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Original article

Synthesis and biological evaluation of a novel series of 1,5-benzothiazepine derivatives as potential antimicrobial agents

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ABSTRACT

Two series of novel 1,5-benzothiazepine derivatives (23 compounds) were efficiently synthesized and evaluated for antibacterial and antifungal activities. The results indicated that the compounds possessed a broad spectrum of activity against the tested microorganisms and showed higher activity against fungi than bacteria. Compound **2e** exhibited the greatest antimicrobial activity. Preliminary study of the structure–activity relationship revealed that substituents in phenyl rings had a great effect on the antimicrobial activity of these compounds.

1. Introduction

Benzothiazepines play a unique role in drug discovery programs, as they display a wide spectrum of biological activities such as antibacterial [1], antifeedant [2], analgesic [3], anticonvulsant [4], and calcium antagonism [5]. Therefore, their beneficial properties have prompted several groups to study these compounds. We have been interested in 1,5-benzothiazepine derivatives for a few years and have prepared several series of 1,5-benzothiazepines. The synthetic method has been developed and the spectroscopic properties of these compounds have been investigated. Recently, biological screening has shown that some compounds display excellent antimicrobial activity. Encouraged by the results, two series of 1,5-benzothiazepine derivatives (23 compounds) have been designed and studied as potential antimicrobial agents. Here, we report the synthesis, biological evaluation and preliminary structure–activity relationships (SAR) of these new compounds whose structures are shown in Fig. 1. The experimental results showed that most of the compounds had good antimicrobial activity against *Candida albicans*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

2. Results and discussion

2.1. Chemistry

One of the most widely employed methods for the preparation of 1,5-benzothiazepines involves the reaction of *o*-aminothiophenol with chalcones under acidic or basic conditions [6]. The synthesis of compounds **1a**–**r** was accomplished in three steps as shown in Scheme 1. Knoevenagel condensation of aromatic aldehydes with 2,4-pentandione in dry benzene catalyzed by piperidine gave compound **3**. Then Michael addition of *o*-aminothiophenol to compound **3** yielded the corresponding pentandione derivatives **4**. Finally, the intramolecular cyclisation of **4**, followed by dehydration in acetic acid/methanol provided compounds **1**, which were purified by crystallization from dry methanol. To the best of our knowledge, this is the first report of the synthesis of compounds **1**.

The target compounds **2** (2,5-dihydro-2-aryl-3-ethoxycarbonyl-4-methyl-1,5-benzothiazepines) were prepared by the same reaction sequence as previously described (Scheme 2) [7]. It is worth noting that when 4-hydroxybenzaldehyde was used as the starting material, compound **2e** was obtained as the final product, whose structure had an unusual imine (–N=C–C–) rather than enamine (–N–C=C–), as demonstrated by its ^{1}H NMR spectrum. The ^{1}H NMR spectra of **2a**–**d** showed the enamine methyl (–N–C=C–*Me*) group at 2.60–2.65 ppm, the NH group at 6.19–6.23 ppm and S–CH group at the C-2 position as a singlet at 5.89–6.14 ppm. However, in ^{1}H NMR spectra of **2e**, the imine methyl (–N=C–*Me*) group went up

Abbreviations: SAR, structure–activity relationships; TLC, thin-layer chromatography; rt, room temperature.

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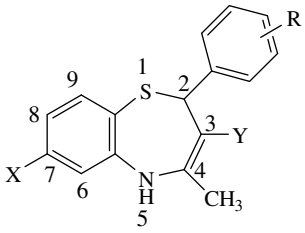
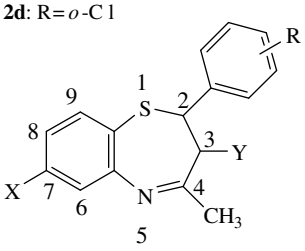


