

SYNTHESIS OF NOVEL 1, 4-BENZOTHIAZEPINES AND IN VITRO SCREENING OF THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Objective: In search of new potential antimicrobial agents, the aim of the present study was to synthesize the series of 1, 4-Benzothiazepine analogs by a simple and accessible approach and evaluate for their antimicrobial activity.

Methods: Synthetic methodology involves the reaction of an α , β -unsaturated ketones (**3** and **6**) with 2-aminothiophenol and 3-4 drops of conc. HCl in methanol at 160°C, which afforded a series of novel 1,4-thiazepine derivatives (**4a-f** and **7a-c**) in good yields.

Results: The structures of the synthesized compounds were provided by spectral and elemental analysis, and by single crystal X-ray diffraction studies. The synthesized compounds were tested for their antimicrobial activity against different fungi and bacteria species *in vitro*.

Conclusion: The results of the study reveal that the new compounds possess promising antimicrobial activities.

Keywords: Antibacterial, Antifungal, Chalcones, Ketone, MIC.

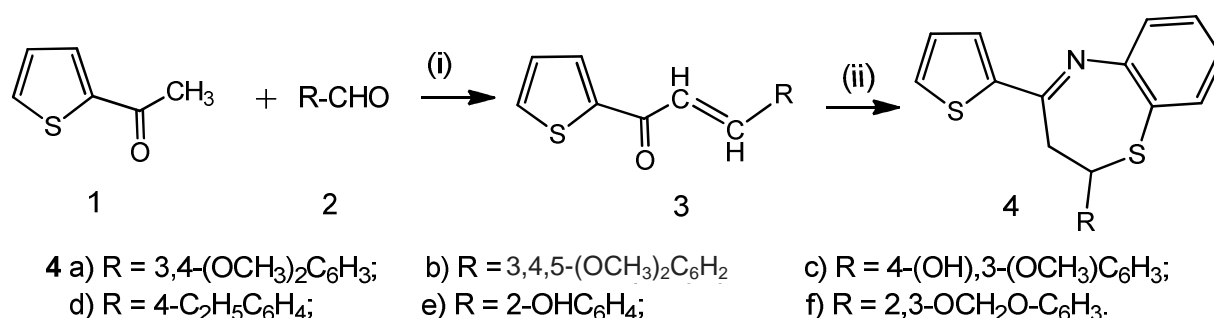
INTRODUCTION

1, 4-Benzothiazepine skeleton is considered as an important moiety in synthetic and pharmaceutical chemistry. Chalcone is an aromatic α , β -unsaturated ketone that forms the central core for a variety of important biological compounds. Chalcones are used as key precursor in the synthesis of biologically important heterocycles such as benzothiazepine [1-4] and pyrazolines [5,6]. The broad spectrum of pharmaceutical importance and commercial success associated with benzothiazepines has led the researchers all over the world to work in this area. 1,4-Benzothiazepine derivatives are known to exhibit biological activities such as antioxidant and cytotoxic [7], antimicrobial [8], and anticonvulsant [9] activities. The diverse biological applications associated with benzothiazepines prompted us to undertake this project of synthesis and biological screening of new benzothiazepine derivatives.

MATERIALS AND METHODS

The precursor chalcones (**3**) were prepared by the condensation of 2-acetyl thiophene (**1**) and with aromatic aldehyde (**2**) in ethyl alcohol under alkali conditions. The compounds (**7**) were obtained by the condensation of aromatic ketone (**5**) and aromatic aldehyde (**2**) under similar conditions.

A mixture of chalcones (**3a-f**) (3mmol), 2-aminothiophenol (3mmol) and 3-4 drops of conc. HCl in methanol (10mL) was refluxed at 160°C for 4h. The progress of the reaction was monitored by TLC (hexane/ethyl acetate). After the completion of reaction, the mixture was extracted into dichloromethane (30mL), washed with dilute hydrochloric acid and then with water. The solvent was evaporated to dryness to obtain products (**4a-f**) in good yields. The reaction pathway is illustrated in scheme-1.



