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Synthesis and *in vitro* evaluation of tetrazole containing 1,5-benzothiazepines as new anticancer, antitubercular, antibacterial, and antifungal agents

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ABSTRACT

Heterocyclic scaffolds have attracted great attention of organic chemists and medicinal chemists, also because of their wide range of synthetic applicability and broad spectrum of biological profile. Therefore, in the present research work, a series of tetrazole containing 1,5-benzothiazepines have been synthesized for evaluation of their biological activities to determine the potential therapeutic profile of these compounds across various medicinal domains. Of the synthesized compounds, five compounds (6f, 8e, 8f, 8g, and 8h) have been screened for anticancer, antitubercular, antibacterial, and antifungal activities. After evaluation of these biological activities, it was found that these compounds possess very limited anticancer activity, moderate antibacterial and antifungal activity, and very strong antitubercular activity, which indicate their great pharmacological applications as subjects for future investigations of novel therapeutic agents for the treatment of tuberculosis.

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1. Introduction

Organic synthetic chemistry is a rapidly growing field among the chemical sciences, and nitrogen and sulphur containing heterocyclic scaffolds have been attracted a great attention of researchers because of their remarkable chemical properties and diverse pharmacological profiles [1-5]. Azepine derivatives are a valuable heterocyclic framework for synthesizing medicinally potent novel heterocycles for drug discovery and development because of their broad pharmacological profiles. Benzo fused azepine and tetrazoles are privileged structures due to their wide range of pharmacological activities and 1,5-benzothiazepines are extremely versatile pharmacophores found in drug molecules [6,7]. Diltiazem and clentiazem, two well-known 1,5-benzothiazepines (BTA)-based drugs, are prescribed as angina-relieving calcium channel blockers, angiotensin conversion enzyme (ACE) inhibitors, coronary vasodilators to treat cardiovascular disorders [8-10]. Thiazesim and quetiapine fumarate are another class of 1,5-benzothiazepine derivatives that are used as psychotropic agents in the treatment of Central Nervous System (CNS) disorders [11]. Other interesting biological properties including anticancer [12-14], anti-HIV [15], enzyme inhibitors [16], anti-diabetic [17], anticonvulsant [18], antimicrobial [19,20] activities have also been explored for the 1,5-benzothiazepine derivatives. Due to the importance of 1,5-benzothiazepines, several reviews have been published previously with a focus on chemistry including novel, mild, green, and highly efficient synthetic routes [21-25]. It is found that nucleophilic attack of substituted 2-aminothiophenols on α,β-unsaturated carbonyl compounds is a general method for synthesis of 1,5-benzothiazepines, which has also been employed in the present research work for the synthesis of 4-(4-(1*H*-tetrazol-1-yl)phenyl)-2-phenyl-2,3-dihydrobenzo[b][1, 4]thiazepine, 4-(4-(1*H*-tetrazol-1-yl)phenyl)-2-(*p*-tolyl)-2, 3-dihydrobenzo[b][1, 4]thiazepine, 4-(4-(1*H*-tetrazol-1-yl)phenyl)-2-(4-methoxyphenyl)-2, 3-dihydrobenzo[b][1, 4]thiazepine, 4-(4-(1*H*-tetrazol-1-yl)phenyl)-2-(4-nitrophenyl)-2,3-dihydrobenzo[b][1,4]thiazepine derivatives which are presented in Schemes 1 and 2 for *in vitro* evaluation of the pharmacological profile.

2. Experimental

2.1. Materials and instrumentations

All reagents and solvents were purchased from Sigma-Aldrich and Alfa-Aesar. All solvents were distilled off and dried properly before use.

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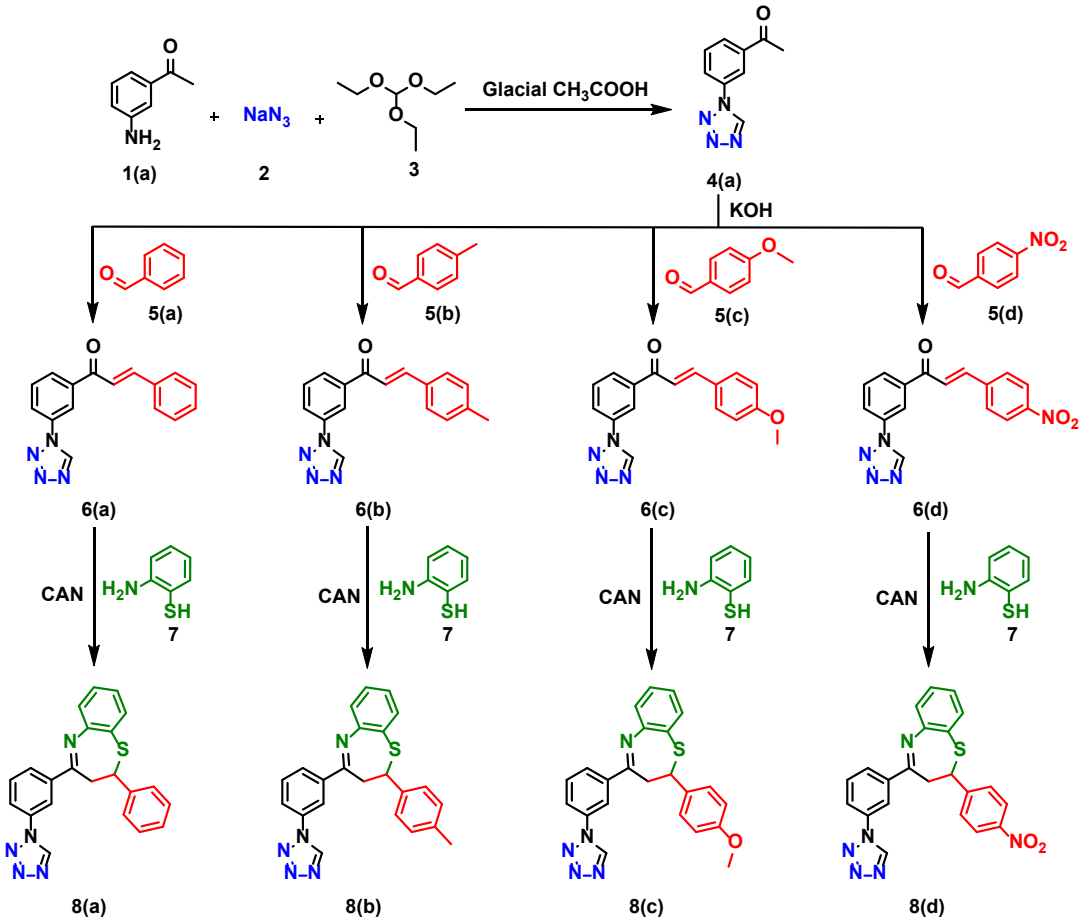


Scheme 1. Synthesis of tetrazole containing 1,5-benzothiazepines (Compounds 4a, 6a-d and 8a-d).