1a-r **2a-d**

1a-j: X=H, Y=$COCH_3$
1a :R= *p*-NO_2
1b: R= *o*-NO_2
1c :R= *p*-Cl
1d: R= *o*-Cl
1e:R= *p*-CH_3
1f: R= *p*-OH
1g: R=H
1h: R= *p*-OCH_3
1i: R= *p*-F
1j: R=2,4-dichloro
1k-r: X=Cl,Y=$COCH_3$
1k: R=*p*-NO_2
1l: R= *o*-NO_2
1m: R=*p*-Cl
1n: R= *o*-Cl
1o: R= H
1p: R= *p*-F
1q: R= p-Br
1r: R= 2,4-dichloro

2a-d: X=H, Y=$COOCH_2CH_3$
2a: R=*p*-CH_3
2b: R=*p*-F
2c: R= *p*-NO_2
2d: R= *o*-Cl



2e: X=H , Y=$COOCH_2CH_3$, R= *p*-OH

Fig. 1. Structures of compounds **1** and **2**.

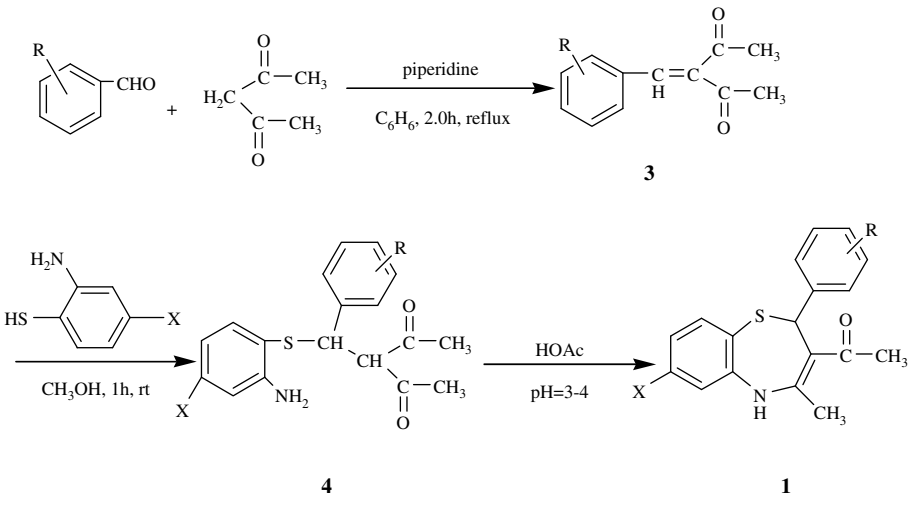
field at 2.29 ppm, the NH signal disappeared, the S–CH group at the C-2 position appeared as doublets at 5.20–5.23 ($J = 9.2$ Hz) and the CH group at the C-3 position in the seven-membered ring of benzothiazepine appeared as doublets at 3.70–3.72 ppm ($J = 9.2$ Hz), owing to their vicinal coupling.

Monitoring the cyclization progress of compounds **4** and **6** by ^{1}H NMR spectroscopy, it was revealed that imines were initially formed and gradually transformed to enamines, except for the formation of **2e**. However, we have no detailed knowledge of these reactions, and ongoing work in our laboratory is attempting to selectively prepare enamines or imines by optimizing reaction conditions, and to reveal whether the tautomeric equilibrium was involved in the present study.