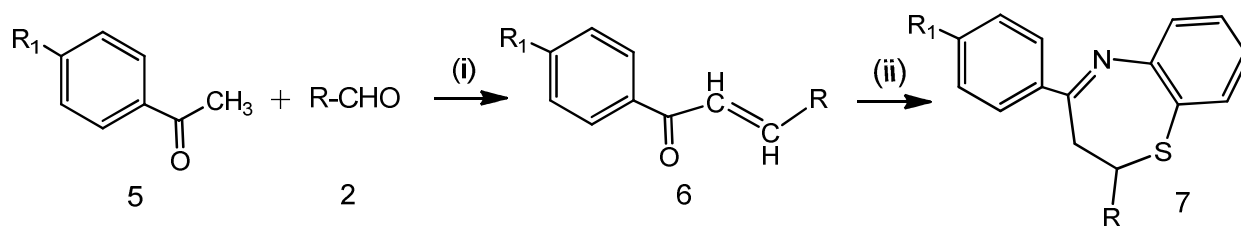
Reagents and conditions: (i) NaOH/C₂H₅OH, RT, 2h.

(ii) 2-Aminothiophenol/CH₃OH/Conc.HCl, 160°C, 4h.

Scheme-1: Synthetic route for the synthesis of thiazepines 4a-f

A mixture of chalcones (**6a-c**) (3mmol), 2-aminothiophenol (3mmol) and 3-4 drops of conc. HCl in methanol (10 mL) was refluxed at 160°C for 4h. Then the mixture was extracted into dichloromethane

(30 mL), washed successively with dilute hydrochloric acid and water. The solvent was evaporated to dryness to obtain products (**7a-c**) in good yields. The reaction pathway is depicted in scheme-2.



7 a) $R_1 = \text{Cl}$, $R = 3,4\text{-(OCH}_3\text{)}_2\text{C}_6\text{H}_3$; b) $R_1 = \text{NH}_2$, $R = 2,4\text{-Cl}_2\text{C}_6\text{H}_3$ c) $R_1 = \text{H}$, $R = \text{CH}_3$.

Reagents and conditions: (i) $\text{NaOH}/\text{C}_2\text{H}_5\text{OH}$, RT, 2h.

(ii) 2-Aminothiophenol/ $\text{CH}_3\text{OH}/\text{Conc.HCl}$, 160°C , 4h.

Scheme-2: Synthetic route for the synthesis of thiazepines 7a-c

In vitro antimicrobial activity of synthesized compounds (**4a-f**) and (**7a-c**) was carried out by paper disc diffusion method [10-13]. The test compounds at the concentration of 50 $\mu\text{g}/\text{mL}$ in methanol on the nutrient agar media were screened for their antibacterial activity against the species *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and antifungal activity against the species *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium*. The antibiotics ciprofloxacin and nystatin were used as standard drugs against bacteria and fungi species respectively. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations.

RESULTS AND DISCUSSION

Chemistry:

2-(3,4-Dimethoxyphenyl)-4-(thiophen-2-yl)-2,3-

dihydrobenzo[b][1,4]thiazepine, 4a³: Obtained from 3-(3, 4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **3a** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as yellow crystals in 88% yield. M.P. $96\text{-}98^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.820 (dd, 1H, $\text{C}_3\text{-H}$), 2.112 (dd, 1H, $\text{C}_3\text{-H}$), 3.665 (dd, 1H, $\text{C}_2\text{-H}$), 3.845 (s, 6H, OCH_3), 6.950-7.568 (m, 10H, Ar- & 5m ring-H). $^{13}\text{C NMR}$ (CDCl_3): δ 40.40 (1C), 50.12 (1C), 55.22 (2C), 110.13 (1C), 112.12 (1C), 117.04 (1C), 121.64 (1C), 124.22 (1C), 125.08 (1C), 125.33 (1C), 127.04 (1C), 127.50 (1C), 127.86 (1C), 133.28 (1C), 137.52 (1C), 137.92 (1C), 147.90 (1C), 148.92 (1C), 151.15 (1C), 164.33 (1C). MS (m/z): 382 (M+1, base peak). Anal. Calcd. for $\text{C}_{21}\text{H}_{19}\text{NO}_2\text{S}_2$: C, 66.11; H, 5.02; N, 3.67%; Found C, 66.10; H, 5.08; N, 3.61%.