The melting points were recorded in the open capillaries and are incorrect. Fourier transform-infra-red (FT-IR) spectra were recorded at Materials Research Centre (MRC), Malviya National Institute of Technology (MNIT), Jaipur, on the FT-IR Spectrum 2 (Perkin Elmer) spectrometer using KBr pallets. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were also recorded at MRC, MNIT, Jaipur on ECS 400 MHz (JEOL) NMR spectrometer by using tetramethyl silane (TMS) as an internal standard with peak values shown in δ ppm. The multiplicities of the signals are represented by *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), and *m* (multiplet). MS spectra were recorded at MRC, MNIT, Jaipur as well as at Central Instrumentation Laboratory (CIL), Guru Jambheshwar University of Science & Technology, Hisar (Haryana) on Waters, Q-TOF Micromass (LC-MS) Mass Spectrometers. All compounds were homogeneous and single spotted on thin layer chromatography (TLC) using precoated aluminum sheet E-Merck with GF_{254} silica gel, 0.2 mm layer thickness in various solvent systems. The analytical data of all compounds were found to be consistent with the structures of these molecules.

2.2. Synthesis

2.2.1. Synthesis of compounds 4a-b

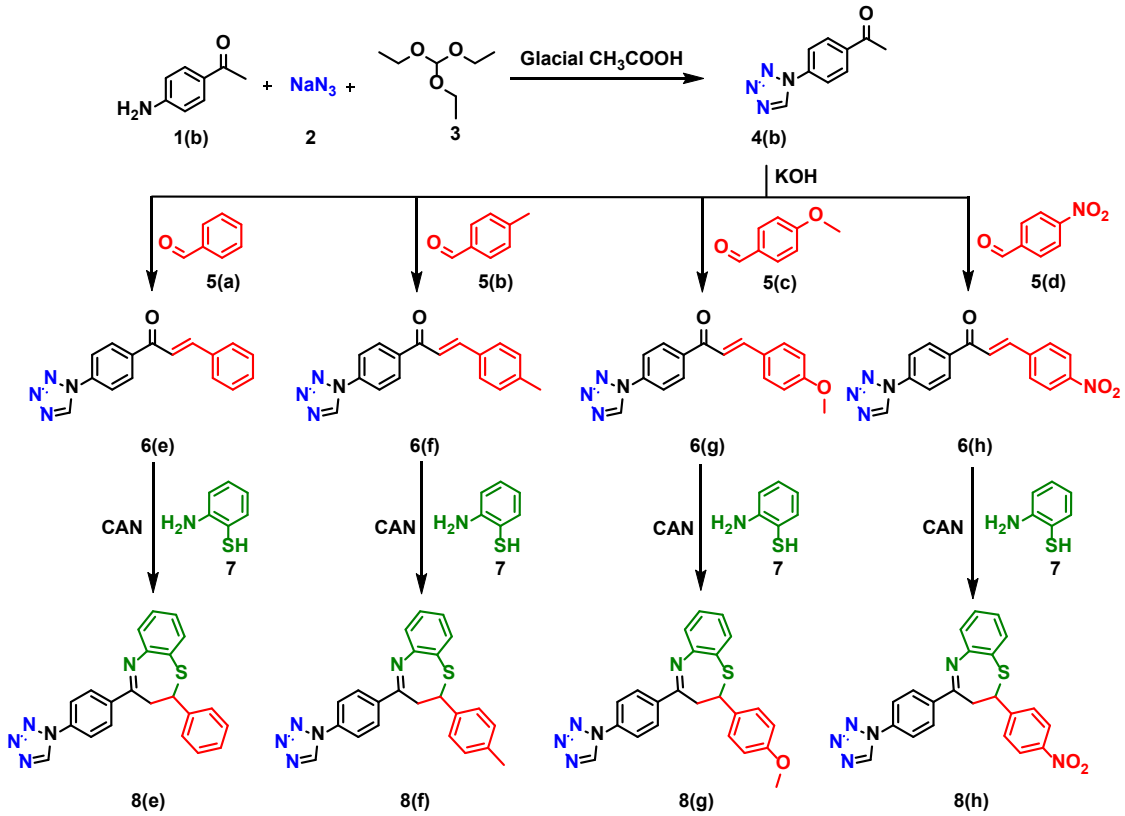
In a round bottom two-neck flask, compounds 1a and b, sodium azide (2), and triethyl orthoformate (3) in the ratio 1:3:1.1 were taken. After taking all these chemicals, glacial acetic acid was added dropwise with continuous stirring at 580-600 rpm. The reaction mixture was refluxed for 7-8 hours at 80-85 °C. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was acidified in ice-cold water, and then the precipitate was filtered through a Buchner funnel. The dried precipitate was recrystallized with ethanol to obtain pure compounds 4a and 4b.

1-(3-(1H-Tetrazol-1-yl)phenyl)ethenone (4a): Color: Pale-yellow. Yield: 91%. M.p.: 189-191 °C. FT-IR (KBr, *v*, cm^{-1}): 3049 (C-H) (Aromatic ring), 1692 (C=O), 1450 (C=N) (Tetrazole ring), 1310 (N-N=N), 1022 (Tetrazole ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.16 (s, 1H, Tetrazole-H), 8.10 (m, 3H, Ar-H), 7.81 (s, 1H, Ar-H), 2.38 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 199.0, 145.4, 141.3, 138.2, 136.0, 132.4, 130.1, 125.2, 35.8. HRMS (ESI, *m/z*) calcd. for $C_9H_8N_4O$, 188.1860; found 188.0546.

1-(4-(1H-Tetrazol-1-yl)phenyl)ethenone (4b): Color: Pale-yellow. Yield: 95 %. M.p.: 190-192 °C. FT-IR (KBr, *v*, cm^{-1}): 3053 (C-H) (Aromatic ring), 1697 (C=O), 1445 (C=N) (Tetrazole ring), 1304 (N-N=N), 1028 (Tetrazole ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.19 (s, 1H, Tetrazole-H), 8.11 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.69 (d, *J* = 8.8 Hz, 2H, Ar-H), 2.49 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 197.0, 144.6, 138.1, 135.0, 130.6, 123.2, 27.7. HRMS (ESI, *m/z*) calcd. for $C_9H_8N_4O$, 188.1860; found 188.0547.

