

Synthesis and Antimicrobial Evaluation of Novel 1,5-Benzothiazepine Derivatives

Afzal B. Shaik

Vignan Pharmacy College
Vadlamudi, Guntur District, Andhra Pradesh, India
Email: bashafoye@gmail.com

Yejella R. Prasad

AU College of Pharmaceutical Sciences
Andhra University, Visakhapatnam, Andhra Pradesh, India.

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Abstract: 1,5-Benzothiazepine is an important heterocyclic ring containing benzene ring fused with a seven- membered thiazepine. This ring structure is not only found in a variety of medicinally important therapeutic agents but also enjoy a broad spectrum of bioactivities. Considering its broad bioactivities, in the present work we synthesized novel 1,5-benzothiazepine derivatives by the reaction of isobutyl chalcones with 2-aminothiophenol. The characterization of the compounds was done by physicochemical and spectral analysis (FT-IR, ^1H NMR, ^{13}C NMR and MS) and evaluated for their antibacterial and antifungal activities. The compounds with halogen atom showed very promising activity when compared to the commercial drugs, ciprofloxacin and fluconazole. Among the halogens, fluorinated compounds i.e., BT6 exhibited superior antimicrobial activity than the standard drugs with MIC 0.4 $\mu\text{g/mL}$ against all the strains whereas it is active on *Escherichia coli* at the MIC 0.8 $\mu\text{g/mL}$. The other fluorinated compound BT5 was the most active compound against *Escherichia coli* with the MIC 0.4 $\mu\text{g/mL}$. BT3 with two chlorine atoms shown potent activity with MIC 0.4 $\mu\text{g/mL}$ and 1.6 $\mu\text{g/mL}$ against *Staphylococcus aureus* and *Candida albicans* respectively. BT5 was also more active than the standard drugs against *Staphylococcus aureus*, *Proteus vulgaris* and *Aspergillus niger* (MIC 0.8 $\mu\text{g/mL}$) and *Candida tropicalis* (1.6 $\mu\text{g/mL}$). Atom-based 3D-QSAR was carried out using the PHASE module of Schrodinger software to assess the validity of the invitro antimicrobial data. The computational studies were more or less in close harmony with the invitro activity data. The invitro biological

activity data and the insilico atom-based 3D-QSAR results provide a set of guiding principles for the designing of compounds with better antibacterial and antifungal properties.

Keywords: 1,5-Benzothiazepine, antimicrobial evaluation, computational studies, atom-based 3D-QSAR.

1. Introduction

The use of antimicrobial drugs is essential in order to treat diseases caused by microorganisms. The currently available antimicrobial agents are not sufficient to overcome the effect of microbes¹⁻³. Resistance to number of antimicrobial agents for clinically significant microbial species emerged as a universal problem⁴. This state of affairs is due to the problems like antimicrobial resistance, narrow antimicrobial spectrum and hypersensitivities and systemic toxicities. Hence, the increasing clinical importance of drug-resistant microbial pathogens had made development of novel antimicrobial agents an urgent need for the humanity⁵. So, there is a greater need for the design and development of newer antimicrobial drugs to combat the microbial infections with better properties including selectivity, potency, shorter length of treatments and less toxicity^{6,7}.

Benzothiazepine is a unique scaffold which is present in a number of drugs used nowadays. Diltiazem (1) and Clentiazem (2) are used for their cardiovascular properties for their cardiovascular properties⁸⁻¹⁰. Other benzothiazepine derivatives quetiapine (3), thiazesim (4), and clothiapine (5) respectively (Figure 1) are employed clinically for the treatment of central nervous system disorders¹¹⁻¹². Many authors had reviewed the biological properties of benzothiazepine derivatives¹³⁻¹⁵. Additionally, the detailed methods of synthesis, characterization, and applications of benzothiazepines had been reviewed¹⁶⁻¹⁸.

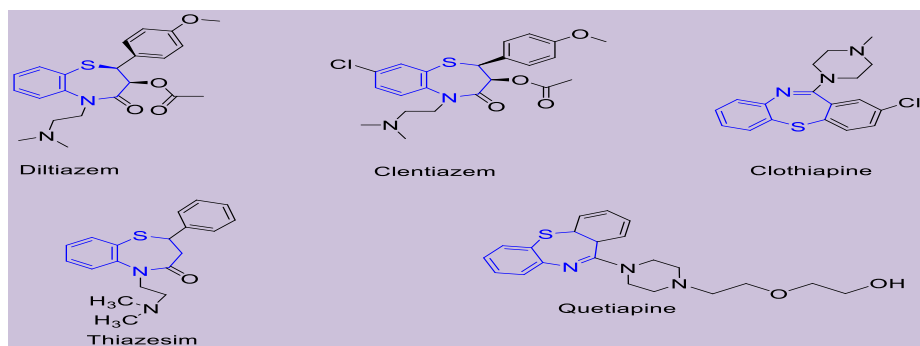


Figure 1. Structures of clinically used benzothiazepine derivatives

Benzothiazepines were reported to possess anticancer¹⁹⁻²⁵, antimicrobial²⁶⁻³¹, ACE inhibitor³², antiplatelet³³, CNS depressant³⁴, bradykinin receptor antagonist³⁵, anti-HIV³⁶ and calmodulin antagonist³⁷ activities. Structures of some of the biologically active antimicrobial benzothiazepines are represented in Figure 2.

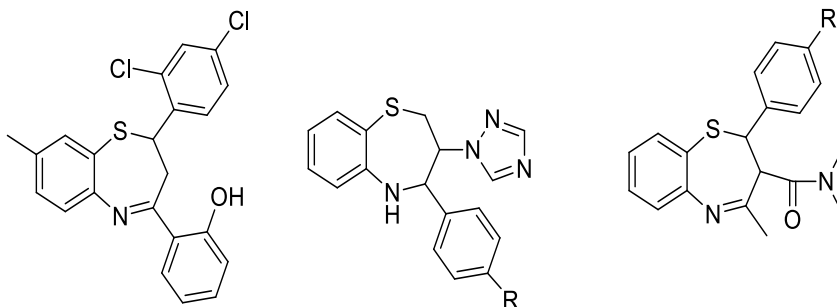


Figure 2. Reported biologically active antimicrobial benzothiazepine derivatives

Based on the above observations, we designed, synthesized and evaluated the antibacterial and antifungal activity of 1,5-benzothiazepines containing an unchanging hydrophobic isobutyl phenyl portion at position-4 and a variable substituted aromatic or a heteroaromatic ring at position-2 (Figure 3) of the benzothiazepine ring.

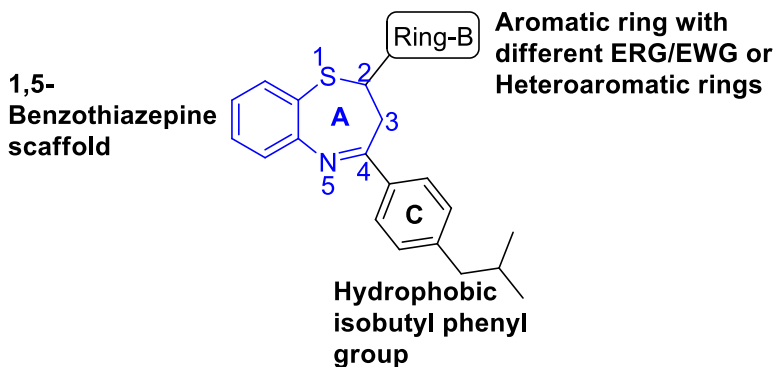


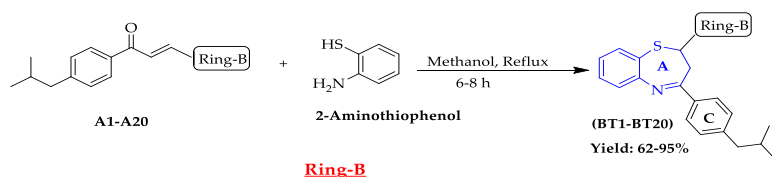
Figure 3. Structural features of the designed molecules

2. Experimental

2.1 Chemistry: 4-isobutyl acetophenone derived chalcones synthesized were used after purification and characterization for the synthesis of the target 1,5-benzothiazepines. The solvents and other reagents were procured

from SD Fine and Himedia. 2-aminothiophenol was purchased from Aldrich Chemical Co. Merck grade Silica gel-G for TLC was used as stationary phase and 25% ethyl acetate in n-hexane as the mobile phase to separate and identify the compounds. Iodine vapors and UV light (254 nm) were used to examine the spots. All the compounds were purified by recrystallization using ethanol as solvent. Boitus melting point apparatus was used to determine the melting points of the purified compounds in open capillaries and are expressed in °C and uncorrected. The IR spectra were recorded using Bruker Vertex 80v spectrometer and the spectrum was scanned by the pressed-pellet technique using KBr discs. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX 400 MHz by dissolving the compounds in deuterated chloroform (CDCl_3) to examine the ^1H NMR spectra. The chemical shift values are given in units as per million, employing Tetramethylsilane (TMS) as the internal standard. Agilent LC-MS spectrometer was used to scan the MS spectra and elemental analyses were carried out using a Carlo Erba 1108 elemental analyzer for C, H, and N.

