diversified molecular design. Benzothiazoles are physiologically active heterocyclic compounds owing to a vast sphere of biological activities, including anticancer [1,2], antidiabetic [3, 4], antiviral, anti-inflammatory [5], antibiotic [6, 7], antileishmanial [8–11], antiprotozoal, antihistamine and antihypertensive [12] properties. Benzothiazoles have been studied as central muscle relaxants and found to impede the glutamate neurotransmission in biochemical, electrophysiological experiments. Triazole derivatives are known to display various pharmacological properties such as, antimicrobial, antitubercular, anticancer, anticonvulsant, anti-inflammatory, analgesic, and antiviral. Many commercial antifungal drugs contain triazole functional moiety. They are used as dyes, biological reagents, photographic chemicals, precursors for the synthesis of peptide mimetics and in the synthesis of polymers. They are also used as agrochemicals, photostablizers in photographic materials used as light stabilizers, fluorescent whiteners and optical seizures.

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Triazoles have also been merged in a wide range of therapeutically stimulating drugs including H1/H histamine receptor blockers and CNS stimulants. Triazoles are highly capable of forming hydrogen bonding, which is responsible for binding biomolecular targets as well as the increased solubility of the compounds. Triazoles can function as an attractive bridging units which could connect two pharmacophores to form a pioneering bifunctional drug. Hence, these compounds are essential in constructing bioactive and functional molecules, which prompted us to promote an organized research into possible chemical transformation of triazole and benzothiazole derivatives. Based on the rich biological reports on triazole derivatives and continuation of our benzothiazole drug development work [13-14], we have planned for synthesis of triazole conjugated benzothiazole side chain to get improved biological applications.

Materials and Methods : Chemistry

The chemicals required for the synthesis of title compounds were procured from Sigma Aldrich Chemical Co, the SD Fine Chem Ltd (India) and solvents were procured from the Rankem Pvt. Ltd (India). TLC was performed on aluminium-backed silica plates and visualized by UV-light. Melting points were found out by the open capillary method and were uncorrected. FT-IR spectra were recorded on Perkin Elmer spectrophotometer version 10.03.09 instrument over the range of 600 to 4000 cm^{-1}, ^1H NMR spectra were recorded on 400 MHz and ^{13}C NMR with 100 MHz from Agilent 400 RDD2 spectrometer using TMS as an internal standard, while $CDCl_3$ and DMSO-d_6 used as solvents. Chemical shifts are given in parts per million downfield from tetramethylsilane and LC-MS was recorded on mass spectrometer (Waters, USA) by positive mode The mass spectra were obtained with a VG70-70H spectrophotometer and the elemental analysis (C, H, and N) was performed on ElementarVario EL III elemental analyzer. The results of elemental analysis were within ±0.4% of the theoretical values.

General procedure for synthesis of substituted 2-amino benzothiazole(compounds 2a-d)

To the stirred solution of potassium thiocyanate(0.019 mol) in 25 cm^3 of acetic acid, substituted aniline (0.020 mol)dissolved in 10 $cm^3$of acetic acid was added was added dropwise at to 0^{o}C and stirred for 30 min. Later, bromine dissolved in 10 $cm^3$of acetic acid was added dropwise to the reaction mixture. The reaction was slowly warmed at room temperature and allowed to react overnight. The mixture was quenched with crushed ice and made alkaline (pH 9-10) by adding dropwise a cold aqueous solution of 35% ammonia. The precipitate was collected through filtration, washed with water and recrystallized from methanol. Compound 2a was taken as a representative example to explain characterization data.

Synthesis of 4-methyl-2-amino benzothiazole (2a,$C_8H_8N_2S_2$)

White solid, yield: 85%; FT-IR (KBr, cm^{-1}): 1368 (C=N), 3000 (NH); ^1H NMR ($CDCl_3$): δ 7.06 (s, 2H, NH_2), 1.98 (s, 3H, Ar-CH_3), 7.49 (t, 1H, Ar-H), 7.36 (d, 1H, Ar-H), 7.87 (d, 1H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 16.5, 119.27, 125.1, 126.08, 127.87, 132.1, 147.6, 163.13; LC-MS m/z 165 $(M+1)^+$. Anal.Calcd. For $C_8H_8N_2S_2$: C, 58.10; H, 4.26; N, 17.85; S, 19.09%. Found: C, 58.50; H, 4.91; N, 17.05; S, 19.59%.

General procedure for synthesis of substituted 2-hydrazino benzothiazole(compounds3a-d)

To 5 $cm^3$of hydrazine hydrate,5 $cm^3$of concentrated HCl dropwise at 5-10^0 C and 25 $cm^3$of ethylene glycol were added, stirred for 20 min. Substituted 2-amino benzothiazole***2a-d***(0.025mol) was added to this mixture in portions which was refluxed for 3 hours and cooled. The mixture was quenched with crushed ice. The solid separated was filtered, washed with water, dried and recrystallized from methanol. Compound 3a was taken as a representative example to explain characterization data.

Synthesis of 4-methyl benzothiazole-2-hydrazine (3a,$C_8H_9N_3S_2$)

Beige colour solid, yield: 83%; FT-IR (KBr, cm^{-1}): 1332 (C=N), 3010 (NH). ^1H NMR ($CDCl_3$): δ 2.06 (s, 2H, NH_2),4.10 (s, 1H, NH), 1.95 (s, 3H, Ar-CH_3), 7.46 (t, 1H, Ar-H), 7.33 (d, 1H, Ar-H), 7.85 (d, 1H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 16.3, 119.17, 125.4, 125.98, 127.17, 132.8, 147.9, 163.33; LC-MS m/z 180 $(M+1)^+$. Anal.Calcd. For $C_8H_9N_3S_2$: C, 53.10; H, 5.26; N, 23.55; S, 18.09%. Found: C, 53.60; H, 5.01; N, 23.45; S, 17.89%.

General procedure for synthesis of benzothiazolyl triazole-2-thiol derivatives (compounds 4a-d)

To a solution of hydrazines**3a-d** (3 mmol) in 60 $cm^3$of ethanol, 3 $cm^3$of carbon disulfide and sodium hydroxide (6mmol) were added and heated under reflux till evolution of hydrogen sulfide was ceased. Afterwards the reaction mixture was cooled to room temperature. The solvent was evaporated at reduced pressure, cold water was poured and acidified with dilute hydrochloric acid solution to bring the pH between 3 and 4. The precipitate thus separated out was allowed to stand overnight. Filtered, washed, dried and recrystallized from ethanol.