2.2.2. Synthesis of compounds 6a-h

To a solution of acetophenone derivative 4b (188 mg, 1.0 mmol) in methanol (15 mL), 4-methylbenzaldehyde 5b (120 mg, 1.0 mmol) was added. To this mixture, methanolic potassium hydroxide (0.056 mg, 1.0 mmol) was poured gradually with constant stirring. After that, the stirring was continued for 24 hours at room temperature.



Scheme 2. Synthesis of tetrazole containing 1,5-benzothiazepines (Compounds 4b, 6e-h and 8e-h).

The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice-cold water and neutralized by 3 N HCl in an ice bath. The separated solid compound was then filtered and washed with ice-cold water until the washing was neutral to litmus. Finally, the compound was recrystallized from ethanol and dried at room temperature to get a pure compound 6f. Other compounds were synthesized following a method similar to that adopted for the synthesis of compound 6f.

1-(3-(1H-Tetrazol-1-yl) phenyl)-3-phenylprop-2-en-1-one (6a): Color: Yellowish. Yield: 91 %. M.p.: 172-174 °C. FT-IR (KBr, *v*, cm^{-1}): 3318 (C-H) (α,β-unsaturated carbonyl group), 3022 (C-H) (aromatic ring), 2231 (C=N) (tetrazole ring), 1665 (C=O) (α,β-unsaturated carbonyl group), 1567 (C=C) (aromatic ring), 1389 (N-N=N) (tetrazole ring), 995-892 (C=C) (α,β-unsaturated carbonyl group). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.31 (s, 1H, tetrazole ring), 8. 34 (s, 1H, Ar-H), 8.20 (m, 3H, Ar-H), 8.10 (d, *J* = 15.4 Hz, 1H, CH), 7.86 (m, 5H, Ar-H), 7.76 (d, *J* = 15.4 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 188.9, 144.6, 144.1, 141.5, 140.1, 139.5, 139.1, 130.3, 129.1, 128.9, 123.2, 121.2, 120.6, 120.1. HRMS (ESI, *m/z*) calcd. for $C_{16}H_{12}N_4O$, 276.2927; found 276.1004.

1-(3-(1H-Tetrazol-1-yl) phenyl)-3-(p-tolyl) prop-2-en-1-one (6b): Color: Yellow. Yield: 89 %. M.p.: 168-170 °C. FT-IR (KBr, *v*, cm^{-1}): 3316 (C-H) (α,β-unsaturated carbonyl group), 3018 (C-H) (aromatic ring), 2980 (C-H) (methyl group), 2230 (C=N) (tetrazole ring), 1668 (C=O) (α,β-unsaturated carbonyl group), 1560 (C=C) (aromatic ring), 1382 (N-N=N) (tetrazole ring), 990-891 (C=C) (α,β-unsaturated carbonyl group). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.36 (s, 1H, tetrazole ring), 8. 34 (s, 1H, Ar-H), 8.20 (m, 3H, Ar-H), 8.11 (d, *J* = 15.4 Hz, 1H, CH), 7.80 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.76 (d, *J* = 15.4 Hz, 1H, CH), 7.20 (d, *J* = 7.9 Hz, 2H, Ar-H), 2.12 (s, 3H, CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): 189.2, 145.8, 144.6, 143.5, 142.1, 141.5, 140.1, 138.9, 138.4, 137.8, 136.4, 130.3, 129.1, 128.9, 21.2. HRMS (ESI, *m/z*) calcd. for $C_{17}H_{14}N_4O$, 290.3193; found 290.1131.

1-(3-(1H-Tetrazol-1-yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (6c): Color: Pale-yellow. Yield: 87 %. M.p.: 173-175 °C. FT-IR (KBr, *v*, cm^{-1}): 3321 (C-H) (α,β-unsaturated carbonyl group), 3014 (C-H) (aromatic ring), 2985 (C-H) (methyl group), 2239 (C=N) (tetrazole ring), 1675 (C=O) (α,β-unsaturated carbonyl group), 1568 (C=C) (aromatic ring), 1389 (N-N=N) (tetrazole ring), 998-896 (C=C) (α,β-unsaturated carbonyl group). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.33 (s, 1H, tetrazole ring), 8. 36 (s, 1H, Ar-H), 8.25 (m, 3H, Ar-H), 8.17 (d, *J* = 15.4 Hz, 1H, CH), 7.82 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.78 (d, *J* = 15.4 Hz, 1H, CH), 7.26 (d, *J* = 7.9 Hz, 2H, Ar-H), 3.76 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 190.6, 146.1, 145.8, 144.2, 143.2, 142.8, 141.1, 139.8, 139.4, 138.8, 137.9, 132.1, 130.4, 126.8, 55.9. HRMS (ESI, *m/z*) calcd. for $C_{17}H_{14}N_4O_2$, 306.3187; found 306.1069.

1-(3-(1H-Tetrazol-1-yl)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (6d): Color: Pale-yellow. Yield: 84 %. M.p.: 178-180 °C. FT-IR (KBr, *v*, cm^{-1}): 3328 (C-H) (α,β-unsaturated carbonyl group), 3019 (C-H) (aromatic ring), 2248 (C=N) (tetrazole ring), 1681 (C=O) (α,β-unsaturated carbonyl group), 1573 (C=C) (aromatic ring), 1391 (N-N=N) (tetrazole ring), 995-898 (C=C) (α,β-unsaturated carbonyl group). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.36 (s, 1H, tetrazole ring), 8.36 (s, 1H, Ar-H), 8.28 (m, 3H, Ar-H), 8.19 (d, *J* = 15.4 Hz, 1H, CH), 7.92 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.88 (d, *J* = 15.4 Hz, 1H, CH), 7.42 (d, *J* = 7.9 Hz, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 1901.3, 147.4, 146.3, 145.9, 144.8, 143.9, 142.8, 140.1, 139.6, 139.1, 138.7, 134.5, 132.7, 130.1. HRMS (ESI, *m/z*) calcd. for $C_{16}H_{11}N_5O_3$, 321.2902; found 321.0821.