2.2 Protocol for the synthesis of 1,5-benzothiazepines: The chalcone intermediates (A1-A20) were synthesized by the base (50% aqueous KOH) catalyzed condensation of equimolar quantities of 4-isobutylacetophenone (1 mmol) and the appropriate aldehyde (1 mmol) dissolved in ethanol (7.5 mL) by stirring for 24 h at room temperature. All the chalcones were purified by recrystallization from ethanol⁴⁰. The chalcone derivatives (1 mmol) and 2-aminothiophenol (1 mmol) were dissolved in dry methanol and to this mixture a catalytic amount of piperidine was added. The mixture was then refluxed which resulted in the separation of crystalline solid (Scheme-1). After cooling, the solid product was collected and washed with diethyl ether and cold methanol. The crude solid was recrystallized from ethanol³⁸.



Ring-B
BT1: 4-chlorophenyl; **BT2:** 4-methylphenyl; **BT3:** 2,4-dichlorophenyl; **BT4:** 2-chlorophenyl; **BT5:** 4-fluorophenyl; **BT6:** 2,4-difluorophenyl; **BT7:** 4-dimethylaminophenyl; **BT8:** 3-bromophenyl; **BT9:** 3-hydroxyphenyl; **BT10:** 4-hydroxyphenyl; **BT11:** 4-nitrophenyl; **BT12:** 4-methoxyphenyl; **BT13:** 3,4-dimethoxyphenyl; **BT14:** 3,4,5-trimethoxyphenyl; **BT15:** 2-pyridinyl; **BT16:** 3-pyridinyl; **BT17:** 4-pyridinyl; **BT18:** 2-pyrrolyl; **BT19:** 2-thienyl; **BT20:** 5-bromofuran-2-yl.

Scheme 1. Protocol for the synthesis of 2,3-dihydro-2-aryl-4-(4-isobutylphenyl)-1,5-benzothiazepine derivatives (**BT1-BT20**).

2,3-dihydro-2-(4-chlorophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT1): Yield 97%; m.p:151-153 °C; $R_f = 0.44$ (25% Ethyl acetate in n-Hexane); IR (KBr, cm^{-1}): 1580 (C=N), 1507 (C=C), 1392 (C-N), 661 (C-S), 839 (C-Cl) 2954 (Alkyl C-H) and 3029 (Ar C-H); ^1H NMR (400 MHz, CDCl_3 , ppm): 5.09 (dd, $J_{2,3a} = 5.1$ Hz, $J_{2,3b} = 12$ Hz, 1H, C₂-H), 5.6 (dd, $J_{3a,3b} = 14.4$ Hz, $J_{3a,2} = 9.9$ Hz, 1H, C₃-H-3a), 3.19 (t, $J_{3b,3a} = J_{3b,2} = 12.9$ Hz, 1H, C₃-H-3b), 7.25-8.19 (12H, Ar-H), 0.95 (6H, d, $J = 8\text{Hz}$, $-(\text{CH}_3)_2$), 1.95-2.03 (1H, m, -CH-), 2.55 (2H, d, $J = 8\text{Hz}$, $-\text{CH}_2-$); ^{13}C NMR (100 MHz, CDCl_3 , ppm): 162.65 (C-4), 155.6, 142.7, 139.8, 133.4, 132.7, 130.8, 129.2, 128.9, 128.7, 128.3, 128.1, 127.5, 126.5, 122.5 (Ar-C's), 50.10 (C-2), 45.74 ($-\text{CH}_2-$, C of isobutyl group at C-4"), 40.10 (C-3), 29.12 ($-\text{CH}-$, C of isobutyl group at C-4") and 22.83 ($-\text{CH}_3$, C of isobutyl group at C-4"); MS (m/z, %): 406.9 (M+1, 99.01); Anal. Calcd for: $\text{C}_{25}\text{H}_{24}\text{ClNS}$: C, 73.96; H, 5.96; N, 3.45; Found: C, 74.01; H, 5.99; N, 3.49.

2,3-dihydro-2-(4-methylphenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT2):Yield 86%; m.p. 143-145 °C; $R_f = 0.53$ (25% Ethyl acetate in n-Hexane); IR (KBr, cm^{-1}): 1589 (C=N), 1503 (C=C), 1397 (C-N), 659 (C-S) 2923 (Alkyl C-H) and 3022 (Ar C-H); ^1H NMR (400 MHz, CDCl_3 , ppm): 2.35 (3H, s, Ar- CH_3), 5.10 (dd, $J_{2,3a} = 5.1$ Hz, $J_{2,3b} = 12$ Hz, 1H, C₂-H), 3.52 (dd, $J_{3a,3b} = 14.4$ Hz, $J_{3a,2} = 9.9$ Hz, 1H, C₃-H-3a), 3.28 (t, $J_{3b,3a} = J_{3b,2} = 12.9$ Hz, 1H, C₃-H-3b), 7.19-8.02 (12H, Ar-H), 0.86 (6H, d, $J = 8\text{Hz}$, $-(\text{CH}_3)_2$), 1.71-1.83 (1H, m, -CH-), 2.13 (2H, d, $J = 8\text{Hz}$, $-\text{CH}_2-$); ^{13}C NMR (100 MHz, CDCl_3 , ppm): 162.55 (C-4), 155.2, 141.7, 139.5, 133.1, 132.4, 130.5, 129.1, 128.5, 128.5, 127.6, 127.3, 126.7, 125.7, 122.3 (Ar-C's), 50.07 (C-2), 45.54 ($-\text{CH}_2-$, C of isobutyl group at C-4), 40.20 (C-3), 29.22 ($-\text{CH}-$, C of isobutyl group at C-4) and 22.53 ($-\text{CH}_3$, C of isobutyl

group at C-4"); MS (m/z, %): 386.56 (M+1, 99.26); Anal. Calcd for: C₂₆H₂₇NS: C, 80.99; H, 7.06; N, 3.63; Found: C, 81.02; H, 7.11; N, 3.65.

2,3-dihydro-2-(2,4-dichlorophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT3): Yield 91%; m.p:159-161 °C; R_f = 0.66 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1592 (C=N), 1512 (C=C), 1391 (C-N), 677 (C-S), 885 (C-Cl), 2993 (Alkyl C-H) and 3076 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.53 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.77 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.35 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.80-8.82 (11H, Ar-H), 0.96 (6H, d, J = 8Hz, -(CH₃)₂), 2.32-2.39(1H, m, -CH-), 2.59 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.25 (C-4), 155.8, 142.1, 139.6, 133.1, 132.2, 130.6, 129.0, 128.6, 128.5, 128.2, 128.0, 126.3, 126.2, 122.5 (Ar-C's), 51.09 (C-2), 45.54 (-CH₂-, C of isobutyl group at C-4), 40.15 (C-3), 29.20 (-CH-, C of isobutyl group) and 22.52 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 441.43 (M+1, 99.36); Anal. Calcd for: C₂₅H₂₃Cl₂NS: C, 68.18; H, 5.26; N, 3.18; Found: C, 68.21; H, 5.28; N, 3.20.

2,3-dihydro-2-(2-chlorophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT4): Yield 77%; m.p:160-162 °C; R_f = 0.37 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1596 (C=N), 1510 (C=C), 1365 (C-N), 688 (C-S), 815 (C-Cl), 2850 (Alkyl C-H) and 3056 (Ar C-H). ¹H NMR (400 MHz, CDCl₃, ppm): 5.55 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 4.06 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.72 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.74-8.53 (12H, Ar-H), 0.99 (6H, d, J = 8Hz, -(CH₃)₂), 1.99-2.03 (1H, m, -CH-), 2.60 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.35 (C-4) 155.3, 142.8, 139.5, 133.1, 132.5, 131.1, 129.9, 129.3, 128.9, 128.3, 127.1, 126.5, 123.0, 121.5 (Ar-C's), 49.10 (C-2), 44.74 (-CH₂-, C of isobutyl group at C-4), 41.10 (C-3), 28.12 (-CH-, C

of isobutyl group at C-4), and 22.73 (-CH₃, C of isobutyl group at C-4); MS (m/z, %): 406.98 (M+1, 98.16); Anal. Calcd for: C₂₅H₂₄ClNS: C, 73.96; H, 5.96; N, 3.45; Found: C, 74.01; H, 5.99; N, 3.49.