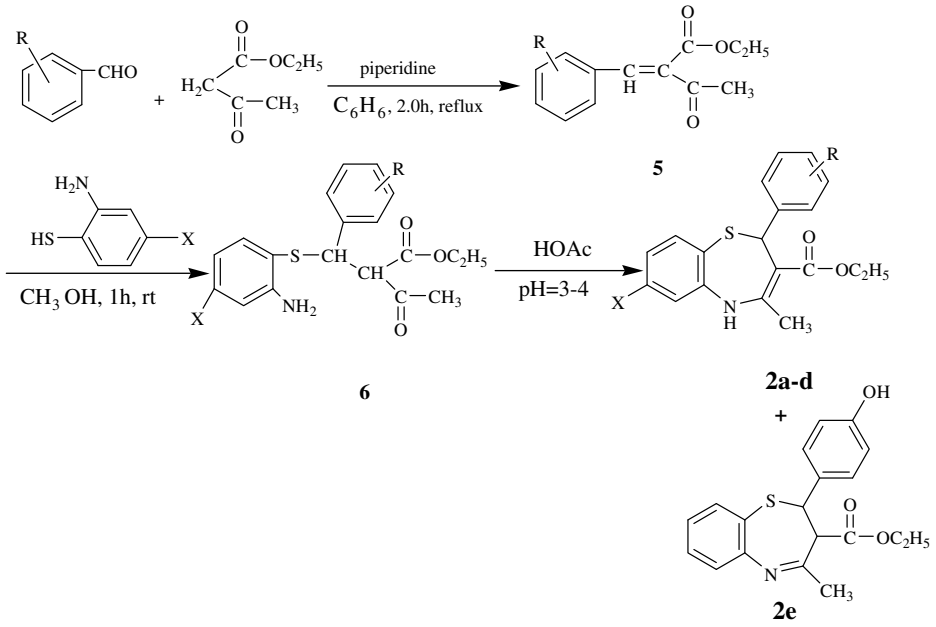
2.2. Biological activity

In the present study, all the synthesized compounds **1** and **2** were tested against microbial strains such as *C. albicans* (ATCC 10231), *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 26069) and *Escherichia coli* (ATCC 44752) using disk diffusion methods. Fluconazole was used as a standard drug against fungi and vancomycin against bacteria. For the purpose of easier visualization, the zone date from these assays indicates the average diameter (from 3 trails) of the growth inhibition zones. The margin of error of these measurements is ±1 mm. The antibacterial activity was classified as highly active (>14 mm), moderately active (10–14 mm), slightly active (6–10 mm) and less than 6 mm was regarded as inactive. The results of our antibacterial and antifungal studies of all the synthesized compounds are depicted in Tables 1 and 2. From Tables 1 and 2, it can be observed that compounds **1** exhibited better activity than compounds **2**, with the exception of **2e**. These results indicated that the acetyl group at C-3 was required for enhancing antimicrobial activity. Compounds **1** and **2e** exhibited higher antifungal activity against *C. albicans* than antibacterial activity against *S. aureus*, *S. epidermidis* and *E. coli*. The results indicated that the compounds seemed to have higher sensitivity for fungi than bacteria. It was also found that none of the compounds tested inhibited the growth of *E. coli*, even at highest tested dose of 200 μg/disc. Among the tested compounds, compounds **1c**, **1e** and **2e** showed significant activity, and the others showed moderate activity.

Table 2 gives the inhibition zone data for compounds **2**. Although most of the compounds did not show antifungal and antibacterial activity, compound **2e** showed good biological activity against bacteria *S. aureus* (inhibition zone 18 mm) and *S. epidermidis* (inhibition zone 20 mm) and fungus *C. albicans* (inhibition zone 30 mm) at 200 μg/disc. This suggests that compound **2e** is the most potent compound among all those synthesized in the present



Scheme 1. Synthesis of benzothiazepines **1a–r**.



Scheme 2. Synthesis of benzothiazepines **2a**–**e**.

study, and the imine structure (–N═C–C–) seems to play an important role in the antimicrobial activity. Another reason for stronger antimicrobial activity of **2e** may be the improved solubility of the compound in agar, mediated by the hydroxyl group in the molecule. A similar result was reported by Seefeld and co-workers [8].

Substitution at the C-2 position of compounds **1a**–**r** showed that the antimicrobial activity changed with the position of the substituents on the phenyl ring at the C-2 position and with the variety of peripheral substituents on the phenyl ring in the same pattern of benzothiazepines. For example, compounds **1a** and **1c**, with *para*-substituents (*p*-NO_2, *p*-Cl), exhibited higher antimicrobial activity than compounds **1b** and **1d**, with the same *ortho*-substituents (*o*-NO_2, *o*-Cl). The results indicated that *para*-substituents on the C-2 aryl ring increased the inhibitory effect of the compounds. Analysis of the SAR in compounds **1a**–**i** also revealed that replacement of H by R on the C-2 aryl ring enhanced antimicrobial activity with the exception of compound **1h**. The introduction of *p*-Cl groups to the C-2 aryl ring caused a marked increase in antimicrobial activity. Comparison of biological activity showed that compounds **1c** and **d** exhibited higher antifungal activity against *C. albicans* than **1j**, which suggested that the multi-chlorine substituents on the C-2 aryl ring of compounds **1** were unfavorable for enhancing antifungal activity.