1-(4-(1H-Tetrazol-1-yl) phenyl)-3-phenylprop-2-en-1-one (6e): Color: Yellowish. Yield: 92 %. M.p.: 176-178 °C. FT-IR (KBr, *v*, cm^{-1}): 3320 (C-H) (α,β-unsaturated carbonyl group), 3019 (C-H) (aromatic ring), 2232 (C=N) (tetrazole ring), 1668 (C=O)

(α,β-unsaturated carbonyl group), 1540 (C=C) (aromatic ring), 1381 (N-N=N) (tetrazole ring), 985-898 (C=C) (α,β-unsaturated carbonyl group). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.32 (s, 1H, tetrazole ring), 8.32 (d, *J* = 7.9 Hz, 2H, Ar-H), 8.16 (d, *J* = 7.9 Hz, 2H, Ar-H), 8.12 (d, *J* = 15.4 Hz, 1H, CH), 7.86 (m, 5H, Ar-H), 7.78 (d, *J* = 15.4 Hz, 1H, CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 190.2, 145.2, 144.8, 142.8, 141.6, 140.2, 139.8, 132.4, 130.7, 129.5, 125.5, 124.8. HRMS (ESI, *m/z*) calcd. for $C_{16}H_{12}N_4O$, 276.2927; found 276.1014.

1-(4-(1H-Tetrazol-1-yl) phenyl)-3-(p-tolyl) prop-2-en-1-one (6f): Color: Pale-yellow. Yield: 94 %. M.p.: 175-177 °C. FT-IR (KBr, *v*, cm^{-1}): 3321 (C-H) (α,β-unsaturated carbonyl group), 3043 (C-H) (aromatic ring), 2915 (C-H) (methyl group), 2233 (C=N) (tetrazole ring), 1682 (C=O) (α,β-unsaturated carbonyl group), 1600 (C=C) (aromatic ring), 1403 (N-N=N) (tetrazole ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.14 (s, 1H, tetrazole ring), 8.31 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.10 (d, *J* = 15.4 Hz, 1H, CH), 7.80 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.76 (d, *J* = 15.4 Hz, 1H, CH), 7.20 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.18 (d, *J* = 7.9 Hz, 2H, Ar-H), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 188.9, 144.6, 144.1, 141.5, 138.1, 135.5, 130.6, 130.3, 129.1, 126.9, 123.2, 121.2, 21.6. HRMS (ESI, *m/z*) calcd. for $C_{17}H_{14}N_4O$, 290.3193; found 290.1042.

1-(4-(1H-Tetrazol-1-yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (6g): Color: Pale-yellow. Yield: 92 %. M.p.: 178-180 °C. FT-IR (KBr, *v*, cm^{-1}): 3322 (C-H) (α,β-unsaturated carbonyl group), 3033 (C-H) (aromatic ring), 2960 (C-H) (methyl group), 2238 (C=N) (tetrazole ring), 1681 (C=O) (α,β-unsaturated carbonyl group), 1610 (C=C) (aromatic ring), 1408 (N-N=N) (tetrazole ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.12 (s, 1H, tetrazole ring), 8.32 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.12 (d, *J* = 15.4 Hz, 1H, CH), 7.81 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.78 (d, *J* = 15.4 Hz, 1H, CH), 7.32 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.19 (d, *J* = 7.9 Hz, 2H, Ar-H), 3.75 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 189.3, 143.8, 142.9, 142.1, 139.9, 136.6, 131.4, 130.7, 129.8, 126.2, 123.8, 121.5, 55.6. HRMS (ESI, *m/z*) calcd. for $C_{17}H_{14}N_4O_2$, 306.3187; found 306.1088.

1-(4-(1H-Tetrazol-1-yl) phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (6h): Color: Pale-yellow. Yield: 89.2 %. M.p.: 180-182 °C. FT-IR (KBr, *v*, cm^{-1}): 3328 (C-H) (α,β-unsaturated carbonyl group), 3045 (C-H) (aromatic ring), 2987 (C-H) (methyl group), 2246 (C=N) (tetrazole ring), 1685 (C=O) (α,β-unsaturated carbonyl group), 1618 (C=C) (aromatic ring), 1424 (N-N=N) (tetrazole ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.18 (s, 1H, tetrazole ring), 8.36 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.16 (d, *J* = 15.4 Hz, 1H, CH), 7.87 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.74 (d, *J* = 15.4 Hz, 1H, CH), 7.38 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.22 (d, *J* = 7.9 Hz, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 190.2, 144.6, 143.7, 143.5, 140.6, 138.2, 133.1, 132.3, 130.2, 128.1, 125.6, 122.9. HRMS (ESI, *m/z*) calcd. for $C_{16}H_{11}N_5O_3$, 321.2902; found 321.0752.

2.2.3. Synthesis of compounds 8a-h

The chalcone derivative 6f (290 mg, 1.0 mmol) and *o*-aminothiophenol (109 mg, 1.0 mmol) were taken in a round bottom flask and then the minimum amount of ethanol was added. Ceric ammonium nitrate (CAN) (10 mole %) was added as a catalyst and irradiated with ultrasound at 60-65 °C for 45-50 minutes. The progress of the reaction was monitored by TLC. After the reaction was complete, the reaction mixture was poured into ice-cold water. The precipitate obtained was then filtered, dried, and finally the crude product was purified by column chromatography to give the pure compound 8f. Other compounds were synthesised following a method similar to that adopted for the synthesis of compound 8f.

4-(3-(1H-Tetrazol-1-yl)phenyl)-2-phenyl-2, 3-dihydrobenzo [b][1,4]thiazepine (8a): Color: Yellow. Yield: 82 %. M.p.: 194-196 °C. FT-IR (KBr, *v*, cm^{-1}): 3022 (C-H) (aromatic ring), 2238 (C=N) (tetrazole ring), 1602 (C=N) (benzothiazepine ring), 1534 (C=C) (aromatic ring), 1379 (N-N=N) (tetrazole ring), 740 and 610 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.18 (s, 1H, tetrazole ring), 8.28 (s, 1H, Ar-H), 8.19 (m, 3H, Ar-H), 8.04 (m, 4H, Ar-H), 7.82 (m, 5H, Ar-H), 5.46 (d, *J* = 12.81 Hz, 1H, thiazepine ring), 5.24 (d, *J* = 8.62 Hz, 1H, thiazepine ring), 3.38 (d, *J* = 12.81 Hz, 1H, thiazepine ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 187.8, 144.5, 144.2, 143.6, 142.8, 141.4, 140.8, 140.2, 139.8, 138.7, 138.2, 137.8, 136.4, 135.9, 135.2, 134.8, 134.1, 58.7, 57.6, 47.6. HRMS (ESI, *m/z*) calcd. for $C_{22}H_{17}N_5S$, 383.4689; found 383.1108.