4-(Thiophen-2-yl)-2-(3,4,5-trimethoxyphenyl)-2,3-

dihydrobenzo[b][1,4]thiazepine, 4b: Obtained from 1-(thiophen-2-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, **3b** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as light yellow crystals in 76% yield. M.P. $82\text{-}84^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.888 (dd, 1H, $\text{C}_3\text{-H}$), 2.118 (dd, 1H, $\text{C}_3\text{-H}$), 3.620 (dd, 1H, $\text{C}_2\text{-H}$), 3.850 (s, 9H, OCH_3), 6.840-7.520 (m, 9H, Ar- & 5m ring-H). $^{13}\text{C NMR}$ (CDCl_3): δ 40.44 (1C), 50.52 (1C), 55.50 (2C), 58.92 (1C), 102.34 (2C), 117.14 (1C), 124.33 (1C), 125.20 (1C), 125.62 (1C), 127.18 (2C), 127.46 (1C), 133.32 (1C), 136.06 (1C), 137.44 (1C), 137.88 (1C), 151.24 (1C), 152.92 (2C), 164.02 (1C). MS (m/z): 412 (M+1, base peak). Anal. Calcd. for $\text{C}_{22}\text{H}_{21}\text{NO}_3\text{S}_2$: C, 64.21; H, 5.14; N, 3.40%; Found C, 64.16; H, 5.10; N, 3.45%.

2-Methoxy-4-(4-(thiophen-2-yl)-2,3-

dihydrobenzo[b][1,4]thiazepin-2-yl)phenol, 4c: Obtained from 3-(4-hydroxy-3-methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **3c** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as brown solid in 80% yield. M.P. $142\text{-}144^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.802 (dd, 1H, $\text{C}_3\text{-H}$), 2.109 (dd, 1H, $\text{C}_3\text{-H}$), 3.688 (dd, 1H, $\text{C}_2\text{-H}$), 3.852 (s, 3H, OCH_3), 5.382 (s, 1H, OH), 6.955-7.654 (m, 10H, Ar- & 5m ring-H). $^{13}\text{C NMR}$ (CDCl_3): δ 40.40 (1C), 50.41 (1C), 55.82 (1C), 112.64 (1C), 117.10 (1C), 124.26 (2C), 125.26 (1C), 125.88 (2C), 127.22 (2C), 127.42 (1C), 133.30 (1C), 136.12 (1C), 137.24 (1C), 137.52 (1C), 151.02 (1C), 151.58 (1C), 164.00 (1C). MS (m/z): 368 (M+1, base peak).

Anal. Calcd. for $\text{C}_{20}\text{H}_{17}\text{NO}_2\text{S}_2$: C, 65.37; H, 4.66; N, 3.81%; Found C, 65.30; H, 4.56; N, 3.78%.

2-(4-Ethylphenyl)-4-(thiophen-2-yl)-2,3-

dihydrobenzo[b][1,4]thiazepine, 4d: Obtained from 3-(4-ethylphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **3d** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as yellow solid in 82% yield. M.P. $98\text{-}100^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.212 (t, 3H, CH_3), 1.814 (dd, 1H, $\text{C}_3\text{-H}$), 2.104 (dd, 1H, $\text{C}_3\text{-H}$), 2.580 (q, 2H, CH_2), 3.545 (dd, 1H, $\text{C}_2\text{-H}$), 7.121-7.618 (m, 11H, Ar- & 5m ring-H). $^{13}\text{C NMR}$ (CDCl_3): δ 13.9 (1C), 28.2 (1C), 40.1 (1C), 49.2 (1C), 116.4 (1C), 124.1 (1C), 125.3 (1C), 125.7 (1C), 126.2 (2C), 127.1 (1C), 127.6 (2C), 133.0 (1C), 136.9 (1C), 140.8 (1C), 142.1 (1C), 151.6 (1C), 164.0 (1C). MS (m/z): 350 (M+1, base peak). Anal. Calcd. for $\text{C}_{21}\text{H}_{19}\text{NS}_2$: C, 72.16; H, 5.48; N, 4.01%; Found C, 72.11; H, 5.46; N, 4.00%.