2,3-dihydro-2-(4-fluorophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT5): Yield 75%; mp: 163-165 °C; R_f = 0.51 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1606 (C=N), 1516 (C=C), 1399 (C-N), 697 (C-S), 919 (C-F) 2893 (Alkyl C-H) and 3055 (Ar C-H). ¹H NMR (400 MHz, CDCl₃, ppm): 5.19 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.71 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.45 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.61-8.55 (12H, Ar-H), 1.21 (6H, d, J = 8Hz, -(CH₃)₂), 2.20-2.33(1H, m, -CH-), 2.82 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm):, 161.65 (C-4), 154.6, 142.5, 138.8, 133.8, 132.5, 130.5, 129.6, 128.5, 128.4, 128.1, 127.5, 126.5, 125.5, 121.5 (Ar-C's), 50.07 (C-2), 44.64 (-CH₂-, C of isobutyl group at C-4), 40.20 (C-3), 29.22 (-CH-, C of isobutyl group at C-4") and 22.73 (-CH₃-, C of isobutyl group at C-4); MS (m/z, %): 390.53 (M+1, 99.18); Anal. Calcd for: C₂₅H₂₄FNS: C, 77.08; H, 6.21; N, 3.60; Found: C, 77.12; H, 6.23; N, 3.61.

2,3-dihydro-2-(2,4-difluorophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT6): Yield 86%; m.p. 169-171 °C; R_f = 0.67 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1585 (C=N), 1522 (C=C), 1386 (C-N), 684 (C-S), 912 (C-F), 945 (C-F), 2842 (Alkyl C-H) and 3012 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.74 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.94 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.58 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.95-8.94 (11H, Ar-H), 1.18 (6H, d, J = 8Hz, -(CH₃)₂), 2.41-2.51(1H, m, -CH-), 2.68 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 163.65 (C-4), 154.6, 141.7, 138.8, 133.5, 132.4, 131.7, 128.1, 127.8, 127.5, 127.3, 126.4, 125.6, 125.1, 123.5 (Ar-

C's), 49.15 (C-2), 45.64 (-CH₂-, C of isobutyl group at C-4"), 41.10 (C-3), 29.12 (-CH-, C of isobutyl group at C-4") and 22.83 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 408.52 (M+1, 99.32); Anal. Calcd for: C₂₅H₂₃F₂NS: C, 73.68; H, 5.69; N, 3.44; Found: C, 73.72; H, 5.71; N, 3.45.

2,3-dihydro-2-(4-dimethylaminophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT7): Yield 87%; m.p: 155-157 °C; R_f = 0.56 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1589 (C=N), 1512 (C=C), 1384 (C-N), 662 (C-S), 1199(-N(CH₃)₂), 2919 (Alkyl C-H) and 3019 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.21 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.47 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.22 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.18-8.15 (12H, Ar-H), 0.76 (6H, d, J = 8Hz, -(CH₃)₂), 1.99-2.13 (1H, m, -CH-), 2.36 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.55 (C-4), 154.6, 141.7, 138.8, 133.8, 132.6, 130.5, 129.1, 128.5, 128.5, 128.4, 128.2, 127.5, 127.1, 121.5 (Ar-C's), 50.05 (C-2), 44.74 (-CH₂-, C of isobutyl group at C-4"), 40.08 (C-3), 28.12 (-CH-, C of isobutyl group at C-4") and 21.83 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 415.61 (M+1, 99.32); Anal. Calcd for: C₂₇H₃₀N₂S: C, 78.22; H, 7.29; N, 6.76; Found: C, 78.24; H, 7.30; N, 6.79.

2,3-dihydro-2-(3-bromophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT8): Yield 76%; m.p:188-190 °C; R_f = 0.72 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1588 (C=N), 1508 (C=C), 1388 (C-N), 672 (C-S), 789 (C-Br), 2919 (Alkyl C-H) and 3019 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 4.91 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.29 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.07 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.16-8.05 (12H, Ar-H), 0.89 (6H, d, J = 8Hz, -(CH₃)₂), 1.91-1.99 (1H, m, -CH-), 2.45 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.35 (C-4), 155.5, 141.7, 138.8, 132.4, 131.8, 131.7,

128.8, 128.5, 128.2, 128.2, 127.9, 127.9, 126.3, 122.6 (Ar-C's), 50.13 (C-2), 44.64 (-CH₂-, C of isobutyl group at C-4"), 41.12 (C-3), 28.12 (-CH-, C of isobutyl group at C-4") and 22.73 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 451.43 (M+1, 97.16); Anal. Calcd for: C₂₅H₂₄BrNS: C, 66.66; H, 5.37; N, 3.11; Found: C, 66.68; H, 5.38; N, 3.13.

2,3-dihydro-2-(3-hydroxyphenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT9): Yield 66%; m.p. 205-207 °C; R_f = 0.30 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1587 (C=N), 1517 (C=C), 1392 (C-N), 659 (C-S), 3452 (Ar-OH), 2961 (Alkyl C-H) and 3045 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 4.91 (s, 1H, Ar-OH), 5.08 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.51 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.23 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 6.46-7.79 (12H, Ar-H), 0.92 (6H, d, J = 8Hz, -(CH₃)₂), 2.12-2.21 (1H, m, -CH-), 2.46 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 161.65 (C-4), 155.5, 142.6, 139.4, 133.3, 132.5, 130.5, 129.1, 128.7, 128.5, 128.5, 128.3, 127.6, 125.5, 123.5 (Ar-C's), 51.11 (C-2), 45.70 (-CH₂-, C of isobutyl group at C-4"), 40.12 (C-3), 29.10 (-CH-, C of isobutyl group at C-4") and 22.80 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 388.54 (M+1, 99.52); Anal. Calcd for: C₂₅H₂₅NOS: C, 77.48; H, 6.50; N, 3.61; Found: C, 77.52; H, 6.51; N, 3.62.

2,3-dihydro-2-(4-hydroxyphenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT10): Yield 73%; m.p. 252-254 °C; R_f = 0.54 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1599 (C=N), 1509 (C=C), 1390 (C-N), 661 (C-S), 3426 (Ar-OH), 2932 (Alkyl C-H) and 3051 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 4.72 (s, 1H, Ar-OH), 4.99 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.29 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.06 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 6.66-8.04 (12H, Ar-H), 0.90 (6H,

d, $J = 8\text{ Hz}$, $-(\text{CH}_3)_2$), 1.94-2.02(1H, m, $-\text{CH}-$), 2.24 (2H, d, $J = 8\text{ Hz}$, $-\text{CH}_2-$); ^{13}C NMR (100 MHz, CDCl_3 , ppm): 163.65 (C-4), 155.8, 142.5, 139.7, 133.5, 132.6, 130.7, 129.3, 128.8, 128.5, 128.5, 127.3, 127.1, 125.5, 121.5 (Ar-C's), 51.12 (C-2), 44.74 ($-\text{CH}_2-$, C of isobutyl group at C-4"), 41.10 (C-3), 29.22 ($-\text{CH}-$, C of isobutyl group at C-4"), and 22.73 ($-\text{CH}_3$, C of isobutyl group at C-4"); MS (m/z, %): 388.54 (M+1, 98.26); Anal. Calcd for: $\text{C}_{25}\text{H}_{25}\text{NOS}$: C, 77.48; H, 6.50; N, 3.61; Found: C, 77.52; H, 6.51; N, 3.62.