Synthesis of 5-methylbenzo[4,5]thiazolo[2,3-c][1,2,4]triazole-3-thiol(4a,$C_9H_7N_3S_2$)

Yellow solid, yield: 88%; FT-IR (KBr, cm^{-1}): 1356 (C=N), 2560 (SH);^{1}H NMR ($CDCl_3$): δ 13.09 (s, 1H, SH), 1.93 (s, 3H, Ar-CH_3), 7.49 (t, 1H, Ar-H), 7.36 (d, 1H, Ar-H), 7.88 (d, 1H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 16.3, 119.17, 125.98, 127.17, 132.8, 135.23, 153.9, 162.33, 168.45; LC-MS m/z 222 $(M+1)^+$. Anal.Calcd. For $C_9H_7N_3S_2$: C, 48.10; H, 3.26; N, 19.55; S, 28.88%. Found: C, 48.80; H, 3.19; N, 19.01; S, 28.89%

General procedure for synthesis of benzothiazolyl triazole-2-ylsulfanyl-ethyl piperidine derivatives (compounds 5a-d)

To a solution of benzothiazolyltriazole-2-thiol derivatives **4a-d** (1 mmol) in 15 $cm^3$of DMF, 1-(2-chloroethyl)-piperidine hydrochloride (1 mmol) and anhydrous potassium carbonate (3 mmol) were added and heated under reflux for 5-6 h. The reaction mixture was checked by TLC using petroleum ether:ethyl acetate (3:1). After completion of the reaction, the reaction mixture was cooled, excess of solvent was evaporated under reduced pressure. The residual crude was poured into crushed ice to remove potassium carbonate. The solid precipitate was filtered to get the desired target compound.

Synthesis of 5-methyl-3-((2-(piperidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole. (5a,$C_{16}H_{20}N_4S_2$)

Yellow gummy solid, yield: 82%; mp 50-53°C; FT-IR (KBr, cm^{-1}): 1351 (C=N), 1450 (CH_2);^{1}H NMR ($CDCl_3$): δ 2.52 (t, 4H, CH_2NCH_2 of piperidine ring), 2.74 (t, 2H, N CH_2), 3.44 (t, 2H, CH_2S), 1.54 (m, 4H, $CH_2CH_2CH_2$ of piperidine ring), 1.44 (t, 2H, CH_2 of piperidine ring), 1.25 (s, 3H, Ar-CH_3), 7.61 (t, *J=8.8*, *J=10.8*Hz,1H, Ar-H), 7.79 (d, *J=2.4*Hz, 1H, Ar-H), 8.013 (d, *J=8.8* Hz, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 15.9, 24.66, 26.14, 30.9, 56.69, 57.80, 119.27, 126.1, 126.98, 131.87, 135.1, 146.9, 153.71, 161.13; LC-MS m/z 333 $(M+1)^+$. Anal.Calcd. For $C_{16}H_{20}N_4S_2$: C, 57.10; H, 6.26; N, 16.85; S, 19.09%. Found: C, 57.80; H, 6.06; N, 16.85; S, 19.29%.

Synthesis of 7-methyl-3-((2-(piperidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole (5b,$C_{16}H_{20}N_4S_2$)

Yellow gummy solid, yield: 86%;mp 48-50°C; FT-IR (KBr, cm^{-1}):1343 (C=N), 1468 (CH_2); ^{1}H NMR ($CDCl_3$): δ 2.53 (t, 4H, CH_2NCH_2 of piperidine ring), 2.75 (t, 2H, N CH_2), 3.45(t, 2H, CH_2S), 1.52 (m, 4H, $CH_2CH_2CH_2$ of piperidine ring), 1.48 (t, 2H, CH_2 of piperidine ring), 1.21 (s, 3H, Ar-CH_3), 7.98 (d, *J=8.8* Hz, 1H, Ar-H), 7.77 (d, *J=2* Hz 1H, Ar-H), 7.57 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 21.3, 24.76, 26.54, 30.8, 56.9, 57.90, 121.27,121.34, 126.9, 134.87,134.9, 135.1, 146.9, 161.13; LC-MS m/z 333 $(M+1)^+$. Anal.Calcd. For $C_{16}H_{20}N_4S_2$: C, 57.10; H, 6.26; N, 16.85; S, 19.09%. Found: C, 57.80; H, 6.06; N, 16.85; S, 19.29%.

Synthesis of 7-bromo-3-((2-(piperidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole (5c,$C_{15}H_{17}BrN_4S_2$)

Brown gummy solid, yield: 84%; mp 45-46°C; FT-IR (KBr, cm^{-1}):1335 (C=N), 1438 (CH_2); ^{1}H NMR ($CDCl_3$): δ 2.44 (t, 4H, CH_2NCH_2 of piperidine ring), 2.79 (t, 2H, N CH_2), 3.49(t, 2H, CH_2S), 1.45 (m, 4H, $CH_2CH_2CH_2$ of piperidine ring), 1.23 (t, 2H, CH_2 of piperidine ring), 7.99 (d, *J=8.4* Hz, 1H, Ar-H), 7.76 (d, *J=2.2* Hz 1H, Ar-H), 7.56 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 24.6, 26.2, 30.7, 56.5, 57.80, 117.27, 123.9, 124.2 128.98, 136.17, 137.8, 146.3, 161.3; LC-MS m/z 397 $(M+1)^+$, 399 $(M+3)^+$. Anal.Calcd. For $C_{15}H_{17}BrN_4S_2$: C, 45.10; H, 4.26; N, 14.15; S, 16.09%. Found: C, 45.34; H, 4.31; Br, 20.11; N, 14.10; S, 16.14%.