Table 1
Antimicrobial activity of compounds **1**.

Compd	X	R	Dose (μg/disc)											
			200	100	50	200	100	50	200	100	50	200	100	50
			Zone of inhibition[a] (mm)											
			C. albicans			*S. aureus*			*S. epidermidis*			*E. coli*		
1a	H	*p*-NO_2	20	18	16	13	12	10	13	12	10	–	–	–
1b	H	*o*-NO_2	16	13	11	12	11	8	10	9	7	–	–	–
1c	H	*p*-Cl	24	21	18	15	14	12	19	17	17	–	–	–
1d	H	*o*-Cl	15	15	12	9	8	8	9	7.5	7.5	–	–	–
1e	H	*p*-CH_3	23	16	16	10	9	7	7	7	7	–	–	–
1f	H	*p*-OH	17	17	15	10	9.5	7	12	11	11	–	–	–
1g	H	H	10	7	–	11	8	6.5	10	7	–	–	–	–
1h	H	*p*-OCH_3	11	9	8	10	7	6.5	10	7	–	–	–	–
1i	H	*p*-F	20	20	18	10	9	7	11	11	10	–	–	–
1j	H	2,4-Dichlo-rine	14	10	9	10	7	7	11	8	7	–	–	–
1k	Cl	*p*-NO_2	14	11	9	11	7	7	11	11	10	–	–	–
1l	Cl	*o*-NO_2	–	–	–	8	7	7	8	8	7	–	–	–
1m	Cl	*p*-Cl	13	11	10	10	7.5	7	10	7.5	7	–	–	–
1n	Cl	*o*-Cl	15	12	10	11	8	7	10	7	7	–	–	–
1o	Cl	H	13	9	7	10	7	7	11	8	7	–	–	–
1p	Cl	*p*-F	10	9	8	9	9	8	8	8	7	–	–	–
1q	Cl	*p*-Br	15	11	10	11	10	9	11	10	9	–	–	–
1r	Cl	2,4-Dichlorine	–	–	–	10	7	–	–	–	–	–	–	–

[a] The values indicate the average diameters in mm (of three trials) for the zone of growth inhibition observed after 24 h of incubation at 37 °C. Inhibition zones of standard drugs: Fluconazole, 23 mm against *C. albicans* at 25 μg/disc, vancomycin: 18, 18, and 8 mm against *S. aureus*, *S. epidermidis* and *E. coli* at 30 μg/disc, respectively. Error values are within ±1 mm.

Table 2
Antimicrobial activity of compounds **2**.

Compd	X	R	Dose (μg/disc)											
			200	100	50	200	100	50	200	100	50	200	100	50
			Zone of inhibition[a] (mm)											
			C. albicans			*S. aureus*			*S. epidermidis*			*E. coli*		
2a	H	*p*-CH_3	–	–	–	–	–	–	–	–	–	–	–	–
2b	H	*p*-F	–	–	–	–	–	–	–	–	–	–	–	–
2c	H	*p*-NO_2	7.0	6.5	–	7.0	6.5	–	–	–	–	–	–	–
2d	H	*o*-Cl	–	–	–	–	–	–	–	–	–	–	–	–
2e	H	*p*-OH	30	24	14	18	15	7	20	16	10	–	–	–

[a] The values indicate the average diameters in mm (of three trials) for the zone of growth inhibition observed after 24 h of incubation at 37 °C. Inhibition zones of standard drugs: Fluconazole, 23 mm against *C. albicans* at 25 μg/disc, vancomycin: 18, 18, and 8 mm against *S. aureus*, *S. epidermidis* and *E. coli* at 30 μg/disc, respectively. Error values are within ±1 mm.

Finally, we noted that the substitution at the C-7 position of compounds **1** also had an effect on the biological activity. The C-7 chloro-substituted analogues **1k–r** were less active than compounds **1a–j**, respectively. The result showed that the chlorine substituent at the C-7 position on the benzothiazepine ring was unfavorable for improving antimicrobial activity.