2,3-dihydro-2-(4-nitrophenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT11): Yield 91%; m.p: 175-177 $^{\circ}\text{C}$; $R_f = 0.68$ (25% Ethyl acetate in n-Hexane); IR (KBr, cm^{-1}): 1648 (C=N), 1555 (N=O, asymmetric), 1515 (C=C), 1386 (C-N), 1338 (N=O, symmetric), 668 (C-S), 2839 (Alkyl C-H) and 3053 (Ar C-H); ^1H NMR (400 MHz, CDCl_3 , ppm): 5.31 (dd, $J_{2,3a} = 5.1\text{ Hz}$, $J_{2,3b} = 12\text{ Hz}$, 1H, C₂-H), 3.63 (dd, $J_{3a,3b} = 14.4\text{ Hz}$, $J_{3a,2} = 9.9\text{ Hz}$, 1H, C₃-H-3a), 3.45 (t, $J_{3b,3a} = J_{3b,2} = 12.9\text{ Hz}$, 1H, C₃-H-3b), 6.61-8.02 (12H, Ar-H), 1.18 (6H, d, $J = 8\text{ Hz}$, $-(\text{CH}_3)_2$), 2.32-2.42(1H, m, $-\text{CH}-$), 2.46 (2H, d, $J = 8\text{ Hz}$, $-\text{CH}_2-$); ^{13}C NMR (100 MHz, CDCl_3 , ppm): 164.65 (C-4), 155.5, 141.7, 139.7, 133.3, 132.5, 130.5, 129.7, 129.1, 128.5, 128.3, 127.3, 126.5, 124.5, 123.5 (Ar-C's), 49.15 (C-2), 45.72 ($-\text{CH}_2-$, C of isobutyl group at C-4"), 39.40 (C-3), 29.10 ($-\text{CH}-$, C of isobutyl group at C-4") and 22.80 ($-\text{CH}_3$, C of isobutyl group at C-4"); MS (m/z, %): 417.54 (M+1, 99.23); Anal. Calcd for: $\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_2\text{S}$: C, 72.09; H, 5.81; N, 6.73; Found: C, 72.12; H, 5.83; N, 6.75.

2,3-dihydro-2-(4-methoxyphenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT12): Yield 81%; m.p: 143-145 $^{\circ}\text{C}$; $R_f = 0.32$ (25% Ethyl acetate in n-Hexane); IR (KBr, cm^{-1}): 1613 (C=N), 1511 (C=C), 1326 (C-N), 1215 ($-\text{O}-\text{CH}_3$), 678 (C-S) 2853 (Alkyl C-H) and 3091 (Ar C-H); ^1H NMR (400

MHz, CDCl₃, ppm): 3.85 (s, 3H, Ar-OCH₃), 4.99 (dd, $J_{2,3a} = 5.1$ Hz, $J_{2,3b} = 12$ Hz, 1H, C₂-H), 3.28 (dd, $J_{3a,3b} = 14.4$ Hz, $J_{3a,2} = 9.9$ Hz, 1H, C₃-H-3a), 3.06 (t, $J_{3b,3a} = J_{3b,2} = 12.9$ Hz, 1H, C₃-H-3b), 6.82-8.04 (12H, Ar-H), 0.88 (6H, d, $J = 8$ Hz, -(CH₃)₂), 1.62-1.72 (1H, m, -CH-), 2.02 (2H, d, $J = 8$ Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.55 (C-4), 155.0, 142.5, 138.8, 132.4, 132.4, 130.4, 129.3, 129.0, 128.5, 128.4, 128.2, 127.9, 126.8, 122.7 (Ar-C's), 50.07 (C-2), 45.54 (-CH₂-, C of isobutyl group at C-4"), 40.09 (C-3), 29.22 (-CH-, C of isobutyl group at C-4"), and 22.73 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 402.56 (M+1, 99.98); Anal. Calcd for: C₂₆H₂₇NOS: C, 77.77; H, 6.78; N, 3.49 Found: C, 77.79; H, 6.80; N, 3.53.

2,3-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-isobutylphenyl)-1,5-

benzoth- iazepine (BT13): Yield 75%; m.p: 215-217 °C; R_f = 0.49 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1597 (C=N), 1510 (C=C), 1376 (C-N), 1232 (-OCH₃), 689 (C-S), 2811 (Alkyl C-H) and 3004 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 3.72 (s, 6H, 2x Ar-OCH₃), 3.94 (s, 3H, Ar-OCH₃), 5.01 (dd, $J_{2,3a} = 5.1$ Hz, $J_{2,3b} = 12$ Hz, 1H, C₂-H), 3.23 (dd, $J_{3a,3b} = 14.4$ Hz, $J_{3a,2} = 9.9$ Hz, 1H, C₃-H-3a), 3.06 (t, $J_{3b,3a} = J_{3b,2} = 12.9$ Hz, 1H, C₃-H-3b), 6.72-8.06 (11H, Ar-H), 1.00 (6H, d, $J = 8$ Hz, -(CH₃)₂), 1.92-2.13 (1H, m, -CH-), 2.33 (2H, d, $J = 8$ Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.45 (C-4), 154.6, 141.7, 139.9, 133.3, 132.4, 130.5, 129.0, 128.7, 128.5, 128.3, 128.1, 127.2, 126.3, 122.0 (Ar-C's), 50.03 (C-2), 45.64 (-CH₂-, C of isobutyl group at C-4"), 40.07 (C-3), 29.22 (-CH-, C of isobutyl group at C-4") and 22.80 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 432.59 (M+1, 98.56); Anal. Calcd for: C₂₇H₂₉NO₂S: C, 75.14; H, 6.77; N, 3.25; Found: C, 75.15; H, 6.79; N, 3.27.

2,3-dihydro-2-(3,4,5-trimethoxyphenyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT14): Yield 67%; m.p: 227-229 °C; $R_f = 0.55$ (25% Ethyl acetate in n-Hexane); IR (KBr, cm^{-1}): 1598 (C=N), 1513 (C=C), 1390 (C-N), 667 (C-S), 1126 (-O-CH₃), 2966 (Alkyl C-H) and 3021 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 3.82 (s, 3H, Ar-OCH₃), 3.89 (s, 6H, Ar-OCH₃), 4.99 (dd, $J_{2,3a} = 5.1$ Hz, $J_{2,3b} = 12$ Hz, 1H, C₂-H), 3.26 (dd, $J_{3a,3b} = 14.4$ Hz, $J_{3a,2} = 9.9$ Hz, 1H, C₃-H-3a), 3.09 (t, $J_{3b,3a} = J_{3b,2} = 12.9$ Hz, 1H, C₃-H-3b), 6.80-8.09 (10H, Ar-H), 1.01 (6H, d, $J = 8$ Hz, -(CH₃)₂), 1.95-2.12 (1H, m, -CH-), 2.31 (2H, d, $J = 8$ Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.45 (C-4), 155.5, 142.6, 139.7, 133.3, 132.5, 130.7, 129.1, 128.4, 128.1, 127.5, 127.1, 126.5, 126.4, 122.0 (Ar-C's), 50.16 (C-2), 44.74 (-CH₂-, C of isobutyl group at C-4'') 40.20 (C-3), 29.02 (-CH-, C of isobutyl group at C-4'') and 22.80 (-CH₃, C of isobutyl group at C-4''); MS (m/z, %): 462.62 (M+1, 98.56); Anal. Calcd for: C₂₈H₃₁NO₃S: C, 72.85; H, 6.77; N, 3.03; Found: C, 72.87; H, 6.79; N, 3.04.

2,3-dihydro-2-(pyridin-2-yl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT15): Yield 62%; m.p: 118-120 °C; $R_f = 0.61$ (25% Ethyl acetate in n-Hexane); IR (KBr, cm^{-1}): 1600 (C=N), 1511 (C=C), 1381 (C-N), 672 (C-S), 2881 (Alkyl C-H) and 3012 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.49 (dd, $J_{2,3a} = 5.1$ Hz, $J_{2,3b} = 12$ Hz, 1H, C₂-H), 3.75 (dd, $J_{3a,3b} = 14.4$ Hz, $J_{3a,2} = 9.9$ Hz, 1H, C₃-H-3a), 3.52 (t, $J_{3b,3a} = J_{3b,2} = 12.9$ Hz, 1H, C₃-H-3b), 7.41-8.44 (12H, Ar-H), 1.02 (6H, d, $J = 8$ Hz, -(CH₃)₂), 2.09-2.22 (1H, m, -CH-), 2.46 (2H, d, $J = 8$ Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.54 (C-4), 155.9, 142.3, 139.5, 132.4, 131.7, 130.5, 129.8, 128.5, 128.5, 128.3, 127.7, 127.0, 126.2, 122.3 (Ar-C's), 50.21 (C-2), 45.84 (-CH₂-, C of isobutyl group at C-4''), 40.13 (C-3), 29.52 (-CH-, C of isobutyl group at C-4'') and 22.73 (-CH₃, C of isobutyl group at C-4'');

MS (m/z, %): 373.53 (M+1, 98.56); Anal. Calcd for: C₂₄H₂₄N₂S: C, 77.38; H, 6.49; N, 7.52; Found: C, 77.41; H, 6.51; N, 7.53.