2-(4-(Thiophen-2-yl)-2,3-dihydrobenzo[b][1,4]thiazepin-2-

yl)phenol, 4e: Obtained from 3-(2-hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one, **3e** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as dark brown solid in 80% yield. M.P. $180\text{-}184^\circ\text{C}$. MS (m/z): 338 (M+1, base peak). Anal. Calcd. for $\text{C}_{19}\text{H}_{15}\text{NOS}_2$: C, 67.62; H, 4.48; N, 4.15%; Found C, 67.57; H, 4.44; N, 4.12%.

2-(Benzo[d][1,3]dioxol-4-yl)-4-(thiophen-2-yl)-2,3-

dihydrobenzo[b][1,4]thiazepine, 4f: Obtained from 3-(benzo[d][1,3]dioxol-4-yl)-1-(thiophen-2-yl)prop-2-en-1-one, **3f** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as yellow solid in 74% yield. M.P. $160\text{-}162^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.84 (dd, 1H, $\text{C}_3\text{-H}$), 2.04 (dd, 1H, $\text{C}_3\text{-H}$), 3.45 (dd, 1H, $\text{C}_2\text{-H}$), 6.08 (s, 2H, O- $\text{CH}_2\text{-O}$), 6.88-7.16 (m, 7H, Aromatic-H), 7.30-7.49 (m, 3H, 5m ring). $^{13}\text{C NMR}$ (CDCl_3): δ 40.8 (1C), 44.2 (1C), 103.8 (1C), 113.9 (1C), 115.3 (1C), 120.0 (1C), 121.8 (1C), 123.3 (1C), 124.1 (1C), 125.1 (1C), 125.8 (1C), 127.0 (1C), 127.5 (1C), 128.2 (1C), 132.0 (1C), 135.2 (1C), 147.3 (1C), 149.1 (1C), 152.2 (1C), 164.3 (1C). MS (m/z): 366 (M+1, Base peak). Anal. Calcd. for $\text{C}_{20}\text{H}_{15}\text{NO}_2\text{S}_2$: C, 65.73; H, 4.14; N, 3.83%; Found C, 65.70; H, 4.09; N, 3.81%.

4-(4-Chlorophenyl)-2-(3,4-dimethoxyphenyl)-2,3-

dihydrobenzo[b][1,4]thiazepine, 7a: Obtained from 1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one, **6a** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as creamy white solid in 83% yield. M.P. $110\text{-}112^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.87 (dd, 1H, $\text{C}_3\text{-H}$), 2.11 (dd, 1H, $\text{C}_3\text{-H}$), 3.55 (dd, 1H, $\text{C}_2\text{-H}$), 3.850 (s, 6H, OCH_3), 6.940-7.421 (m, 7H, Ar-H), 7.882 (dd, 2H, Ar-H), 8.026 (dd, 2H, Ar-H). $^{13}\text{C NMR}$ (CDCl_3): δ 40.2 (1C), 49.3 (1C), 55.4 (2C), 110.1 (1C), 112.9 (1C), 116.5 (1C), 122.1 (1C), 124.7 (1C), 127.6 (1C), 128.2 (2C), 128.8 (2C), 132.9 (1C), 135.6 (2C), 136.9 (1C), 137.8 (1C), 148.1 (2C), 154.1 (1C), 162.9 (1C). MS (m/z): 412 (M+1, ^{37}Cl , 33), 410 (M+1, ^{35}Cl , base peak). Anal. Calcd. for $\text{C}_{23}\text{H}_{20}\text{ClNO}_2\text{S}_2$: C, 67.39; H, 4.92; N, 3.42%; Found C, 67.34; H, 4.91; N, 3.45%.