2.1.4.4 Synthesis of 7-nitro-3-((2-(piperidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole (5d, $C_{15}H_{17}N_5O_2S_2$)

Browngummy solid, yield: 87%; mp 48-49°C; FT-IR (KBr, cm^{-1}): 1335 (C=N),1452 (CH_2); ^{1}H NMR ($CDCl_3$): δ 2.46 (t, 4H, CH_2NCH_2 of piperidine ring), 2.74 (t, 2H, N CH_2), 3.45 (t, 2H, CH_2S), 1.44 (m, 4H, $CH_2CH_2CH_2$ of piperidine ring), 1.20 (t, 2H, CH_2 of piperidine ring), 7.93 (d, *J=8.4* Hz, 1H, Ar-H), 7.73 (d, *J=2.4* Hz 1H, Ar-H), 7.59 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ24.9, 26.44, 30.4, 56.3, 57.50, 119.27, 121.9, 122.8, 136.1, 143.4, 144.8, 146.9, 161.8; LC-MS m/z 364 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{17}N_5O_2S_2$: C, 49.10; H, 4.66; N, 19.15; O, 8.83; S, 11.09%. Found: C, 49.57; H, 4.71; N, 19.27; O, 8.80; S, 17.64%.

General procedure for synthesis of benzothiazolyl triazole-2-ylsulfanyl)-ethyl morpholine derivatives(compounds6a-d)

To a solution of benzothiazolyl triazole-2-thiol derivatives **4a-d** (1 mmol) in 15 cm^3 of DMF, 4-(2-chloroethyl)-morpholine hydrochloride (1 mmol) and anhydrous potassium carbonate (3 mmol) were added and heated under reflux for 5-6 h. The reaction mixture was checked by TLC using petroleum ether:ethyl acetate (3:1). After completion of the reaction, the reaction mixture was cooled, excess of solvent was evaporated under reduced pressure. The residual crude was poured into crushed ice to remove potassium carbonate. The solid precipitate was filtered to get the desired target compound.

Synthesis of 4-(2-((5-methylbenzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)ethyl)morpholine. (6a, $C_{15}H_{18}N_4OS_2$,)

Yellow gummy solid, yield: 78%; mp 58-60 °C; FT-IR (KBr, cm^{-1}): 1343 (C=N),1272 (C-O-C), 1470 (CH_2);^{1}H NMR ($CDCl_3$): δ 2.52 (t, 4H, CH_2NCH_2 of morpholine ring), 2.82 (t, 2H, NCH_2), 3.47 (t, 2H, CH_2S), 3.69 (t, 4H, CH_2OCH_2 of morpholine ring), 1.242 (s, 3H, Ar-CH_3),7.65 (t, *J=8.4, J=10.4* Hz, 1H, Ar-H), 7.74 (d, *J=2* Hz, 1H, Ar-H), 8.015 (d, *J=8.4* Hz, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 29.66, 53.14, 56.69, 59.80, 59.97, 66.54, 76.99, 77.31, 116.20, 127.28, 129.67, 156.01, 162.93, 166.54; LC-MS m/z 335 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{18}N_4OS_2$: C, 53.10; H, 5.49; N, 16.81; S, 19.19%. Found: C, 53.87; H, 5.42; N, 16.75; O, 4.78; S, 19.17%.

Synthesis of 4-(2-((7-methylbenzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)ethyl)morpholine (6b, $C_{15}H_{18}N_4OS_2$,)

Yellow gummy solid,yield: 77%; mp 56-58 °C; FT-IR (KBr, cm^{-1}): 1352 (C=N),1276 (C-O-C), 1466 (CH_2); ^{1}H NMR ($CDCl_3$): δ 2.53 (t, 4H, CH_2NCH_2 of morpholine ring), 2.83 (t, 2H, NCH_2), 3.46 (t, 2H, CH_2S), 3.69 (t, 4H, CH_2OCH_2 of morpholine ring), 1.32 (s, 3H, Ar-CH_3), 7.92 (d, *J=9.2* Hz, 1H, Ar-H), 7.77 (d, *J=2.8* Hz 1H, Ar-H), 7.56 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 21.3, 30.8, 55.9, 57.90, 66.9, 121.37, 121.54, 126.9, 134.7,134.9, 135.1, 147.4, 162.13; LC-MS m/z 335 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{18}N_4OS_2$: C, 53.10; H, 5.49; N, 16.81; S, 19.19%. Found: C, 53.87; H, 5.42; N, 16.75; O, 4.78; S, 19.17%

Synthesis of 4-(2-((7-bromobenzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)ethyl)morpholine (6c, , $C_{14}H_{15}BrN_4OS_2$)

Browngummy solid, yield: 78%; mp 53-55°C; FT-IR (KBr, cm^{-1}): 1349 (C=N),1254 (C-O-C), 1425 (CH_2); ^{1}H NMR ($CDCl_3$): δ 2.54 (t, 4H, CH_2NCH_2 of morpholine ring), 2.89 (t, 2H, N CH_2), 3.49 (t, 2H, CH_2S), 3.66 (t, 4H, CH_2OCH_2 of morpholine ring), 7.98 (d, *J=9.2* Hz, 1H, Ar-H), 7.75 (d, *J=2* Hz 1H, Ar-H), 7.53 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 30.8, 56.7, 57.7, 66.6, 117.37, 123.7, 124.3 128.9, 136.2, 137.6, 146.9, 161.5;LC-MS m/z 399 $(M+1)^+$, 401 $(M+3)^+$. Anal.Calcd. For $C_{14}H_{15}BrN_4OS_2$: C, 45.10; H, 4.26; N, 14.15; S, 16.09%. Found: C, 42.11; H, 3.79; Br, 20.01; N, 14.03; O, 4.01; S, 16.06%.

Synthesis of 4-(2-((7-nitrobenzo[4,5]thiazolo[2,3-c][1,2,4]triazol-3-yl)thio)ethyl)morpholine (6d, $C_{14}H_{15}N_5O_3S_2$,)

Browngummy solid, yield: 81%; mp 58-60 °C; FT-IR (KBr, cm^{-1}): 1385 (C=N), 1248 (C-O-C), 1458 (CH_2);^{1}H NMR ($CDCl_3$): δ 2.54 (t, 4H, CH_2NCH_2 of morpholine ring), 2.89 (t, 2H, NCH_2), 3.49 (t, 2H, CH_2S), 3.69 (t, 4H, $CH_2CH_2CH_2$ of morpholine ring), 7.96 (d, *J=8.8* Hz, 1H, Ar-H), 7.765 (d, *J=2.4* Hz 1H, Ar-H), 7.58 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 30.7, 55.8, 57.70, 66.9, 119.3, 121.8, 122.6, 136.2, 143.5, 144.7, 146.7, 161.4;