2,3-dihydro-2-(pyridin-3-yl)-4-(4-isobutylphenyl)-1,5-benzothiazepine

(BT16): Yield 68%; m.p: 112-114 °C; R_f = 0.57 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1607 (C=N), 1519 (C=C), 1396 (C-N), 688 (C-S), 2815 (Alkyl C-H) and 3167 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.06 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.45 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.13 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.12-8.24 (12H, Ar-H), 1.03 (6H, d, J = 8Hz, -(CH₃)₂), 1.98-2.07(1H, m, -CH-), 2.46 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.45 (C-4), 155.1, 142.3, 139.5, 133.8, 132.6, 130.9, 128.9, 128.6, 128.5, 128.2, 127.9, 127.3, 126.2, 122.1 (Ar-C's), 50.30 (C-2), 45.54 (-CH₂-, C of isobutyl group at C-4"), 40.31 (C-3), 29.62 (-CH-, C of isobutyl group at C-4") and 22.63 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 373.53 (M+1, 99.21); Anal. Calcd for: C₂₄H₂₄N₂S: C, 77.38; H, 6.49; N, 7.52; Found: C, 77.41; H, 6.51; N, 7.53.

2,3-dihydro-2-(pyridin-4-yl)-4-(4-isobutylphenyl)-1,5-benzothiazepine

(BT17): Yield 65%; m.p:109-111 °C; R_f = 0.35 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1591 (C=N), 1523 (C=C), 1398 (C-N), 686 (C-S), 3321 (N-H), 2734 (Alkyl C-H) and 3012 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.31 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.68 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.41 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.11-8.08 (12H, Ar-H), 0.86 (6H, d, J = 8Hz, -(CH₃)₂), 2.22-2.39(1H, m, -CH-), 2.81 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.55 (C-4), 155.9, 142.8, 139.4, 133.5, 132.3, 130.5, 129.5, 128.6, 128.5, 128.3, 128.2, 127.6, 126.3, 122.6 (Ar-C's), 50.22 (C-2), 45.64 (-CH₂-, C of isobutyl group at C-4"), 40.22 (C-3), 29.22

(-CH-, C of isobutyl group at C-4'') and 22.83 (-CH₃- C of isobutyl group at C-4''); MS (m/z, %): 373.53 (M+1, 98.36); Anal. Calcd for: C₂₄H₂₄N₂S: C, 77.38; H, 6.49; N, 7.52; Found: C, 77.41; H, 6.51; N, 7.53.

2,3-dihydro-2-(2-pyrrolyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine

(BT18): Yield 77%; m.p: 116-118 °C; R_f = 0.43 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1579 (C=N), 1505 (C=C), 1380 (C-N), 694 (C-S), 2911 (Alkyl C-H) and 3127 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.01 (1H, s, -NH), 5.55 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.53 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.31 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 6.92-8.03 (11H, Ar-H), 0.91 (6H, d, J = 8Hz, -(CH₃)₂), 1.89-1.97(1H, m, -CH-), 2.56 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.35 (C-4), 155.1, 142.6, 139.5, 133.1, 132.9, 130.6, 129.0, 128.9, 128.5, 128.5, 128.0, 127.3, 126.4, 122.7 (Ar-C's), 50.25 (C-2), 45.64 (-CH₂-, C of isobutyl group at C-4''), 40.30 (C-3), 29.32 (-CH-, C of isobutyl group at C-4'') and 22.53 (-CH₃, C of isobutyl group at C-4''); MS (m/z, %): 361.52 (M+1, 99.56); Anal. Calcd for: C₂₃H₂₄N₂S: C, 76.63; H, 6.71; N, 7.77; Found: C, 76.64; H, 6.72; N, 7.79.

2,3-dihydro-2-(2-thienyl)-4-(4-isobutylphenyl)-1,5-benzothiazepine

(BT19): Yield 72%; m.p. 133-135 °C; R_f = 0.69 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1587 (C=N), 1522 (C=C), 1396 (C-N), 677 (C-S), 2765 (Alkyl C-H) and 3022 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.23 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.52 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.39 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 6.82-8.11 (11H, Ar-H), 0.99 (6H, d, J = 8Hz, -(CH₃)₂), 2.18-2.31(1H, m, -CH-), 2.54 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.45 (C-4), 154.6, 141.7, 139.7, 133.3, 132.1, 130.5, 129.3, 128.5, 128.3, 128.1, 128.0, 127.8, 126.3, 122.6 (Ar-C's), 50.28 (C-

2), 45.62 (-CH₂-, C of isobutyl group at C-4"), 40.15 (C-3), 29.52 (-CH-, C of isobutyl group at C-4") and 22.63 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 378.57 (M+1, 99.36); Anal. Calcd for: C₂₃H₂₃NS₂: C, 73.17; H, 6.14; N, 3.71; Found: C, 73.20; H, 6.17; N, 3.73.

2,3-dihydro-2-(5-bromofuran-2-yl)-4-(4-isobutylphenyl)-1,5-benzothiazepine (BT20): Yield 68%; m.p:188-190 °C; R_f = 0.45 (25% Ethyl acetate in n-Hexane); IR (KBr, cm⁻¹): 1584 (C=N), 1519 (C=C), 1386 (C-N), 735 (C-Br), 665 (C-S), 2799 (Alkyl C-H) and 3011 (Ar C-H); ¹H NMR (400 MHz, CDCl₃, ppm): 5.52 (dd, J_{2,3a} = 5.1 Hz, J_{2,3b} = 12 Hz, 1H, C₂-H), 3.61 (dd, J_{3a,3b} = 14.4 Hz, J_{3a,2} = 9.9 Hz, 1H, C₃-H-3a), 3.23 (t, J_{3b,3a} = J_{3b,2} = 12.9 Hz, 1H, C₃-H-3b), 7.25-8.35 (10H, Ar-H), 0.94 (6H, d, J = 8Hz, -(CH₃)₂), 1.82-1.93 (1H, m, -CH-), 2.12 (2H, d, J = 8Hz, -CH₂-); ¹³C NMR (100 MHz, CDCl₃, ppm): 162.75 (C-4), 155.1, 142.4, 139.5, 133.3, 132.5, 130.7, 129.0, 128.8, 128.5, 128.3, 128.2, 127.3, 126.7, 122.1 (Ar-C's), 50.16 (C-2), 45.60 (-CH₂-, C of isobutyl group at C-4), 40.19 (C-3), 29.22 (-CH-, C of isobutyl group at C-4") and -22.53 (-CH₃, C of isobutyl group at C-4"); MS (m/z, %): 441.4 (M+1, 99.56); Anal. Calcd for: C₂₃H₂₂BrNOS: C, 62.73; H, 5.04; N, 3.18; Found: C, 62.75; H, 5.08; N, 3.21.

2.3 Antimicrobial evaluation: The antimicrobial activity was performed against four bacterial and two fungal strains. The organisms selected were the gram-positive *Bacillus subtilis* (NCIM-2079, Bs), *Staphylococcus aureus* (NCIM-2063, Sa); gram negative *Escherichia coli* (NCIM-2068, Ec), and *Proteus vulgaris* (NCIM-2027, Pv) and the fungal strains *Aspergillus niger* (ATCC-6275, An) and *Candida tropicalis* (ATCC-1369, Ca). Serial tube dilution method was employed and the minimum inhibitory concentration was determined³⁹ for each compound. 2.048 mg of each test compound was placed in vials separately and 2 mL of methanol was added and obtained a solution of the concentration, 1.024 mg/mL. The test bacteria which were grown at 37 °C in nutrient agar medium was diluted in sterile nutrient broth medium to get a suspension containing about 10⁷ cells/mL and was used as the inoculum. 11 test tubes were taken, 9 of which were marked 1, 2, 3, 4, 5, 6, 7, 8, 9 and the rest two were assigned as

T_M (medium), and T_{MI} (medium+inoculum). All the 11 test tubes were poured with 1 mL of nutrient broth medium, cotton plugged and sterilized in an autoclave at 15 lbs/sq.in pressure. After cooling, first test tube was added with 1 mL of the sample solution and mixed well and then 1 mL of this content was transferred to the second test tube and the process of serial dilution was continued upto the ninth test tube. 10 μ L of properly diluted inoculum was added to each of the nine test tubes and mixed well. 10 μ L of the inoculum was added to the test tube T_{MI} to observe the growth of the organism in the medium used. The controlled test tube T_M containing only the medium was used to confirm the sterility of the medium. All the test tubes were incubated at 37 °C for 18 h. A similar experiment with medium, methanol and inoculum without compound was also performed to ensure that the methanol has no inhibitory effect in the dilutions used. The test tube number in which the first sign of growth of the organism observed was noted. The MIC was taken as that concentration used in the test tube number just prior to the test tube number where the first sign of growth observed. This procedure was followed to determine the MIC values for all the compounds. The same procedure was followed for antifungal activity testing except that Potato-Dextrose-Agar medium was used.