4-(2-(2,4-Dichlorophenyl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-

yl)aniline, 7b: Obtained from 1-(4-aminophenyl)-3-(3,5-dichlorophenyl)prop-2-en-1-one, **6b** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as yellow solid in 70% yield. M.P. $215\text{-}217^\circ\text{C}$. $^1\text{H NMR}$ (CDCl_3): δ 1.86 (dd, 1H, $\text{C}_3\text{-H}$), 2.02 (dd, 1H, $\text{C}_3\text{-H}$), 3.44 (dd, 1H, $\text{C}_2\text{-H}$), 6.630 (s, 2H, NH_2), 7.122-7.526 (m, 11H, Ar-

H). MS (m/z): 401 (M+1, ^{37}Cl , 33%), 399 (M+1, ^{35}Cl , base peak). Anal. Calcd. for $\text{C}_{21}\text{H}_{16}\text{Cl}_2\text{N}_2\text{S}$: C, 63.16; H, 4.04; N, 7.01%; Found C, 63.10; H, 4.00; N, 7.06%.

2-Methyl-4-phenyl-2,3-dihydrobenzo[b][1,4]thiazepine, 7c: Obtained from 1-phenylbut-2-en-1-one, **6c** (0.004 mmol) and 2-aminothiophenol (0.004 mmol) as gummy mass in 76% yield. ^1H NMR (CDCl_3): δ 1.322 (s, 3H, CH_3), 1.88 (dd, 1H, $\text{C}_3\text{-H}$), 2.08 (dd, 1H, $\text{C}_2\text{-H}$), 3.48 (dd, 1H, $\text{C}_2\text{-H}$), 7.220-7.598 (m, 9H, Ar-H), 7.882 (dd, 2H, Ar-H). ^{13}C NMR (CDCl_3): δ 21.2 (1C), 40.8 (1C), 48.6 (1C), 116.7 (1C), 126.1 (1C), 127.7 (1C), 128.6 (2C), 128.9 (2C), 130.9 (1C), 132.8 (1C), 137.5 (1C), 137.9 (1C), 154.6 (1C), 162.4 (1C). MS (m/z): 254 (M+1, base peak), 253 (M+, 12%). Anal. Calcd. for $\text{C}_{16}\text{H}_{15}\text{NS}$: C 75.85, H 5.97, N 5.53%; Found C 75.80, H 5.95, N 5.52%.

Designed series of molecules **4a-f** and **7a-c** were characterized by spectral and elemental analysis before being evaluated for their antimicrobial activity. The structural assignments were made by NMR analysis by considering compound (**4a**) as the representative compound. In its ^1H NMR spectra, H_a , H_b and H_c protons of the benzothiazepine ring appeared as a doublet of doublet. The doublet of H_a appeared at δ 1.822 ppm; doublet of H_b appeared at δ 2.112 ppm; and that of H_c appeared at δ 3.665 ppm. Doublets of H_a and H_b are due to diastereotopic nature of methylene protons. Among H_a , H_b and H_c protons, H_c is the most deshielded due to its close proximity to benzene ring. H_c couples not only with H_a but also with H_b and appears as doublet of doublet instead of a triplet i.e., the methylene protons of benzothiazepine ring (H_a and H_b) exhibited a typical ABX spin system with H_c as a doublet of doublets as in diagram-1. Further it showed signals due to substituent and aromatic protons at the expected region. All compounds displayed the signals in the similar pattern.