LC-MS m/z 366 $(M+1)^+$. Anal.Calcd. For $C_{14}H_{15}N_5O_3S_2$: C, 49.10; H, 4.66; N, 19.15; O, 8.83; S, 11.09%. Found: C, 46.01; H, 4.14; N, 19.16; O, 13.13; S, 17.55%

2.1.6 General procedure for synthesis of benzothiazolyltriazole-2-ylsulfanyl)-ethylpyrrolidine derivatives (compounds 7a-d)

To a solution of benzothiazolyl triazole-2-thiol derivatives **4a-d** (1 mmol) in 15 cm^3 of DMF, 1-(2-chloroethyl)-pyrrolidine hydrochloride (1 mmol) and anhydrous potassium carbonate (3 mmol) were added and heated under reflux for 5-6 h. The reaction mixture was checked by TLC using petroleum ether:ethyl acetate (3:1).After completion of the reaction, the reaction mixture was cooled, excess of solvent was evaporated under reduced pressure. The residual crude was poured into crushed ice to remove potassium carbonate. The solid precipitate was filtered to get the desired target compound.

Synthesis of 5-methyl-3-((2-(pyrrolidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole (7a,$C_{15}H_{18}N_4S_2$)

Yellow gummy solid, yield: 83%; mp 52-54 °C; FT-IR (KBr, cm^{-1}): 1358 (C=N), 1454 (CH_2)^{1}H NMR ($CDCl_3$): δ 2.56 (t, 4H, CH_2NCH_2 of pyrrolidine ring), 2.82 (t, 2H, NCH_2), 3.47 (t, 2H, CH_2S), 1.65 (t, 4H, CH_2CH_2 of pyrrolidine ring), 1.92 (s, 3H, Ar-CH_3), 7.66 (t, *J=8.8, J=10.8* Hz, 1H, Ar-H), 7.75 (d, *J=2.4* Hz, 1H, Ar-H), 8.018 (d, *J=8.8* Hz, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 29.66, 53.14, 56.69, 59.80, 59.97, 66.54, 76.99, 77.31, 116.20, 127.28, 129.67, 156.01, 162.93, 166.54; LC-MS m/z 319 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{18}N_4S_2$: C, 57.10; H, 5.69; N, 17.81; S, 20.19%. Found: C, 56.57; H, 5.70; N, 17.59; S, 20.14%.

Synthesis of 7-methyl-3-((2-(pyrrolidin-1-yl)ethyl)thio)benzo[4,5]thiazolo [2,3c][1,2,4] triazole (7b, $C_{15}H_{18}N_4S_2$,)

Yellow gummy solid, yield: 78%; mp 48-50 °C; FT-IR (KBr, cm^{-1}): 1333 (C=N), 1450 (CH_2);^{1}H NMR ($CDCl_3$): δ 2.52 (t, 4H, CH_2NCH_2 of pyrrolidine ring), 2.87 (t, 2H, NCH_2), 3.48 (t, 2H, CH_2S), 1.66 (t, 4H, CH_2CH_2 of pyrrolidine ring), 2.34 (s, 3H, Ar-CH_3), 7.91 (d, *J=8.4* Hz, 1H, Ar-H), 7.75 (d, *J=2.4* Hz 1H, Ar-H), 7.49 (s, 1H, Ar-H);^{13}C-NMR ($CDCl_3$): δ 21.3, 24.76, 26.54, 30.8, 56.9, 57.90, 121.27,121.34, 126.9, 134.87,134.9, 135.1, 146.9, 161.13; LC-MS m/z 319 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{18}N_4S_2$: C, 56.70; H, 5.72; N, 17.71; S, 20.19%. Found: C, 56.57; H, 5.70; N, 17.59; S, 20.14%.

Synthesis of 7-bromo-3-((2-(pyrrolidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4] triazole (7c, $C_{14}H_{15}BrN_4S_2$)

Browngummy solid, yield 80%; mp 43-45 °C; FT-IR (KBr, cm^{-1}): 1357 (C=N),1485 (CH_2); ^{1}H NMR ($CDCl_3$): δ 2.56 (t, 4H, CH_2NCH_2 of pyrrolidine ring), 2.82 (t, 2H, NCH_2), 3.47 (t, 2H, CH_2S), 1.65 (t, 4H, CH_2CH_2 of pyrrolidine ring), 7.94 (d, *J=8.4* Hz, 1H, Ar-H), 7.75 (d, *J=2* Hz 1H, Ar-H), 7.52 (s, 1H, Ar-H);^{13}C-NMR ($CDCl_3$): δ 24.6, 26.2, 30.7, 56.5, 57.80, 117.27, 123.9, 124.2 128.98, 136.17, 137.8, 146.3, 161.3; LC-MS m/z 383 $(M+1)^+$, 385 $(M+3)^+$ Anal. Calcd. For $C_{14}H_{15}BrN_4S_2$: C, 43.90; H, 3.96; Br, 20.89; N, 14.65; S, 16.69%. Found: C, 43.87; H, 3.94; Br, 20.84; N, 14.62; S, 16.73%

Synthesis of 7-nitro-3-((2-(pyrrolidin-1-yl)ethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole (7d, $C_{14}H_{15}N_5O_2S_2$)

Browngummy solid, yield 82%; mp 47-49°C; FT-IR (KBr, cm^{-1}): 1354 (C=N), 1421 (CH_2);^{1}H NMR ($CDCl_3$): δ 2.45 (t, 4H, CH_2NCH_2 of pyrrolidine ring), 2.81 (t, 2H, NCH_2), 3.51 (t, 2H, CH_2S), 1.69(t, 4H, CH_2CH_2 of pyrrolidine ring), 7.99 (d, *J=8* Hz, 1H, Ar-H), 7.79 (d, *J=2* Hz 1H, Ar-H), 7.51 (s, 1H, Ar-H);^{13}C-NMR ($CDCl_3$): δ 24.9, 26.44, 30.4, 56.3, 57.50, 119.27, 121.9, 122.8, 136.1, 143.4, 144.8, 146.9, 161.8; LC-MS m/z 350 $(M+1)^+$. Anal.Calcd. For $C_{14}H_{15}N_5O_2S_2$: C, 48.10; H, 4.36; N, 20.15; O, 9.13; S, 18.09%. Found: C, 48.12; H, 4.33; N, 20.04; O, 9.16; S, 18.35 %.