2.4 Atom based 3D-QSAR studies of benzothiazepines: The employed methodology deals with development of atom-based 3D-QSAR models to predict the antibacterial and antifungal activity for the synthesized benzothiazepines. By these studies, it is possible to understand how the compounds interact with the target. The results emerging out of these studies can be used to identify new active ligands. For this reason, PHASETM v 3.1 (Schrödinger LLC, Portland, Oregon, USA; <http://www.schrodinger.com/>) was used to carry out the defined studies.

2.4.1 Data set selection: The data set consists of structurally diverse compounds having antibacterial and antifungal activities against *B.subtilis*, *S.aureus*, *E.coli*, *P.vulgaris*, *A.niger* and *C.tropicalis*. The selected series of benzothiazepines (BT1-BT20) and their antibacterial and antifungal activities are given in Table 1. The MIC (μ g/mL) values were taken into account to assess the antibacterial and antifungal activities of these compounds. The biological activities used in the present computational studies were represented as $-\log(\text{MIC})$.

2.4.2 Molecular Modeling (Energy minimization): The structures of all the 20 synthesized benzothiazepines were modelled using Chemdraw ultra 10.0 (Cambridge software), and then the modelled structure was copied to Chem3D ultra 10.0 to create a 3D model which was subjected to energy

minimization using molecular mechanics (MM2). The minimization was executed until the root mean square gradient value reached a value smaller than 0.001 kcal/mol. Such type of energy minimized structures was considered for molecular docking and 3D QSAR studies. However, the corresponding MDL MOL files were prepared using Chem3D ultra 10.0 integral options (saved as /MDL MOL).

2.4.3 Generation of 3D-QSAR models using PHASE™: In order to understand the structural differences between the active and inactive compounds, it was thought proper to do a visual inspection of the aligned ligands and quantified these differences by building a 3D-QSAR model that identifies which functional groups contribute, either positively or negatively, to activity. PHASE™ generated individual atom-based 3D-QSAR models by using a set of 20 benzothiazepines, which all have been aligned in a three-dimensional space with reference of their antibacterial and antifungal activity data. 20 compounds were selected to generate the 3D-QSAR model (i.e. the training set; see supplementary data for tables) and 5 compounds were selected to validate it (i.e. the test set). The criterion used to select these sets was purely based on a random selection in the percent ratio of 80:20.

2.4.4 PHASE'S steps to build the atom-based 3D-QSAR models: PHASE™ can use two alternative ways to generate the structural components that constitute the 3D-QSAR models i.e., the pharmacophore model and atom-based 3D-QSAR. This model is more useful for elucidating the structure activity relationships. In atom-based 3D-QSAR models, a regression is performed by using the partial least squares (PLS) method (where the independent variables are the binary-valued occupancies (i.e. the values are either 0 or 1) of the cubes by the different structural components). Thus, in this model, the number of independent variables used are the 6N occupancies of the N cubes by the six available atom classes (i.e. each variable corresponds to a given cube and a given atom class) and the value for each variable can be 0 or 1. Therefore, the regression involves finding a linear least-squares relationship between the activity data (i.e. the dependent variable) and a special set of orthogonal factors that are linear combinations of the bit value variables (i.e. the independent variables). The accuracy of the 3D-QSAR models increases with increasing the number of PLS factors until over-fitting starts to occur (where the maximum number of PLS factors is $N/5$ and N is the number of ligands). Thus, the PLS facilitates the identification of specific chemical features that tend to increase or decrease the estimated activity. The number of PLS used in the present study was 4. After that, the statistics for

the training and test set were analysed in order to produce a 3D-QSAR model with the best predictive power. Since the training sets employed in the present study have reduced structural diversity, the better model was obtained by using the atom-based 3D-QSAR models. PHASE™ provides the means to build atom-based 3D QSAR models using the activities of the ligands without having a pharmacophore hypothesis in the Individual QSAR Model panel.

2.4.5 3D-QSAR validation and statistics: PHASE™ supports only the use of a true external test set (i.e. compounds which have not been used to build the model) to validate the 3D-QSAR model. For this reason, it is necessary to analyze the statistics obtained from the training and test sets. The main statistical properties that describe the 3D-QSAR model when the training set data is used are: (a) the R-squared or R^2 (i.e. the coefficient of determination; which never can be negative); (b) the standard deviation of regression or SD; (c) the F statistic (i.e. the overall significance of the model); and (d) the statistical significance or P (i.e. the probability that the correlation could occur by chance). Thus, in the case that the independent variables have no statistical relationship with the activity, R^2 would be 0. On the other hand, the main statistical quantities describing the test set prediction are: (a) the Q-squared or Q^2 (i.e. equivalent to R^2 but now, using the predicted and experimental test set activity values; in contrast to R^2 , it can take negative values); (b) the Pearson value or R (i.e. the Pearson correlation coefficient); and (c) the root-mean-square error or RMSE. At this point, it is worth to remark that there is no any single parameter that allows choosing the best model. In this sense, we have to consider all the statistic parameters reported by PHASE™ to evaluate the different 3D-QSAR models.

2.4.6. 3D-QSAR model visualization: Once the 3D-QSAR models have been generated, we have to visualize and analyze them. Thus, to understand how the structures of the ligands contribute, either positively or negatively to the computed activity, the three-dimensional aspects of the 3D-QSAR model were examined. The visualization allows viewing the cubic volume elements occupied by one specific ligand or all the cubes in the 3D-QSAR model shows the volume occlusion maps (i.e. the union of the cubes occupied by all the compounds from the set). In this visualization, the maps represent the regions of favourable and unfavourable interactions. The volume occlusion maps describe the spatial arrangement of favourable interactions to accept groups of the target protein. In figures, 2-7 red

regions indicate unfavourable region for substitution and blue region indicates favourable region for substitution.

3. Results and Discussions

3.1 Chemistry : The condensation of different 1-(4-isobutylphenyl) ethanone derived chalcones with 2-aminothiophenol in the presence of piperidine had resulted in the formation of different 2,3-dihydro-2-aryl-4-(4-isobutylphenyl)-1,5-benzothiazepine derivatives (BT1-BT20). Results of the spectral data including IR, ^1H NMR, ^{13}C NMR and MS untangled the structures of the compounds and were consistent with the expected structures. Three diagnostic absorption bands in the IR spectra of the compounds around the wave numbers 1580-1650 (C=N), 1320-1395 (C-N) and 650-690 (C-S), and additional stretching bands around 2700-3000 (Alkyl C-H) and 3000-3100 (Ar C-H) confirmed the important functional groups present in the benzothiazepine derivatives. The disappearance of two characteristic doublets of chalcones in the region of δ 6.7-7.4 and δ 7.3-7.8 ppm and the appearance of two distinguishing doublets of doublets for $\text{C}_2\text{-H}$ and $\text{C}_3\text{-H-3a}$ around 5.01- 5.60 (dd, $J_{2,3a}$ = 5.1 Hz, $J_{2,3b}$ = 12 Hz, 1H, $\text{C}_2\text{-H}$) and 3.50-4.10 (dd, $J_{3a,3b}$ = 14.4 Hz, $J_{3a,2}$ = 9.9 Hz, 1H, $\text{C}_3\text{-H-3a}$) and a triplet for the proton of $\text{C}_3\text{-H-3b}$ at 3.05-3.45 (t, $J_{3b,3a}$ = $J_{3b,2}$ = 12.9 Hz, 1H, $\text{C}_3\text{-H-3b}$) in the ^1H NMR spectrum have established the formation of target compounds. The spectra also accounted for the other aliphatic and aromatic protons. The ^{13}C NMR spectrum of the compounds showed characteristic signals around δ 50-52 (C-1), 40-42 (C-2) and 160-164 (C-3). The M+1 peak in the mass spectrum further confirmed the structures of 1,5-benzothiazepine derivatives. The composition of the molecules was confirmed by elemental analysis and the results were within ± 0.4 % of the calculated values.