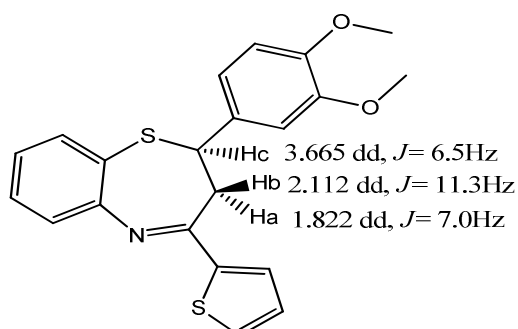


Diagram-1: Proton chemical shifts and couplings of **4a**

In ^{13}C NMR spectra, all compounds showed the signals due to aromatic and substituent carbons at the expected region. The mass spectrum of all the synthesised benzothiazepines showed M+1 molecular ion peak as base peak. Further all showed satisfactory elemental analysis. These observations confirmed the formation of these compounds. Further, the structure of one of the series of compounds synthesised (**4a**) was confirmed by single crystal X-ray diffraction studies [3].

Antimicrobial activity

The results of antibacterial activity of the synthesized compounds tested against bacterial species were shown in fig-1.

The experimental results revealed that all compounds displayed moderate to good antibacterial activity with reference to the standard against the tested organisms. The compound **4f** having methenedioxy substituent on the aromatic ring found less active, while **4d** and **4e** found moderately active against the organisms tested. The compound **4a-c**, and **7c** exhibited good activity against the all species tested. It is interesting to note that the compounds **7a**, **7b** exhibited excellent antibacterial activity against all the organisms tested. This may be attributed to the presence of chloro substitution on the aromatic ring.

The results of antifungal activity of the synthesised new benzothiazepines against different fungal species were summarised in fig-2.

The results revealed that the compound **4e** having hydroxyl substitution on the aromatic ring found less active, while **4d**, **4f** and **7c** found moderately active and **4c** showed good activity against the organisms tested. The compounds **4a**, **4b**, **7a**, and **7b** displayed excellent activity against the organisms tested in comparison with the standard. From the results of the study, it is evident that the presence of electron donating methoxy substituents in **4a**, **4b**, and the electronegative chloro substitution in **7a**, and **7b** caused these molecules to exhibit greater antifungal activity.

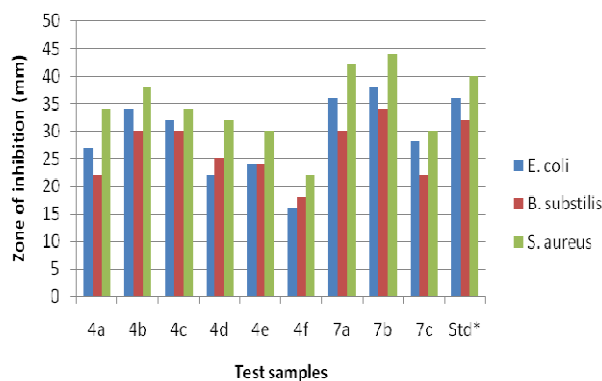


Fig. 1: Antibacterial activity of the synthesised benzothiazepines

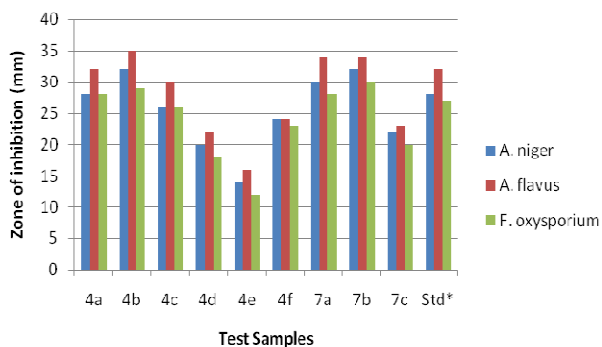


Fig. 2: Antifungal activity of the synthesised benzothiazepines

CONCLUSION

An accessible approach for the synthesis of 1,4-benzothiazepines was presented. The potential antimicrobial activity of the synthesised compounds validates the significance of this study. Among the synthesised compounds, 4-(4-chlorophenyl)-2-(3,4-dimethoxyphenyl)-2,3-dihydrobenzo[b][1,4]thiazepine and 4-(2-(3,5-dichlorophenyl)-2,3-dihydrobenzo[b][1,4]thiazepin-4-yl)aniline acts as potential antifungal and antibacterial agents.