In order to explore the biological activity of 1,5-benzothiazepines and identify promising lead compounds, **1c** and **2e**, with remarkable activity in the present study, were selected to investigate the effect of dose levels on antimicrobial activity. Figs. 2 and 3 show the relationship between compound activity and dose level. The zones of inhibition values suggested the antimicrobial activities of compounds (benzothiazepine) **1c** and **2e** at a broad range from 12.5 to 200 μg/disc against *C. albicans*, *S. aureus* and *S. epidermidis*. It was also observed that the antimicrobial activity was dose-related and declined with decreasing dose level. The compound exhibited inactivity at doses <12.5 μg/disc. From Figs. 2 and 3, it was also found that the antimicrobial capacity for the above microorganisms was in the order *C. albicans* > *S. epidermidis* > *S aureus* > *E. coli*. The same tendency was also observed for most of the compounds in our present study.

3. Conclusion

In conclusion, two series of novel 1,5-benzothiazepine derivatives were synthesized and their biological activities evaluated. The results showed that most of the compounds exhibited moderate to good activity against *C. albicans*, *S. epidermidis* and *S. aureus* and displayed their antimicrobial capacity in the order *C. albicans* > *S. epidermidis* > *S. aureus* > *E. coli*. Three compounds, **1c**, **1e** and **2e**, have significant antimicrobial properties. Thus, the promising activities and easy access of benzothiazepine derivatives render them as very attractive antimicrobial leads. The extensive study of the SAR of this series of compounds will provide information for the design and development of new antimicrobial drugs based on 1,5-benzothiazepine derivatives. Further detailed SAR studies are the subject of future studies and will be reported in due course.

4. Experimental

Melting points (°C) were determined in a PXT-4 digital melting point apparatus and elemental analysis was performed on a Vario ELIII Elemental Analyzer. The IR spectra (in KBr pellets) were recorded on a PE-M-1730 IR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker ARX spectrometer; chemical shifts (δ) are reported in ppm using $SiMe_4$ as the internal standard when measured in $CDCl_3$ or DMSO-d_6. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), m (multiplet), and q (quartet). Low-resolution mass spectra were recorded on a Micromass ZAC-HS mass spectrometer. The substituted benzaldehyde and *o*-aminothiophenol were purchased from Sigma–Aldrich, and the other chemicals were used as commercial products of analytical

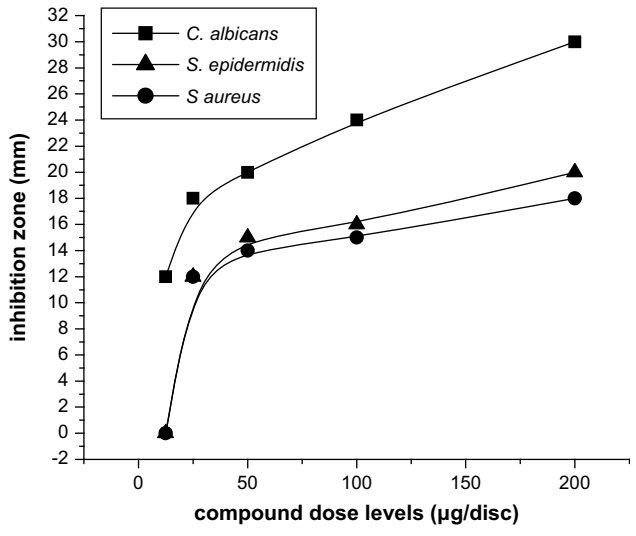


Fig. 2. Inhibition zones at different dose levels for compound **1c**.