4-(3-(1H-Tetrazol-1-yl)phenyl)-2-(p-tolyl)-2,3-dihydrobenzo [b][1,4]thiazepine (8b): Color: Pale-yellow. Yield: 80 %. M.p.: 192-194 °C. FT-IR (KBr, *v*, cm^{-1}): 3018 (C-H) (aromatic ring), 2236 (C=N) (tetrazole ring), 1604 (C=N) (benzothiazepine ring), 1560 (C=C) (aromatic ring), 1382 (N-N=N) (tetrazole ring), 742 and 602 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.09 (s, 1H, tetrazole ring), 8.22 (s, 1H, Ar-H), 8.14 (m, 3H, Ar-H), 8.06 (m, 4H, Ar-H), 7.93 (dd, 4H, Ar-H), 5.48 (d, *J* = 12.81 Hz, 1H, thiazepine ring), 5.23 (d, *J* = 8.62 Hz, 1H, thiazepine ring), 3.36 (d, *J* = 12.81 Hz, 1H, thiazepine ring), 2.24 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 187.2, 144.3, 144.1, 143.5, 142.6, 141.2, 140.5, 140.1, 139.4, 138.3, 138.1, 137.8, 136.4, 135.9, 135.2, 134.8, 134.1, 58.3, 57.6, 48.1, 22.8. HRMS (ESI, *m/z*) calcd. for $C_{23}H_{19}N_5S$, 397.4955; found 397.1283.

4-(3-(1H-Tetrazol-1-yl)phenyl)-2-(4-methoxyphenyl)-2, 3-di hydrobenzo[b][1,4]thiazepine (8c): Color: Pale-yellow. Yield: 78 %. M.p.: 196-198 °C. FT-IR (KBr, *v*, cm^{-1}): 3024 (C-H) (aromatic ring), 2241 (C=N) (tetrazole ring), 1606 (C=N) (benzothiazepine ring), 1565 (C=C) (aromatic ring), 1384 (N-N=N) (tetrazole ring), 744 and 604 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.10 (s, 1H, tetrazole ring), 8.23 (s, 1H, Ar-H), 8.15 (m, 3H, Ar-H), 8.08 (m, 4H, Ar-H), 7.96 (dd, 4H, Ar-H), 5.51 (d, *J* = 12.81 Hz, 1H, thiazepine ring), 5.25 (d, *J* = 8.61 Hz, 1H, thiazepine ring), 3.39 (d, *J* = 12.81 Hz, 1H, thiazepine ring), 3.65 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 189.2, 145.2, 144.8, 144.2, 143.4, 142.8, 142.3, 141.8, 141.4, 140.9, 140.3, 139.7, 139.2, 138.8, 138.2, 137.8, 137.1, 58.8, 55.6, 46.9, 24.9. HRMS (ESI, *m/z*) calcd. for $C_{23}H_{19}N_5OS$, 413.4949; found 413.1209.

4-(3-(1H-Tetrazol-1-yl)phenyl)-2-(4-nitrophenyl)-2, 3-dihyd robenzo[b][1,4]thiazepine (8d): Color: Pale-yellow. Yield: 73 %. M.p.: 198-200 °C. FT-IR (KBr, *v*, cm^{-1}): 3028 (C-H) (aromatic ring), 2246 (C=N) (tetrazole ring), 1610 (C=N) (benzothiazepine ring), 1568 (C=C) (aromatic ring), 1389 (N-N=N) (tetrazole ring), 746 and 610 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.18 (s, 1H, tetrazole ring), 8.24 (s, 1H, Ar-H), 8.16 (m, 3H, Ar-H), 8.10 (m, 4H, Ar-H), 7.98 (dd, 4H, Ar-H), 5.56 (d, *J* = 12.81 Hz, 1H, thiazepine ring), 5.23 (d, *J* = 8.60 Hz, 1H, thiazepine ring), 3.48 (d, *J* = 12.81 Hz, 1H, thiazepine ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 190.3, 146.8, 146.2, 145.7, 144.1, 143.8, 143.1, 142.6, 142.2, 141.8, 141.4, 140.6, 140.2, 139.8, 138.2, 137.8, 137.1, 59.2, 58.5, 48.7. HRMS (ESI, *m/z*) calcd. for $C_{22}H_{16}N_6O_2S$, 428.4664; found 428.1046.

4-(4-(1H-Tetrazol-1-yl)phenyl)-2-phenyl-2, 3-dihydrobenzo [b][1,4]thiazepine (8e): Color: Yellow. Yield: 78 %. M.p.: 198-200 °C. FT-IR (KBr, *v*, cm^{-1}): 3024 (C-H) (aromatic ring), 2239 (C=N) (tetrazole ring), 1608 (C=N) (benzothiazepine ring), 1560 (C=C) (aromatic ring), 1380 (N-N=N) (tetrazole ring), 742 and 602 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.26 (s, 1H, tetrazole ring), 8.28 (d, *J* = 8.46 Hz, 2H, Ar-H), 8.18 (d, *J* = 8.46 Hz, 2H, Ar-H), 8.04 (m, 4H, Ar-H), 7.80 (m, 5H, Ar-H), 5.44 (d, *J* = 12.80 Hz, 1H, thiazepine ring), 5.22 (d, *J* = 8.61 Hz, 1H, thiazepine ring), 3.36 (d, *J* = 12.80 Hz, 1H, thiazepine ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 189.8, 144.5, 144.2, 143.6, 142.8, 141.4, 140.8, 140.2, 139.8, 138.7, 138.2, 137.8, 136.4, 135.9, 135.2, 58.2, 57.5, 41.7. HRMS (ESI, *m/z*) calcd. for $C_{22}H_{17}N_5S$, 383.4689; found 383.1106.