General procedure for synthesis of benzothiazolyl triazole-2-ylsulfanyl)-methyl pyridine derivatives (compounds 8a-d)

To a solution of benzothiazolyl triazole-2-thiol derivatives **4a-d** (1 mmol) in 15 cm^3 of DMF, 2-(chloromethyl)pyridine hydrochloride (1 mmol) and anhydrous potassium carbonate (3 mmol) were added and

heated under reflux for 5-6 h. The reaction mixture was checked by TLC using petroleum ether:ethyl acetate (3:1).After completion of the reaction, the reaction mixture was cooled, excess of solvent was evaporated under reduced pressure. The residual crude was poured into crushed ice to remove potassium carbonate. The solid precipitate was filtered to get the desired target compound.

Synthesis of 5-methyl-3-((pyridin-2-ylmethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole(8a, $C_{15}H_{12}N_4S_2$)

Yellow gummy solid, yield 85%; mp 48-50°C; FT-IR (KBr, cm^{-1}): 1338 (C=N), 1458 (CH_2);^{1}H NMR ($CDCl_3$): δ 7.26 (t, 1H, CHCHCH of pyridine ring), 7.66 (t, 1H, CHCHCH of pyridine ring), 7.16 (d, 1H, CHCHCH of pyridine ring), 8.51 (d, 1H, CHN of pyridine ring), 5.41 (t, 2H, CH_2S), 1.98 (s, 3H, Ar-CH_3), 7.67 (t, *J=8, J=10* Hz, 1H, Ar-H), 7.79 (d, *J=2* Hz, 1H, Ar-H), 8.013 (d, *J=8.4* Hz, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 15.66, 40.81, 118.20, 120.9, 124.4, 125.98, 126.67, 131.4, 135.32, 136.4, 146.81, 148.8, 153.61, 158.8, 160.93; LC-MS m/z 313 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{12}N_4S_2$: C, 57.70; H, 3.9; N, 17.81; S, 20.29%. Found: C, 57.67; H, 3.87; N, 17.93; S, 20.53%.

Synthesis of 7-methyl-3-((pyridin-2-ylmethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole(8b, $C_{15}H_{12}N_4S_2$)

Yellow gummy solid, $C_{15}H_{12}N_4S_2$, yield 87%; mp 50-52°C; FT-IR (KBr, cm^{-1}): 1333 (C=N), 1470 (CH_2);^{1}H NMR ($CDCl_3$): δ 7.29 (t, 1H, CHCHCH of pyridine ring), 7.68 (t, 1H, CHCHCH of pyridine ring), 7.18 (d, 1H, CHCHCH of pyridine ring), 8.58 (d, 1H, CHN of pyridine ring), 5.47 (t, 2H, CH_2S), 2.39 (s, 3H, Ar-CH_3), 7.98 (d, *J=8.8* Hz, 1H, Ar-H), 7.78 (d, *J=2.8* Hz 1H, Ar-H), 7.52 (s, 1H, Ar-H);^{13}C-NMR ($CDCl_3$): δ 21.3, 40.71, 120.9, 121.30, 121.57, 124.6, 126.87, 134.4, 134.5, 135.42, 136.7, 146.90, 148.9, 158.8, 161.43; LC-MS m/z 313 $(M+1)^+$. Anal.Calcd. For $C_{15}H_{12}N_4S_2$: C, 57.70; H, 3.9; N, 17.81; S, 20.29%. Found: C, 57.67; H, 3.87; N, 17.93; S, 20.53%.

Synthesis of 7-bromo-3-((pyridin-2-ylmethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole(8c, $C_{14}H_9BrN_4S_2$)

Browngummy solid, , yield 88%; mp 49-51 °C; FT-IR (KBr, cm^{-1}): 1389 (C=N), 1454 (CH_2);^{1}H NMR ($CDCl_3$): δ 7.29 (t, 1H, CHCHCH of pyridine ring), 7.69 (t, 1H, CHCHCH of pyridine ring), 7.18 (d, 1H, CHCHCH of pyridine ring), 8.56 (d, 1H, CHN of pyridine ring), 7.97 (d, *J=8* Hz, 1H, Ar-H), 7.75 (d, *J=2.4* Hz 1H, Ar-H), 7.59 (s, 1H, Ar-H);^{13}C-NMR ($CDCl_3$): δ 40.71, 120.9, 121.30, 121.57, 124.6, 126.87, 134.4, 134.5, 135.42, 136.7, 146.90, 148.9, 158.8, 161.43; LC-MS m/z 377 $(M+1)^+$, 379 $(M+3)^+$. Anal.Calcd. For $C_{14}H_9BrN_4S_2$: C, 45.10; H, 4.26; N, 14.15; S, 16.09%. Found: C, 45.34; H, 4.31; Br, 20.11; N, 14.10; S, 16.14%.

Synthesis of 7-nitro-3-((pyridin-2-ylmethyl)thio)benzo[4,5]thiazolo[2,3-c][1,2,4]triazole(8d, $C_{14}H_9N_5O_2S_2$)

Browngummy solid, , yield 78%; mp 58-60 °C; FT-IR (KBr, cm^{-1}): 1389 (C=N), 1489 (CH_2);^{1}H NMR ($CDCl_3$): δ 7.28 (t, 1H, CHCHCH of pyridine ring), 7.68 (t, 1H, CHCHCH of pyridine ring), 7.19 (d, 1H, CHCHCH of pyridine ring), 8.53 (d, 1H, CHN of pyridine ring), 7.90 (d, *J=8.4* Hz, 1H, Ar-H), 7.75 (d, *J=2* Hz 1H, Ar-H), 7.50 (s, 1H, Ar-H); ^{13}C-NMR ($CDCl_3$): δ 40.71, 120.9, 121.30, 121.57, 124.6, 126.87, 134.4, 134.5, 135.42, 136.7, 146.90, 148.9, 158.8, 161.43; LC-MS m/z 344 $(M+1)^+$. Anal.Calcd. For $C_{14}H_9N_5O_2S_2$: C, 49.10; H, 4.66; N, 19.15; O, 8.83; S, 11.09%. Found: C, 49.57; H, 4.71; N, 19.27; O, 8.80; S, 17.64%.