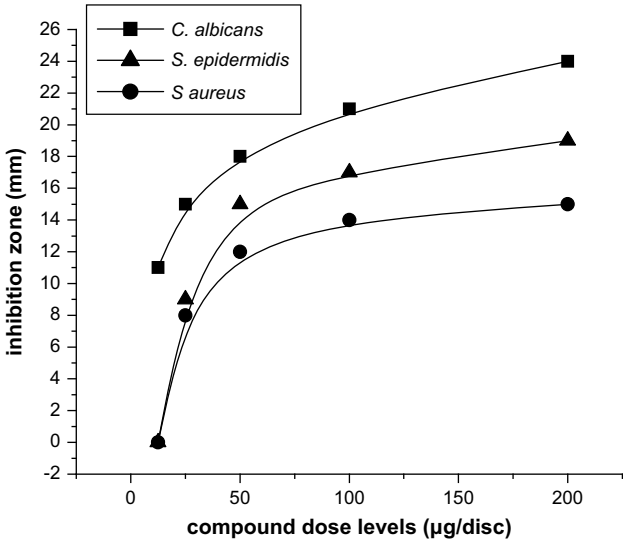


Fig. 3. Inhibition zones at different dose levels for compound **2e**.

grade. Solvents were dried and purified according to the literature when necessary. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates.

4.1. General method for the synthesis of 2,5-dihydro-4-methyl-2-aryl-3-acetyl-1,5-benzothiazepine (**1a**–**r**)

4.1.1. Synthesis of 3-benzylidene-2,4-pentandione (**3**)

2,4-Pentandione (15.3 mL, 0.15 mol) and piperidine (0.9 mL) were dissolved in 150 mL dry benzene. Then substituted benzaldehyde (0.15 mol) was added dropwise at room temperature over 20 min. The reaction mixture was slowly brought to boil and refluxed for 2 h with stirring (TLC monitoring). After cooling, the organic layer was washed with cold aqueous 10% sodium carbonate, water and aqueous 5% acetic acid. Then, the organic layer was dried and evaporated under reduced pressure and the crude product was purified by crystallization from ether.

4.1.2. Synthesis of 3-(1-aryl-1-o-aminophenylthio methyl)-2,4-pentandione (**4**)

A mixture of 3-benzylidene-2,4-pentandione (**3**) (25 mmol) and *o*-aminothiophenol (3.1 g, 25 mmol) in dry methanol (50 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, cooled, and the solid was collected by filtration, washed with water and cold methanol. The crude products were purified by crystallization from the appropriate solvent.

4.1.3. Synthesis of 2,5-dihydro-4-methyl-2-aryl-3-acetyl-1,5-benzothiazepine (**1a**–**r**)

To a solution of 3-(1-aryl-1-*o*-aminophenylthio methyl)-2,4-pentandione (**4**) (20 mol) in dry methanol (30 mL), acetic acid was added until it reached pH 4, and the mixture was stirred at room temperature for 12 h. The precipitate was collected by filtration, washed well with cold methanol, and crystallized from methanol.

The overall yield and characterization (Mp, IR, NMR, MS and elementary analysis data) of 2,5-dihydro-4-methyl-2-aryl-3-acetyl-1,5-benzothiazepine (**1a**–**r**) were as follows:

*4.1.3.1. 2,5-Dihydro-4-methyl-2-(4-nitrophenyl)-3-acetyl-1,5-benzothiazepine (**1a**).* Yield 32%; mp 161 °C; MS $[M+H^+]$: 341; $C_{18}H_{16}N_2O_3S$, found: C, 63.40; H, 4.87; N, 8.10; Calcd: C, 63.51; H, 4.74; N, 8.23; IR KBr (ν cm^{-1}) 1712 (C=O), 1635 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.13–8.36 (8H, m, $-C_6H_4$), 6.33 (1H, s, NH), 5.60 (1H, s, SCH), 2.29 (3H, s, –CO–CH_3), 1.93 (3H, s, $-CH_3$).

*4.1.3.2. 2,5-Dihydro-4-methyl-2-(2-nitrophenyl)-3-acetyl-1,5-benzothiazepine (**1b**).* Yield 31%; mp 127 °C; MS $[M+H^+]$: 341; $C_{18}H_{16}N_2O_3S$: found: C, 63.40; H, 4.87; N, 8.10; Calcd: C, 63.51; H, 4.74; N, 8.23; IR KBr (ν cm^{-1}) 1718 (C=O), 1635 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.13–8.36 (8H, m, $-C_6H_4$), 6.20 (1H, s, NH), 5.81 (1H, s, SCH), 2.32 (3H, s, –CO–CH_3), 2.00 (3H, s,–CH_3).