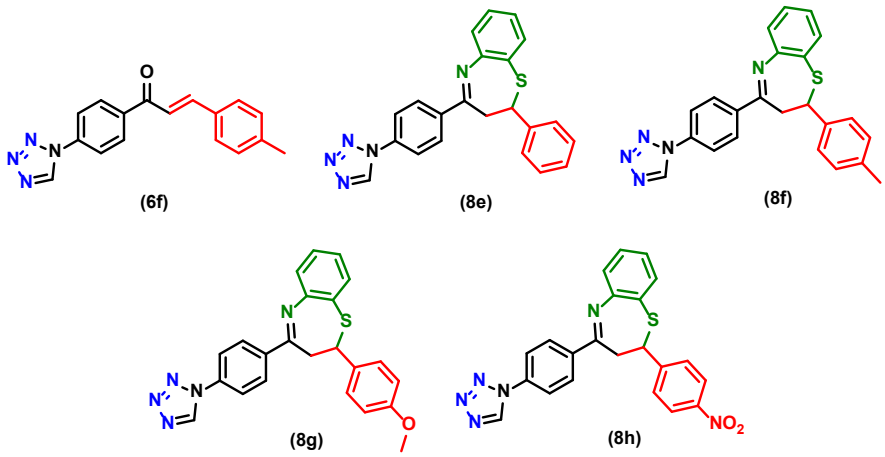


Figure 1. Compounds selected for *in vitro* evaluation of biological activities.

4-(4-(1H-Tetrazol-1-yl)phenyl)-2-(p-tolyl)-2,3-dihydrobenzo [b][1,4]thiazepine (8f): Color: Pale-yellow. Yield: 85 %. M.p.: 190-192 °C. FT-IR (KBr, *v*, cm^{-1}): 3053 (C-H) (aromatic ring), 2929 (C-H) (methyl group), 1596 (C=N) (benzothiazepine ring), 1531 and 1445 (C=C) (aromatic ring), 1328 (N-N=N) (tetrazole ring), 742 and 602 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.78 (s, 1H, tetrazole-H), 8.06 (d, *J* = 8.46 Hz, 2H, Ar-H), 7.89 (d, *J* = 8.46 Hz, 2H, Ar-H), 7.87 (m, *J* = 6.5 Hz, 4H, Ar-H), 7.62 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.38 (d, *J* = 7.7 Hz, 2H, Ar-H), 5.37 (d , *J* = 12.7, 4.8 Hz, 1H, thiazepine ring), 5.21 (d, *J* = 8.61 Hz, 1H, thiazepine ring), 3.43 (d, *J* = 12.7 Hz, 1H, thiazepine ring), 2.42 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm):181.48, 152.48, 141.01, 136.00, 132.67, 131.68, 130.80, 130.75, 130,09, 129.34, 126.62, 123.43, 121.82, 121.53, 117.53, 58.21, 56.51, 40.72, 21.63. HRMS (ESI, *m/z*) calcd. for $C_{23}H_{19}N_5S$, 397.4955; found 397.1438.

4-(4-(1H-Tetrazol-1-yl)phenyl)-2-(4-methoxyphenyl)-2, 3-di hydrobenzo[b][1,4]thiazepine (8g): Color: Pale-yellow. Yield: 79 %. M.p.: 196-198 °C. FT-IR (KBr, *v*, cm^{-1}): 3052 (C-H) (aromatic ring), 2928 (C-H) (methyl group), 1598 (C=N) (benzothiazepine ring), 1532 and 1448 (C=C) (aromatic ring), 1329 (N-N=N) (tetrazole ring), 744 and 610 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.72 (s, 1H, tetrazole-H), 8.08 (d, *J* = 8.46 Hz, 2H, Ar-H), 7.88 (d, *J* = 8.46 Hz, 2H, Ar-H), 7.84 (m, *J* = 6.5 Hz, 4H, Ar-H), 7.66 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.39 (d, *J* = 7.7 Hz, 2H, Ar-H), 5.38 (d , *J* = 12.7, 4.8 Hz, 1H, thiazepine ring), 5.23 (d, *J* = 8.60 Hz, 1H, thiazepine ring), 3.45 (d, *J* = 12.7 Hz, 1H, thiazepine ring), 3.47 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 184.88, 153.46, 142.89, 139.23, 134.68, 132.66, 131.83, 131.68, 130,89, 130.44, 128.69, 127.41, 123.81, 121.54, 118.51, 58.21, 55.51, 41.73, 22.68. HRMS (ESI, *m/z*) calcd. for $C_{23}H_{19}N_5OS$, 413.4949; found 413.1209.

4-(4-(1H-Tetrazol-1-yl)phenyl)-2-(4-nitrophenyl)-2,3-dihyd-robenzo[b][1,4]thiazepine (8h): Color: Pale-yellow. Yield: 71 %. M.p.: 198-200 °C. FT-IR (KBr, *v*, cm^{-1}): 3056 (C-H) (aromatic ring), 1598 (C=N) (benzothiazepine ring), 1538 and 1453 (C=C) (aromatic ring), 1328 (N-N=N) (tetrazole ring), 746 and 612 (C-S-C) (thiazepine ring). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 11.82 (s, 1H, tetrazole-H), 8.12 (d, *J* = 8.46 Hz, 2H, Ar-H), 7.98 (d, *J* = 8.46 Hz, 2H, Ar-H), 7.86 (m, *J* = 6.5 Hz, 4H, Ar-H), 7.72 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.43 (d, *J* = 7.7 Hz, 2H, Ar-H), 5.49 (d , *J* = 12.7, 4.8 Hz, 1H, thiazepine ring), 5.24 (d, *J* = 8.60 Hz, 1H, thiazepine ring), 3.51 (d, *J* = 12.7 Hz, 1H, thiazepine ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 188.71, 156.56, 147.81, 142.56, 139.79, 136.61, 132.82, 131.45, 130,91, 131.44, 129.12, 128.48, 126.82, 123.45, 121.53, 59.22, 56.11, 45.89. HRMS (ESI, *m/z*) calcd. for $C_{22}H_{16}N_6O_2S$, 428.4664; found 428.1010.

2.3. In vitro evaluation of biological activity

Of the synthesized compounds, some compounds, as shown in Figure 1 were evaluated *in vitro* for their anticancer, anti-tubercular, antibacterial, and antifungal activities.

2.3.1. Anticancer activity

Dulbecco's modified Eagles' medium (MEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline (PBS), fetal bovine serum (FBS), trypsin, *etc.* purchased from Sigma-Aldrich and Alfa-Aesar. *cis*-Platin was used as a standard drug for anticancer activity. HeLa cancer cell lines were purchased from the National Centre for Cell Science (NCCS), Pune, and the cells were kept in MEM supplemented with 10% FBS and penicillin/streptomycin (0.5 1/mL) in an atmosphere of 5% CO_2 / 95% air at 37 °C. For the MTT assay, each test compound was weighed separately and dissolved in dimethyl sulfoxide (DMSO). To make the final concentration 1.0 molar, the cells were treated with a series of concentrations from 5.0 to 100 µM. Measurements of cell viability and proliferation form the basis for numerous *in vitro* assays of a cell population's response to external factors. The MTT cell proliferation assay measures the cell proliferation rate [26-30].