3.2 Antimicrobial evaluation: The antimicrobial activity of the prepared benzothiazepines was performed by serial tube method and their MIC values are summarized in Table 1. The standard drugs used were amoxicillin and fluconazole for antibacterial and antifungal activity respectively and the activity of all the tested compounds is less in comparison to the standard drugs. The compounds with halogen atom showed very promising activity when compared to the standard drugs. (Table 1). Among the halogens, fluorinated compounds i.e., BT6 exhibited superior antimicrobial activity than the standard drugs with MIC 0.4 $\mu\text{g/mL}$ against all the strains whereas it is active on *Escherichia coli* at the MIC 0.8 $\mu\text{g/mL}$. The other fluorinated compound BT5 was the most active compound against *Escherichia coli* with the MIC 0.4 $\mu\text{g/mL}$. BT3 with two

chlorine atoms shown potent activity with MIC 0.4 µg/mL and 1.6 µg/mL against *Staphylococcus aureus* and *Candida tropicalis* respectively. BT5 was also more active than the standard drugs against *Staphylococcus aureus*, *Proteus vulgaris* and *Aspergillus niger* (MIC 0.8 µg/mL) and *Candida tropicalis* (1.6 µg/mL). The activity of the benzothiazepines is more than the corresponding chalcones indicating the ring closure of the propenone bridge had a great impact on the antimicrobial properties of the proposed compounds.

Table 1 Antibacterial and antifungal activities of chalcones (MIC in µg/mL)

Compound	R	Bs	Sa	Ec	Pv	An	Ct
BT1	4"-chlorophenyl	6.4	6.4	6.4	6.4	3.2	3.2
BT2	4"-methylphenyl	128	128	256	128	32	64
BT3	2",4"-dichlorophenyl	3.2	3.2	3.2	3.2	1.6	1.6
BT4	2"-chlorophenyl	12.8	12.8	6.4	6.4	6.4	6.4
BT5	4"-fluorophenyl	0.8	0.8	0.4	0.8	0.8	1.6
BT6	2",4"-difluorophenyl	0.4	0.4	0.8	0.4	0.4	0.4
BT7	4"dimethylaminophenyl	64	64	128	256	16	32
BT8	3"-bromophenyl	256	128	128	256	32	128
BT9	4"-hydroxyphenyl	64	64	64	128	32	64
BT10	3"-hydroxyphenyl	128	128	128	64	256	128
BT11	4"-nitrophenyl	256	128	256	128	128	64
BT12	4"-methoxyphenyl	128	128	64	128	128	64
BT13	3",4"-dimethoxyphenyl	256	512	128	256	128	64
BT14	3",4",5"-trimethoxyphenyl	32	64	64	128	16	32
BT15	2"-pyridinyl	64	64	128	64	128	64
BT16	3"-pyridinyl	128	256	256	128	128	64
BT17	4"-pyridinyl	256	256	512	256	32	64
BT18	2"-pyrrolyl	64	128	256	128	128	64
BT19	2"-thienyl	128	64	64	128	16	32
BT20	5"-bromofuran-2"-yl	256	128	128	64	32	16
Ampicillin		12.8	12.8	6.4	6.4	--	--
Fluconazole		--	--	--	--	2	2

Bs=Bacillus subtilis; Sa=Staphylococcus aureus; Ec=Escherichia coli; Pv=Proteus vulgaris; An=Asperigillus niger; Ct=Candida tropicalis.

3.3 Atom based 3D-QSAR studies of chalcones: The set of 20 benzothiazepines (BT1-BT20) were subjected to atom-based 3D-QSAR analysis using Partial Least Square (PLS) method to identify the potential antibacterial and antifungal agents. In the figures, 4-9 red regions indicate unfavourable region for substitution, and blue region indicates favourable region for substitution. The orientation in these cubes gives a clue to find favourable and unfavourable functional groups for the biological activity.

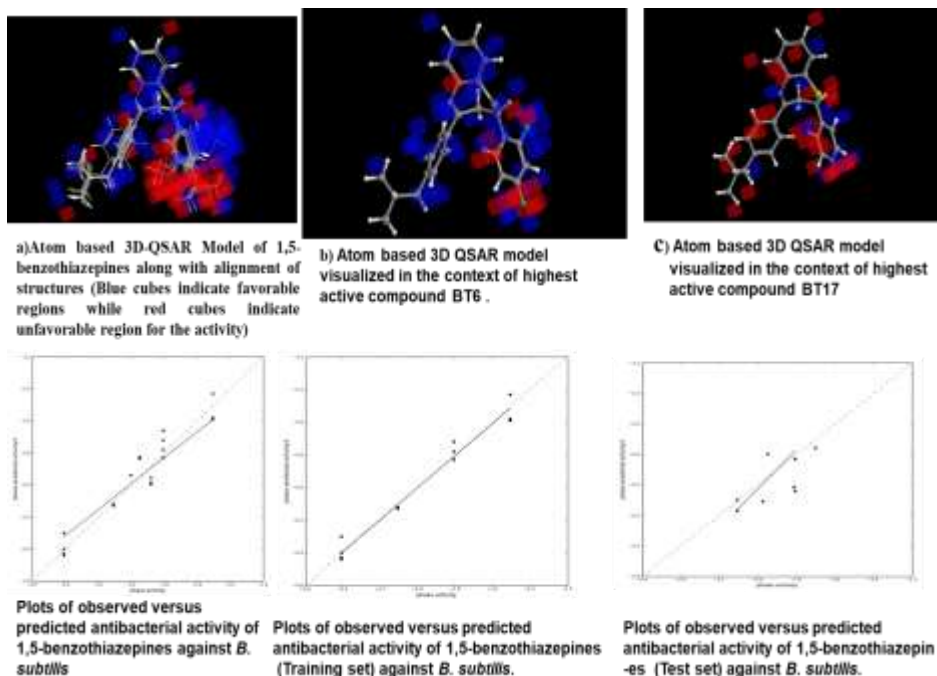


Figure 4. Atom based 3D-QSAR model for antibacterial activity of benzothiazepines against *B. subtilis*

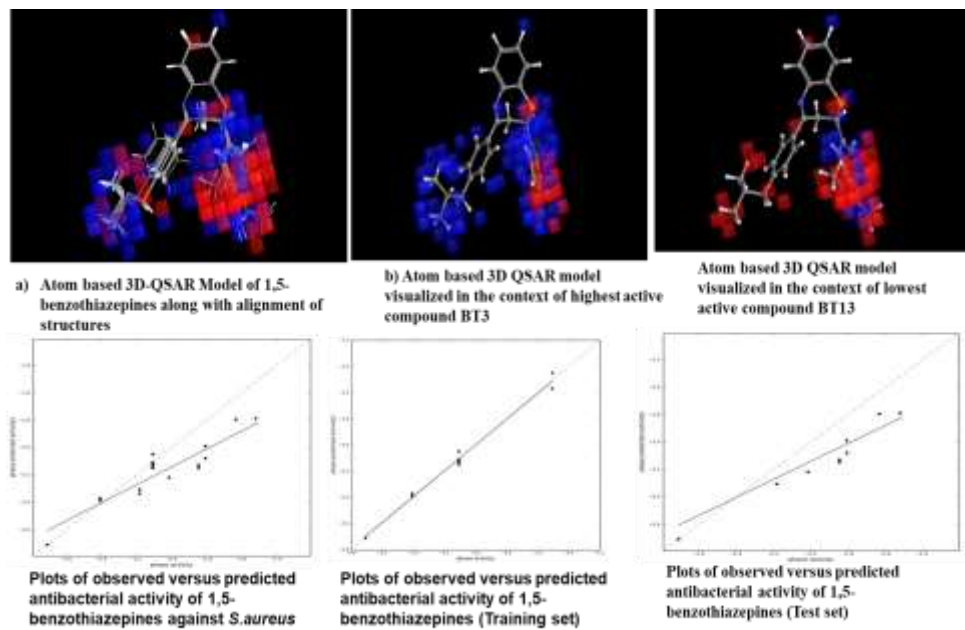


Figure 5. Atom based 3D-QSAR model for antibacterial activity of benzothiazepines against *S.aureus*