*4.1.3.3. 2,5-Dihydro-4-methyl-2-(4-chlorophenyl)-3-acetyl-1,5-benzothiazepine (**1c**).* Yield 42%; mp 117 °C; MS $[M+H^+]$: 330; $C_{18}H_{16}ClNOS$: found: C, 65.42; H, 5.01; N, 4.14; Calcd: C, 65.54; H, 4.89; N, 4.25; IR KBr (ν cm^{-1}) 1708 (C=O), 1635 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.74–7.07 (8H, m, $-C_6H_4$), 6.29 (1H, s, NH), 5.63 (1H, s, SCH), 2.56 (3H, s, –CO–CH_3), 2.18 (3H, s, $-CH_3$).

*4.1.3.4. 2,5-Dihydro-4-methyl-2-(2-chlorophenyl)-3-acetyl-1,5-benzothiazepine (**1d**).* Yield 40%; mp 149 °C; MS $[M+H^+]$: 330; $C_{18}H_{16}ClNOS$: found: C, 65.42; H, 5.01; N, 4.14; Calcd: C, 65.54; H, 4.89; N, 4.25; IR KBr (ν cm^{-1}) 1701 (C=O), 1637 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.51–7.30 (8H, m, $-C_6H_4$), 6.39 (1H, s, NH), 5.86 (1H, s, SCH), 2.57 (3H, s, –CO–CH_3), 2.14 (3H, s, $-CH_3$).

*4.1.3.5. 2,5-Dihydro-4-methyl-2-(4-methylphenyl)-3-acetyl-1,5-benzothiazepine (**1e**).* Yield 47%; mp 139 °C; MS $[M+H^+]$: 310; $C_{19}H_{19}NOS$: found: C, 73.62; H, 6.31; N, 4.39; Calcd: C, 73.75; H, 6.19; N, 4.4.53; IR KBr (ν cm^{-1}) 1724 (C=O), 1635 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.69–7.49 (8H, m, $-C_6H_4$), 6.51 (1H, s, NH), 5.62 (1H, s, SCH), 2.55 (3H, s, –CO–CH_3), 2.17 (3H, s, $-CH_3$), 2.01 (3H, s, $PhCH_3$–).

*4.1.3.6. 2,5-Dihydro-4-methyl-2-(4-hydroxylphenyl)-3-acetyl-1,5-benzothiazepine (**1f**).* Yield 31%; mp 177 °C; MS $[M+H^+]$: 312; $C_{18}H_{17}NO_2S$: found: C, 69.31; H, 5.62; N, 4.36; Calcd: C, 69.43; H, 5.50; N, 4.50; IR KBr (ν cm^{-1}) 1697 (C=O), 1635 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.64–7.53 (8H, m, $-C_6H_4$), 6.39 (1H, s, NH), 5.69 (1H, s, SCH), 5.36 (1H, s, OH), 2.29 (3H, s, $-COCH_3$), 1.87 (3H, s, $-CH_3$).

*4.1.3.7. 2,5-Dihydro-4-methyl-2-phenyl-3-acetyl-1,5-benzothiazepine (**1g**).* Yield 49%; mp 167 °C; MS $[M+H^+]$: 295; $C_{18}H_{17}NOS$: found: C, 73.31; H, 5.70; N, 4.82; Calcd: C, 73.22; H, 5.76; N, 4.75; IR KBr (ν cm^{-1}) 1708 (C=O), 1636 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.70–7.28 (9H, m, $-C_6H_4$,$-C_6H_5$), 6.41 (1H, s, NH), 5.69 (1H, s, SCH), 2.59 (3H, s, $-COCH_3$), 2.20 (3H, s, $-CH_3$).