The MTT assay is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The assay depends on the number of cells present and the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters into the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan crystal. Cells are then solubilized in dimethylsulfoxide, and the formazan reagent is measured spectrophotometrically at 570 nm [26-30].

Cell viability was evaluated by the MTT assay with three independent experiments with six concentrations of compounds in triplicate. Cells were trypsinised and the trypan blue assay was performed to know the viable cells in the cell suspension. Cells were counted by Haemocytometer and seeded at a density of 5.0×10^3 cells/well in 100 µL media in 96 well plate culture media and incubated overnight at 37 °C. After incubation, old media and fresh media were taken with different concentrations of test compounds in the represented wells in 96 plates. After 48 h, the drug solution was discarded and fresh media with 0.5 mg/mL MTT solution was added to each well, and then the plates were incubated at 37 °C for 3 h. At the end of the incubation time, precipitates formed as a result of the reduction of the MTT to chromophore formazan crystals by cells with metabolically active mitochondria. The optical

density of the solubilized crystals in DMSO was measured at 570 nm using a microplate reader. The percentage growth inhibition was calculated by Equation 1 [26-30]:

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Treatment}})}{\text{Abs}_{\text{Control}}} \times 100 \quad (1)$$

The IC_{50} value was determined by using a linear regression equation, *i.e.,* $y = mx + c$. Here, $y = 50$, m and c values were derived from the viability graph.

2.3.2. Antitubercular activity

Becton, Dickinson and Company's Mycobacterial Growth Indicator (BD MGIT) tubes, BD BACTEC™, MGIT™ automated machine, DMSO, positive culture and streptomycin (S), isoniazid (I), rifampin (R) and ethambutol (E), *i.e.* SIRE supplement were used and method was divided in to two parts:

2.3.2.1. Dilution of test compounds

Given that the diluted compounds were used for anti-mycobacterial activity testing, 2.0 mg of each sample was dissolved in 300 µL DMSO solvent to make the compound concentration 0.66 mg/100 µL.

2.3.2.2. Antimycobacterial activity of test compounds

Given compounds were dissolved in DMSO to the required concentration. Parallelly, a total of eight MGIT tubes were labelled and 0.8 mL of supplement was added to each tube. The 1st tube was then kept aside and 100 µL of diluted compounds were added to the respective tubes. The compounds of all tubes were mixed properly and kept aside. 1:100 dilution of inoculum *(M. Tuberculosis* ATCC-25177) for the growth control tube (1st tube) and No dilution for drug susceptibility testing (DST) inoculum *(M. Tuberculosis* ATCC-25177) for mother culture vial were prepared. 0.5 mL of 1:100 dilution was added to the 1st tube (growth control tube) and 0.5 mL from the mother culture vial was added to the other respective tubes and then all tubes were incubated in MGIT-320 [31-36].

Automated antibacterial susceptibility testing of different compounds against *M. tuberculosis* (ATCC-25177) was carried out using the BD BACTEC™ MGIT™ DST rapid culture method [31-36]. Microscopic image of *Mycobacterium tuberculosis* bacilli (ZN staining) was recoded from MGIT positive growth control tube during the testing.

2.3.3. Antibacterial activity

The Agar Well Diffusion method was used as a standard microbial technique for *in vitro* antibacterial assays. The different samples were diluted by using 10% DMSO and 3 different concentrations (50, 100, and 200 mg/mL) of all compounds were prepared. Disinfected Petri dishes holding the nutrient agar medium (NA) were used for the inoculation of microorganisms. This inoculum was spread over the plate using a spreader and kept in place for 30 minutes. Wells of 6mm diameter were prepared in seeded agar plates. At equal distance control, a well was also prepared. All different concentrations of all samples and standard drugs (30 µL) were poured into the preorganized wells of the seeded plates. Plates were kept for incubation at 37 ° C for 24 h. The antibacterial spectrum of the test sample was determined by means of a zone of inhibition (ZI) around each prepared well. The comparison of the diameters of the zone of inhibition developed by the test sample and by the commercial control, *i.e.,* ciprofloxacin (1 mg/mL) was done [37-41].

2.3.4. Antifungal activity

The Agar Well Diffusion method was used as a standard microbial technique for *in vitro* antifungal assays. The different samples were diluted by using 10% DMSO and 3 different concentrations (50, 100, and 200 mg/mL) of all compounds were prepared. Disinfected petri dishes holding the nutrient agar (NA) medium were used for the inoculation of test micro-organisms. This inoculum was spread over the plate using a spreader and kept in place for 30 minutes. Wells of 6mm diameter were prepared in seeded agar plates. At equal distance control, well was also prepared. All different concentrations of all samples and standard drug (30 µL) were poured into the preorganized wells of seeded plates. The plates were kept for incubation at 37 °C for 24 h. The antibacterial spectrum of the test sample was determined via a zone of inhibition (ZI) around each prepared well. The comparison of the diameters of the zone of inhibition developed by the test sample and by the commercial control, *i.e.,* ciprofloxacin (1 mg/mL) was done [37-41].

3. Results and discussion

3.1. Synthesis

The synthetic routes which are applied for the synthesis of the targeted tetrazole containing 1,5-benzothiazepine derivatives are presented in Schemes 1 and 2. In the present research work, 1-(aminophenyl)ethanones (1a and 1b) were treated with sodium azide (2) and triethyl orthoformate (TEOF) (3) in the presence of glacial acetic acid medium to give 1-(4-(1*H*-tetrazol-1-yl)phenyl)ethenones (4a and 4b) [42]. Compounds 6a-h were obtained by condensation reaction between compounds 4a and 4b and various aromatic aldehydes (5a-d) in the presence of potassium hydroxide [43,44]. The tetrazole containing 1,5-benzothiazepines 8a-h were synthesized from the chalcones 6a-h with the reaction of *o*-aminothiophenol (7) by using ceric ammonium nitrate (CAN) [45]. The formation of various compounds was confirmed by thin-layer chromatography (TLC), and the compounds were characterized by spectral data. The formation of compounds 4a and 4b was confirmed by FT-IR and ^{1}H NMR spectra, in which the two IR peaks of the NH_2 group of compounds 1a and 1b at around 3450 and 3370 cm^{-1} and the ^{1}H NMR signals of the NH_2 groups around δ 4.3 ppm disappeared and the new IR peaks of C=N of the tetrazole ring around 1450 cm^{-1} and the new ^{1}H NMR signals of tetrazole-H of compounds 4a and 4b at δ 10.16 ppm appeared. Similarly, the formation of the compounds 6a-h from compounds 4a-b and the compounds 8a-h from compounds 6a-h have also been confirmed by the comparison peaks and signals of the FT-IR and ^{1}H NMR spectra of these compounds. The spot and R_f values, starting materials, and reaction mixtures were also compared on the TLC to confirm the formation of these compounds.