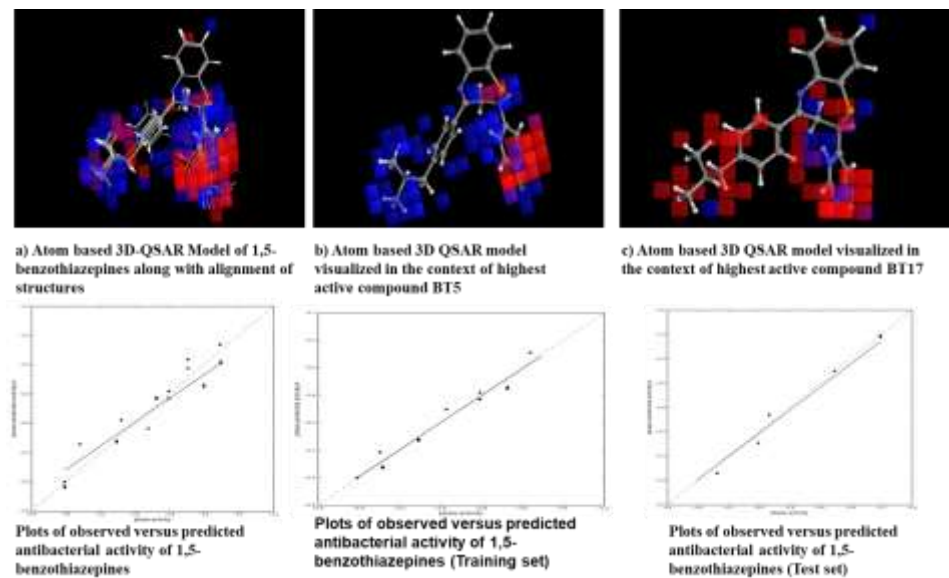
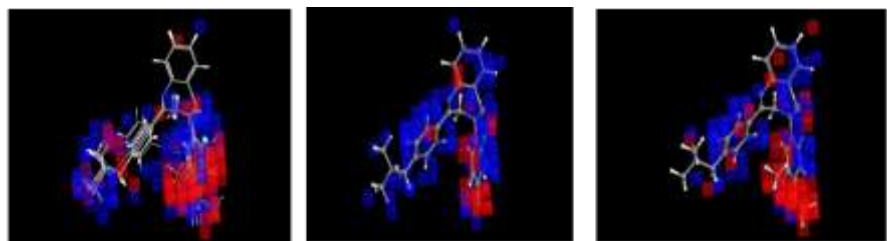


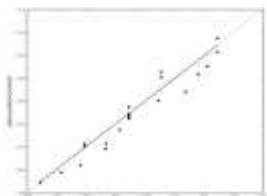
Figure 6. Atom based 3D-QSAR model for antibacterial activity of chalcones against *E.coli*



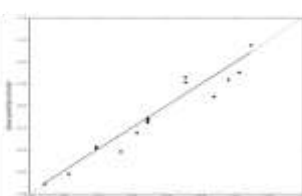
a) Atom based 3D-QSAR Model of 1,5-benzothiazepines along with alignment of structures

b) Atom based 3D QSAR model visualized in the context of highest active compound BT6 against *P.vulgaris*.

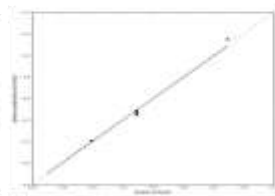
c) Atom based 3D QSAR model visualized in the context of lowest active compound BT13 against *P.vulgaris*.



Plots of observed versus predicted antibacterial activity of 1,5-benzothiazepines against *P.vulgaris*

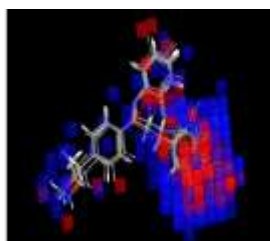


Plots of observed versus predicted antibacterial activity of 1,5-benzothiazepines (Training set)

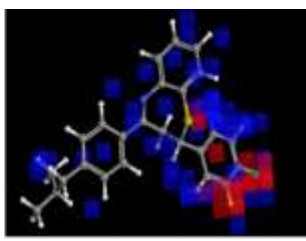


Plots of observed versus predicted antibacterial activity of 1,5-benzothiazepines (Test set)

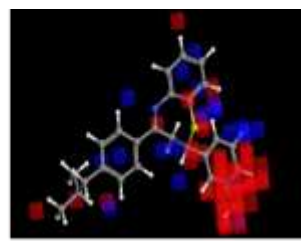
Figure 7. Atom based 3D-QSAR model for antibacterial activity of chalcones against *P.vulgaris*



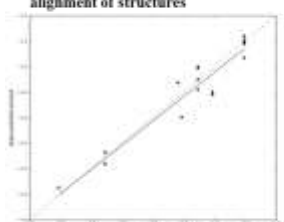
a) Atom based 3D-QSAR Model of 1,5-benzothiazepines along with alignment of structures



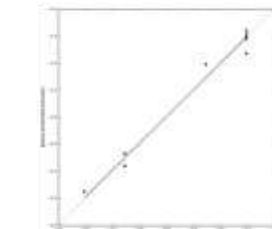
b) Atom based 3D QSAR model visualized in the context of highest active compound BT5



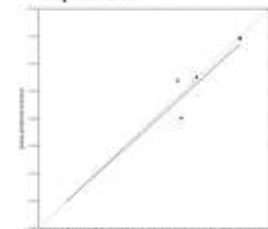
c) Atom based 3D QSAR model visualized in the context of highest active compound BT10



Plots of observed versus predicted antifungal activity of 1,5-benzothiazepines against *A.niger*.



Plots of observed versus predicted antifungal activity of 1,5-benzothiazepines (Training set) against *A.niger*



Plots of observed versus predicted antifungal activity of 1,5-benzothiazepines (Training set) against *A.niger*

Figure 8. Atom based 3D-QSAR model for the antifungal activity of chalcones against *A.niger*

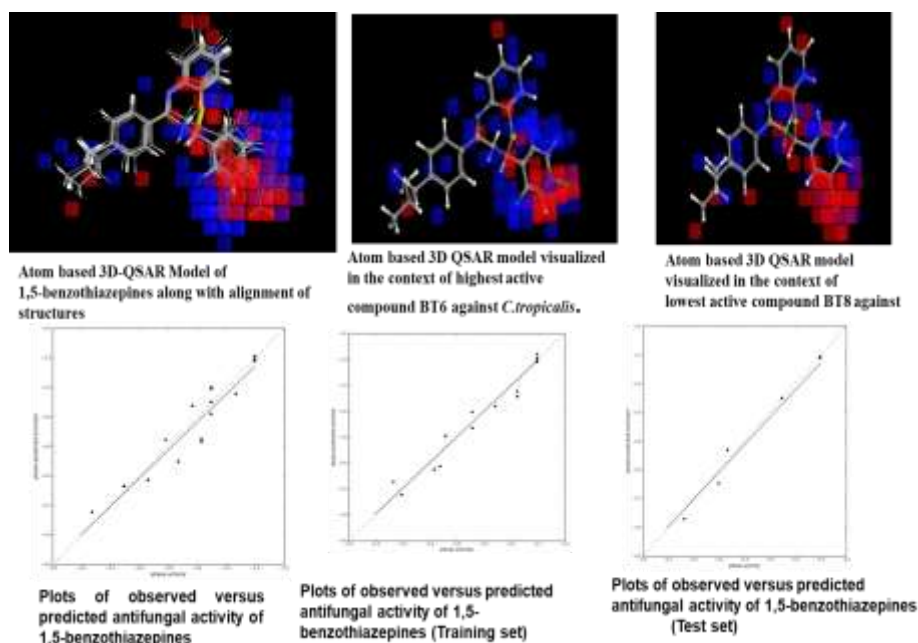


Figure 9. Atom based 3D-QSAR model for the antifungal activity of chalcones against *C. tropicalis*.

4. Conclusion

1,5-benzothiazepines having a variety of pharmacophores could be successfully synthesized in good yield, purified and characterized by spectral studies and evaluated for antimicrobial activity by biological and computational means. 1,5-Benzothiazepines possess potential antibacterial and antifungal activities. The observed – log MIC values from the actual results and the predicted – log MIC values through PLS method were well correlated supporting the usefulness of these computational studies. The invitro biological activity data and the Insilco atom-based 3D-QSAR results provide a set of guiding principles for the designing of compounds with better antibacterial and antifungal properties. Further optimization on the leads can be attempted by introducing substituents on the 4-isobutylacetophenone moiety. Studies are under progress to establish the mode of action of these potential compounds.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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