Biological Activity

Anti- inflammatory activity study using Gelatin Zymography:

Gelatin zymography is the most appropriate assay system to quantify the secretion of MMP-2 and MMP-9. Electrophoresis apparatus with large plate, two spacers and small plate on top were taken. The plates were assembled with the help of agarose gel to seal the bottom surface.10 % resolving gel was prepared by mixing reagents of Acryl amide – Bisacrylamide 3.5 ml,10 % SDS, 1.5 % APS and TEMED .5 % stacking gel was prepared by Acrylamide – Bisacrylamide 1.7 ml, 5 % SDS, 0.75 % APS and TEMED. 80 % of the plates were filled with 10 % resolving gel and were allowed to set for 45 minutes. Later stacking gel was poured in, allowed to set for about 30 minutes and assembled gels onto the electrode/gasket section of the gel apparatus. Added 50 µl of MMP sample and 50 µl of the extract/compound and incubated for one hour. Mixed this with non reducing buffer in equal volume. 50 µl of MMP sample was taken for negative control and 50 µl of MMP sample + 50 µl of Tetracycline HCl (store for 1hr) was taken for positive control. Load 20 µl of sample in each

well. Run the apparatus at about 50V for 15 min by connecting the electrodes and then 100V until the bromophenol blue reached the bottom of the plates. After electrophoresis, dissemble the apparatus and put the gel into a plastic dish and wash the gel with zymogram 0renaturing buffer i.e.2.5%Triton for one hour and allow proteins to denature. Decant the zymogram renaturing buffer and incubate the gel in zymogram incubation buffer at 37^0C overnight and stained and destained. After staining, the background stains blue with Coomassie stain where the gelatin was degraded, while bands appear indicating the presence of gelatinases. The lower bands were gelatinases-A (MMP-2) which is about 72KD while the upper bands were gelatinases-B (MMP-2) which is about 90KD.

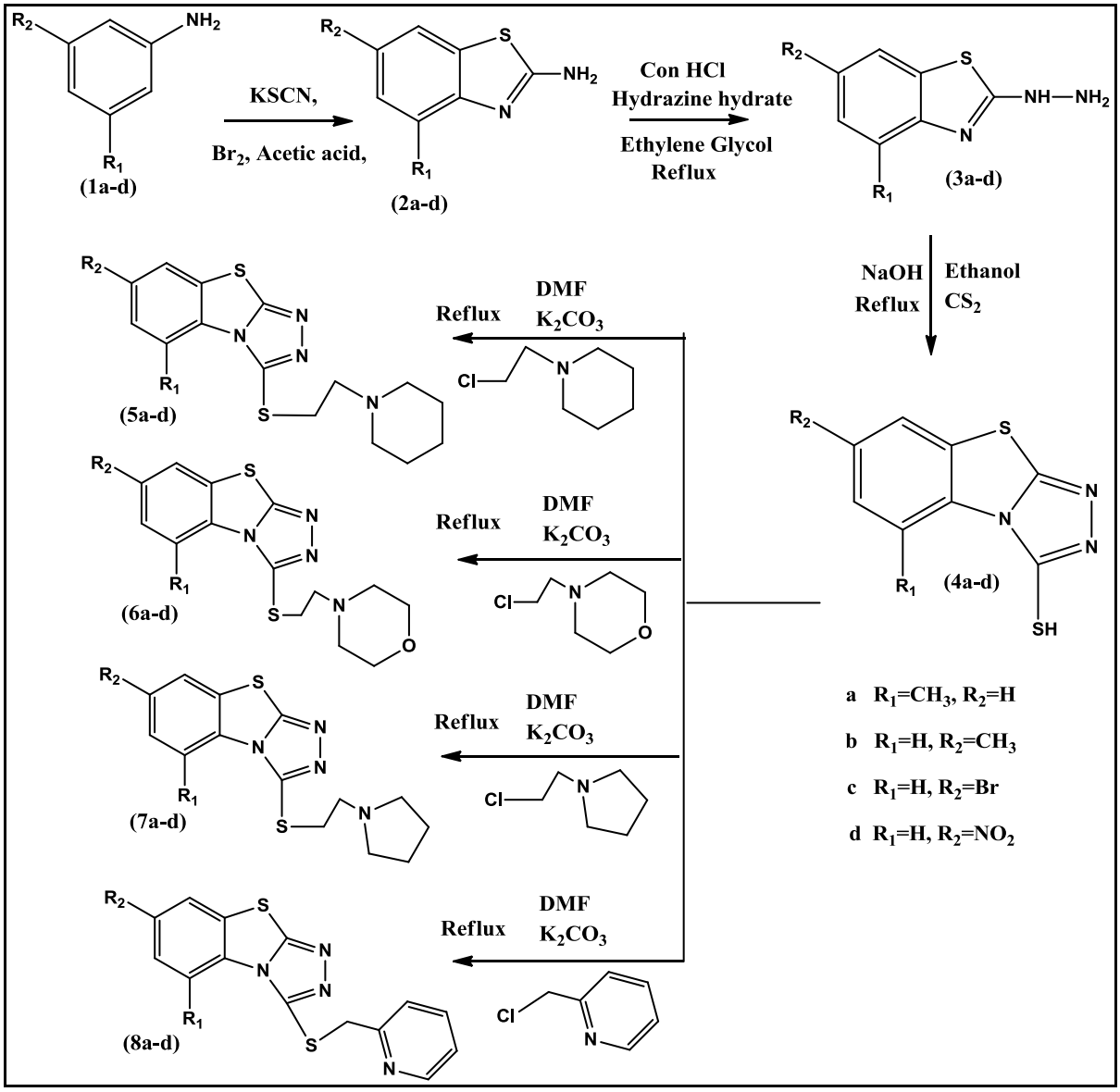
Antioxidant activity:

Free radical scavenging ability by DPPH radical assay [1,1-diphenyl-2-picryl hydrazyl]:

The DPPH radical scavenging activity was determined by different concentrations of test samples in aliquots of 100 µl were mixed with 100 µl of 40 µM methanolic solutions of DPPH (Himedia, Mumbai, India) in a 96-well micro titer plates. The decrease in absorbance was recorded at 517 nm after the incubation at room temperature for 15 min. The absorbance of the DPPH solution with methanol and without sample was used as the control. The ascorbic acid (AA, Himedia, Mumbai) was used as a standard to compare the activity. Appropriate blank readings at 517 nm were recorded for each tested dilutions. The assay was carried out in duplicate. The percentage inhibition of the DPPH radical by the samples was calculated. The IC_{50} value was calculated graphically based on the capacity of compound concentration to scavenge 50% of free radicals.