3.2. In vitro evaluation of biological activity

3.2.1. Anticancer activity

Synthesized compounds 6f, 8e, 8f, 8g, and 8h were evaluated for anticancer activity [26-30] and the IC_{50} values for anticancer activity are given in Table 1. After comparison of the IC_{50} values of the synthesized compounds with the standard, as summarized in Table 1, it is observed that all synthesized compounds 6f, 8e, 8f, 8g and 8h possess very poor anticancer activity.

Table 1. Anticancer activity of the investigated compounds.

Compound	IC_{50} (µM)
6f	81.87
8e	81.32
8f	62.70
8g	33.38
8h	46.63
Standard (*Cis*-platin)	5.62

Table 2. Antitubercular activity of the investigated compounds.

Compound	Extract type	Growth observed *M. tuberculosis* ATCC-25177	No. of days
6f	DMSO	No	-
8e	DMSO	No	-
8f	DMSO	No	-
8g	DMSO	No	-
8h	DMSO	No	-
Control	DMSO	Yes	10

Table 3. Antibacterial activity of the investigated compounds.

Compound	Zone of inhibition (mm)					
	E. coli			*S. aureus*		
	50 mg/L	100 mg/L	200 mg/L	50 mg/L	100 mg/L	200 mg/L
6f	3	4	6	7	10	12
8e	11	16	19	5	11	14
8f	8	13	17	6	8	11
8g	11	13	15	5	8	11
8h	17	20	22	14	17	23
Standard (Ciprofloxacin)	25	27	32	22	25	30

Table 4. Antifungal activity of the investigated compounds.

Compound	Zone of inhibition (mm)					
	A. alternate			*F. solani*		
	50 mg/L	100 mg/L	200 mg/L	50 mg/L	100 mg/L	200 mg/L
6f	7	10	12	3	6	9
8e	5	11	14	7	11	14
8f	6	8	11	11	13	16
8g	5	8	11	10	12	16
8h	14	17	22	13	17	24
Standard (Ketoconazole)	22	25	30	28	30	34

3.2.2. Antitubercular activity

The antitubercular activity of the synthesized compounds 6f, 8e, 8f, 8g, and 8h was also evaluated [31-36]. Observation of the growth of mycobacterium against the compounds tested is summarized in Table 2. After comparison of the observation table, it is observed that all synthesized compounds 6f, 8e, 8f, 8g, and 8h are strongly active against *M. tuberculosis* ATCC-25177 as there is no growth in the MGIT tubes even after 25 days of incubation. This may be due to a strong bonding between the synthesized compounds and the substrate.

3.2.3. Antibacterial activity

The synthesized compounds 6f, 8e, 8f, 8g, and 8h were also evaluated for antibacterial activity against *E. coli* and *S. aureus* [37-41]. The results of the inhibition zone (in mm) are given in Table 3. After comparison of the synthesized compounds with the standard, as summarized in Table 3, it is found that compound 8h possesses moderate activity, compounds 8e, 8f, and 8g possess weak activity, while compound 6f has very weak antibacterial activity against *E. coli* and *S. aureus.* The variation in antibacterial activity is due to the different bond strengths of substituents or groups, *i.e.,* nitro, methoxy, and methyl groups with *E. coli* and *S. aureus*. Compound 8h has moderate antibacterial activity against *E. coli* and *S. aureus* due to the presence of nitro group in its molecular framework, while compound 6f has very weak antibacterial activity due to the absence of a 1,5-benzothiazepines ring.

3.2.4. Antifungal activity

The synthesized compounds 6f, 8e, 8f, 8g, and 8h were also evaluated for antifungal activity against *A. alternate* and *F. solani* [37-41]. The results of the inhibition zone (in mm) are given in Table 4. After comparison of the synthesized compounds with the standard, as summarized in Table 4, it is found that compound 8h has moderate, compounds 8e, 8f, and 8g have weak, while compound 6f has very weak antifungal activity against *A. alternate* and *F. solani.* Variation in antifungal activity is due to different bond strength of substituents or groups, *i.e.,* nitro, methoxy, and methyl groups with *A. alternate* and *F. solani*. Compound 8h has moderate antifungal activity against *A. alternate* and *F. solani* due to the presence of nitro group in its molecular framework, while compound 6f has very weak antifungal activity due to the absence of the 1,5-benzothiazepines ring.

The *meta*- and *para* positions of the tetrazole group in compounds 8d and 8h, respectively, lead to variations mainly in electronic distribution, steric hindrance, and conformational preferences. These main factors collectively contribute to the observed differences in the antibacterial and antifungal activities of compounds having a tetrazole group at the *meta* position (compound 8d) and *para* position (compound 8h).

4. Conclusions

In the present research work, tetrazole containing 1,5-benzothiazepines were synthesized as final compounds for the evaluation of anticancer, antitubercular, antibacterial, and antifungal activities. Of the synthesized compounds, the compounds 6f, 8e, 8f, 8g, and 8h were evaluated for their anti-cancer, antitubercular, antibacterial and antifungal activities and it was concluded that the compounds 6f, 8e, 8f, 8g and 8h have very limited anticancer activity, moderate antibacterial and antifungal activity and very strong antitubercular activity, indicating their great value as subjects for future investigations as novel therapeutic agents for the treatment of tuberculosis.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Bhawani Singh, Ashok Kumar Suman; Methodology: Bhawani Singh, Ashok Kumar Suman; Formal Analysis: Bhawani Singh, Ashok Kumar Suman; Investigation: Bhawani Singh, Ashok Kumar Suman; Resources: Bhawani Singh, Ashok Kumar Suman; Writing - Original Draft: Ashok Kumar Suman, Anu; Writing - Review and Editing: Bhawani Singh; Visualization: Bhawani Singh; Funding acquisition: Bhawani Singh, Ashok Kumar Suman; Supervision: Bhawani Singh.

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