REFERENCES

- Om Prakash, Ajay Kumar, Anil Sadana, Prakash R, Singh SP, Claramunt RM, Sanz D, Alkorta I, Elguero J. Study of the reaction of chalcone analogs of dehydroacetic acid and *o*-aminothiophenol: synthesis and structure of 1,5-benzothiazepines and 1,4-benzothiazines. Tetrahedron, 2005: 61;6642-6651.
- Manjula M, Manjunath BC, Renuka N, Ajay Kumar K, Lokanath NK. 2-(4-Fluorophenyl)-4-(thiophen-2-yl)-2,3-dihydrobenzo[b][1,4]thiazepine, Acta Cryst Sect E, 2013: 69, Part 11; o1608-o1608.
- Manjunath BC, Manjula M, Raghavendra KR, Shashikanth S, Ajay Kumar K, Lokanath NK. 2-(3,4-Dimethoxyphenyl)-4-(thio-

- phen-2-yl)-2, 3-dihydro-1, 5-benzothiazepine. Acta Cryst Sect E. 2014; 70 Part 2;o121-o121.
- Manjunath BC, Manjula M, Raghavendra KR, Ajay Kumar K, Lokanath NK. 4-(Thiophen-2-yl)-2-[4-(trifluoromethyl)-phenyl]-2,3-dihydro-1,5-benzothiazepine. Acta Cryst Sect E. 2014; 70 Part 3;o261-o261.
 - Manjula M, Jayaroopa P, Manjunath BC, Ajay Kumar K, Lokanath NK. 3-Methyl-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole. Acta Cryst Sect E. 2013; 69 Part 4;o602-o602.
 - Jayaroopa P, Ajay Kumar K. Synthesis and antimicrobial activity of 4,5-dihydropyrazoline derivatives. Int J Pharm Pharm Sci. 2013; 5(4);431-433.
 - Shi F, Zeng X-N, Cao X-D, Zhang S, Jiang B, Zheng W-F, Tu S-J. Design and diversity-oriented synthesis of novel 1,4-benzothiazepan-3-ones fused with bioactive heterocyclic skeletons and evaluation of their antioxidant and cytotoxic activities. Bioorg Med Chem Lett. 2012; 22:743-746.
 - Singh G, Kumar N, Yadav AK, Mishra AK. Syntheses of New 1,5-Benzothiazepine Derivatives and Their Ribofuranosides as Antimicrobial Agents. Heteroatom Chemistry. 2002; 13(7):620-625.
 - Pandeya SN, Deepak Kumar, Verma PK. Newer applications of 1,5-benzothiazepines and their anticonvulsant activity. Der Pharma Chemica. 2012; 4(5):1853-1855.
 - Ajay Kumar K, Lokanatha Rai KM, Vasanth Kumar G, Mylarappa BN. A facile route for the synthesis of ethyl *N*-aryl-2,6-dioxo-piperid-3-ene-4-carboxylates and their biological activity. Int J Pharm Pharm Sci. 2012; 4 (Suppl 4):564-568.
 - Ajay Kumar K, Lokanatha Rai KM, Umesha KB. Synthesis and evaluation of antifungal and antibacterial activity of ethyl 3,5-diarylloxazole-4-carboxylates. J Chem Res (S). 2001:436-438.
 - Ajay Kumar K, Lokanatha Rai KM. Synthesis and evaluation of antimicrobial activity of 4,5-dihydro-12,4-oxadiazoles. Bulg Chem Commun. 2004; 36:249-252.
 - Govindaraju M, Mylarappa BN, Ajay Kumar K. Synthesis of novel pyrazole derivatives and their efficacy as antimicrobial agents. Int J Pharm Pharm Sci 2013; 5(4):734-737.