ABTS [2,2'-azino-bis (3-ethyl benzthiazolin-6-sulphonic acid)] radical scavenging activity:

ABTS assay was performed with a modification of the Samaga *et al.* method [19]. The ABTS stock solution was prepared by mixing equal volumes of 7.4 mM ABTS solution and 2.6 mM potassium per sulfate solution followed by incubation for 12 h at room temperature in the dark. The reaction mixture consisted of 1 ml of the purified compound at different concentrations (0.5–500 µg/ml in respective solvents) and 3 ml of standardized ABTS solution. The decrease in absorbance was measured at 734 nm after 15 min, of incubation. Data for each assay were recorded in triplicate. Ascorbic acid (NICE Chemicals Pvt. Ltd, Cochin, India) was used as positive control. The scavenging activity was estimated. The IC_{50} value was calculated graphically based on the capacity of compound concentration to scavenge 50% of free radicals.



Scheme 1.Synthesis of triazole conjugated benzothiazole derivatives.

Results and Discussion: Chemistry

The synthetic protocol of the synthesis of title compounds is represented in the Scheme 1. In this present pursuit, novel 16 compounds of substituted mercaptobenzothiazolyltriazoles are synthesized while unsubstitutedmercaptobenzothiazolyltriazoles were reported earlier. Substituted 2-amino benzothiazoles**2a-d** were obtained by refluxing commercially available substituted aniline with potassium thiocyanate and confirmed by the appearance of C=N and NH_2 stretching bands of benzothiazole amine in the FT-IR spectrum. The compounds **2a-d** on treatment with hydrazine hydrate afforded hydrazine benzothiazoles, which were confirmed by the appearance of NH_2 stretching band in the FT-IR spectra and the appearance of NH_2 and NH protons in NMR spectroscopy. Hydrazino derivatives **3a-d**were then subjected to cyclization with carbon disulfide in the presence of NaOH to yield the corresponding triazol-2-thiol **4a-d,** which proved the disappearance of NH_2 stretching band in the FT-IR spectra. The appearance of SH proton and the disappearance of NH_2 and NH protons of hydrazine in the NMR spectra also confirms the formation of the product. Ultimately, the title compounds were obtained by alkylation of triazol-2-thiol derivatives with heterocyclic compounds in boiling DMF with fused potassium carbonate.

Biology

In the present scenario, synthesized vital molecules have played a prime role in the development of multi target drugs with precise action. Unique heterocyclic compounds with a linked multi component structure were synthesized having benzothiazole and triazole cores as backbone with nucleophillic alkylation of piperidine, morpholine, pyrrolidine and pyridine nuclei.

Structure activity relationship (SAR).

Anti-inflammatory Activity study

Anti-inflammatory activity can be studied by intervening matrix metalloproteinase (MMP)-2 and (MMP)-9 expressions by gelatin zymography as shown in Table l. Anomalous production of MMP-2 and MMP-9 assist cytoskeletal reorganization, migration and invasion of tumor cells.Anti-inflammatory activity was investigated by gelatin zymography with tetracycline hydrochloride as positive control as shown in Figure l. The lower bands were gelatinases-A (MMP-2) which is about 72KD while the upper bands were gelatinases-B (MMP-2) which is about 90KD.

Table 1.Anti- inflammatory activity of the triazole conjugated benzothiazole derivatives.

Name of the Sample	Anti-inflammatory activity against MMP-2	Anti-inflammatory activity against MMP-9
5a	**85%**	**80%**
6a	**75%**	**85%**
7a	**90%**	**75%**
8a	**88%**	**70%**
5b	**85%**	**85%**
6b	**75%**	**88%**
7b	**92%**	**90%**
8b	**92%**	**85%**
5c	**85%**	**65%**
6c	**93%**	**67%**
7c	**88%**	**55%**
8c	**87%**	**60%**
5d	**90%**	**70%**
6d	**89%**	**75%**
7d	**85%**	**78%**
8d	**88%**	**80%**
PC	**95%**	**90%**
NC	**10%**	**Nil**
Positive Control: Tetracycline hydrochloride , NC: MMP sample		

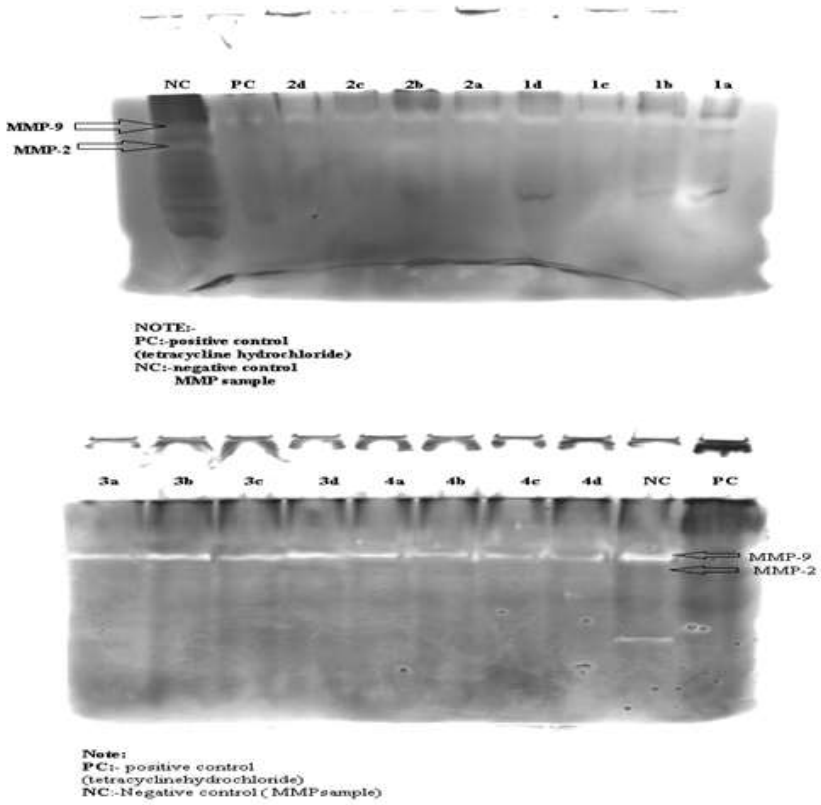


Figure 1. Gelatin Zymography Assay of Anti-inflammatory activity

Evaluation of Anti-inflammatory activity in gelatin zymography revealed that, compound **6c**(*7*-Brwithmorpholine) group exhibited maximum 92**%** against(MMP)-2 and compound **7b**(*7-CH_3* with pyrrolidine) group exhibited maximum 92**%** against(MMP)-9. Compound **6a** and **6b** (*CH_3* with morpholine) group exhibited least 75 **%** against(MMP)-2 and Compound **8c**(*7- NO_2*with pyridine group) exhibited least 60%against(MMP)-9.The compounds**7b**(*7-CH_3*with pyrrolidine) and **8b** *(7-CH_3* with pyridine) exhibited better activity against both MMPs, thereby curtailing the expression of MMP-2 and MMP-9. The structure–activity relationship study discloses that, the compounds with pyrrolidine and pyridine scaffolds having electron donating group exhibited the superior activity against MMP-9 whereas the compounds with morpholineand pyridine having electron withdrawing groups exhibit the least activity.

Antioxidant study

Free radicals play a very important role in the pathogenesis of various human diseases and aging. The antioxidants that scavenge reactive free radicals may be of great value in preventing the onset and propagation of oxidative diseases. Highly reactive free radicals react with lipids, proteins and DNA, evoking irreversible damages in their bio-molecular structure. Hence, Scavenging activity can be studied by DPPH and ABTS methods. The matching reciprocity could be seen in both the DPPH and ABTS *In -vitro* antioxidant activities of benzothiazole coupled triazole derivatives.

In the present study, majority of benzothiazole conjugated triazole compounds displayed excellent activity with concordant IC_{50} values between 120-82 µg/ml concentrations in DPPH and ABTS assay in comparison with standard ascorbic acid. Particularly, compounds **7b** *(7-CH_3*with pyrrolidine) and **5c** *(7-Br* with piperidine) having IC_{50} values 93.25 and 82.25 respectively, were most efficient among the series in Figure 2. The SAR study discloses that, the compounds with pyrrolidine and piperidine scaffolds exhibited the superior activity irrespective of electron donating and pyridine having electron withdrawing groups. Compound 7b showed the IC_{50} value at 93 and 92 µg/ml for ABTS and DPPH, respectively.The remaining compounds showed the moderate to lower activity and have IC_{50} values at higher concentrations.

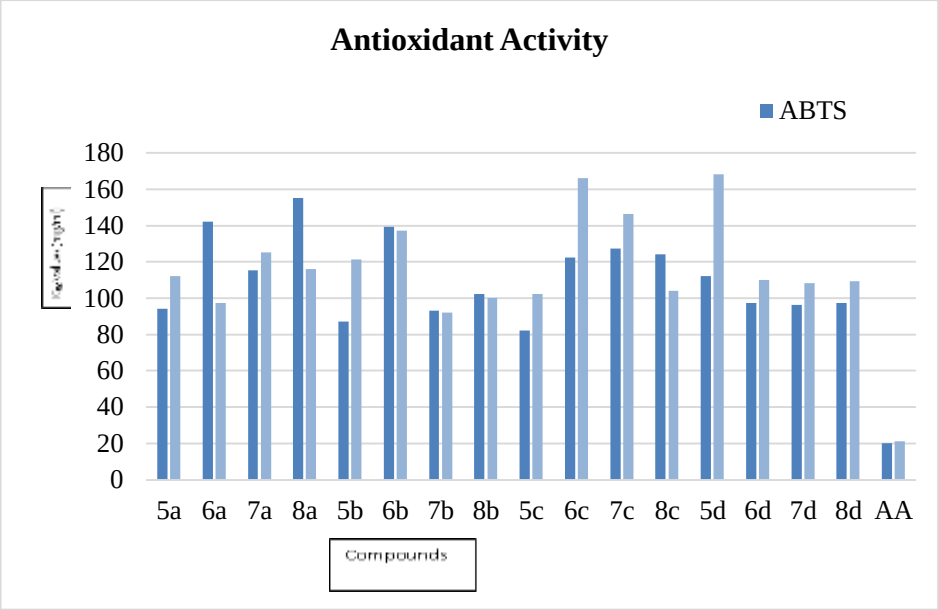
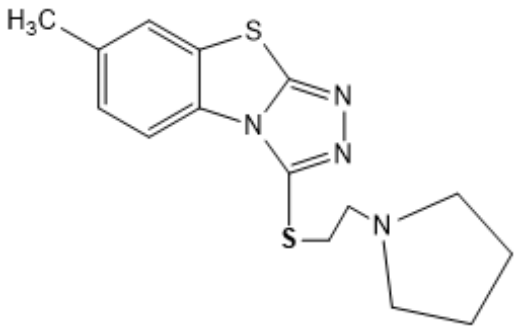


Figure 2. Antioxidant activity of the synthesized compounds.

Compound 7b is the potent multi bioactivities molecule

Based on the *In-vitroscreening of series of synthesized compounds*,compound**7b** showed good biological activity as anti-inflammation and antioxidant agents and it has emerged as the potent compound. Structure–activity relation interrelates the biological activity and chemical structure of the molecule. Structurally, compound *7b* has a pyrrolidine group (five membered ring) attached to two CH_2 groups and methyl substituent at 7^{th} position of the benzothiazole ring.



Compound 7b

7-methyl-3-((2-(pyrrolidin-1-yl)ethyl)thio)benzo[4,5]thiazolo [2,3c][1,2,4] triazole

Conclusions

The synthesized compounds were characterized by spectroscopic techniques. In the present study, structure-based drug design method has been utilized to synthesize the series of benzothiazole conjugated triazole derivatives. In general, the activity of the compound is influenced by the chemical structure, size &shape, molecular arrangements and electron donating/withdrawing groups, *etc*. Compounds with pyrrolidine and piperidine moieties exhibit more superior activity than pyridine and morpholine cores. However, pyridine and morpholine groups showed better activities when Br group was at 7 position. Compound **7b** is the novel multi target molecule acting as anti tubercular, anti-inflammatory, antibacterial and anti fungal activities.

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