*4.1.3.8. 2,5-Dihydro-4-methyl-2-(4-methoxylphenyl)-3-acetyl-1,5-benzothiazepine (**1h**).* Yield 44%; mp 107 °C; MS $[M+H^+]$: 326; $C_{19}H_{19}NO_2S$: found: C, 70.31; H, 5.70; N, 4.42; Calcd: C, 70.15; H, 5.85; N, 4.31; IR KBr (ν cm^{-1}) 1713 (C=O), 1634 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.49–7.28 (8H, m, $-C_6H_4$), 5.63 (1H, s, NH), 3.67 (3H, s, $-OCH_3$), 3.48 (1H, s, SCH), 2.56 (3H, s, $-COCH_3$), 2.19 (3H, s, $-CH_3$).

*4.1.3.9. 2,5-Dihydro-4-methyl-2-(4-flurorophenyl)-3-acetyl-1,5-benzothiazepine (**1i**).* Yield 39%; mp 117 °C; MS $[M+H^+]$: 314; $C_{18}H_{16}FNOS$: found: C, 69.12; H, 5.05; N, 4.52; Calcd: C, 69.01; H, 5.11; N, 4.47; IR KBr (ν cm^{-1}) 1710 (C=O), 1635 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.74–7.07 (8H, m, $-C_6H_4$), 6.25 (1H, s, NH), 5.65 (1H, s, SCH), 2.56 (3H, s, –CO–CH_3), 2.18 (3H, s, $-CH_3$).

*4.1.3.10. 2,5-Dihydro-4-methyl-2-(2,4-dichlorophenyl)-3-acetyl-1,5-benzothiazepine (**1j**).* Yield 37%; mp 145 °C; MS $[M+H^+]$: 364; $C_{18}H_{15}Cl_2NOS$: found: C, 59.31; H, 4.02; N, 3.90; Calcd: C, 59.34; H, 4.12; N, 3.85; IR KBr (ν cm^{-1}) 1712 (C=O), 1600 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.41–7.31 (7H, m, $-C_6H_4$, $-C_6H_3$), 5.79 (1H, s, NH), 3.48 (1H, s, SCH), 2.57 (3H, s, –CO–CH_3), 2.12 (3H, s, $-CH_3$).

*4.1.3.11. 7-Chloro-2,5-dihydro-4-methyl-2-(4-nitrophenyl)-3-acetyl-1,5-benzothiazepine (**1k**).* Yield 30%; mp 161 °C; MS $[M+H^+]$: 376; $C_{18}H_{15}N_2ClO_3S$: found: C, 57.52; H, 4.07; N, 5.70; Calcd: C, 57.60; H, 4.00; N, 5.76; IR KBr (ν cm^{-1}) 1715 (C=O), 1643 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.13–8.36 (7H, m, $-C_6H_4$, $-C_6H_3$), 6.25 (1H, s, NH), 5.60 (1H, s, SCH), 2.29 (3H, s, –CO–CH_3), 1.93 (3H, s, $-CH_3$).

*4.1.3.12. 7-Chloro-2,5-dihydro-4-methyl-2-(2-nitrophenyl)-3-acetyl-1,5-benzothiazepine (**1l**).* Yield 30%; mp 167 °C; MS $[M+H^+]$: 376; $C_{18}H_{15}N_2ClO_3S$: found: C, 57.52; H, 4.07; N, 5.70; Calcd: C, 57.60; H, 4.00; N, 5.76; IR KBr (ν cm^{-1}) 1662 (C=O), 1624 (C=C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.67–7.74 (7H, m, $-C_6H_4$, $-C_6H_3$), 6.57 (1H, s, NH), 6.22 (1H, s, SCH), 2.57 (3H, s, –CO–CH_3), 2.27 (3H, s, $-CH_3$).

*4.1.3.13. 7-Chloro-2,5-dihydro-4-methyl-2-(4-chlorophenyl)-3-acetyl-1,5-benzothiazepine (**1m**).* Yield 29%; mp 139 °C; MS $[M+H^+]$: 364; $C_{18}H_{15}Cl_2NOS$: found: C, 59.31; H, 4.02; N, 3.90; Calcd: C, 59.34; H, 4.12; N, 3.85; IR KBr (ν cm^{-1}) 1716 (C═O), 1553 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.72–7.26 (7H, m, $-C_6H_4$, $-C_6H_3$), 6.17 (1H, s, NH), 5.59 (1H, s, SCH), 2.52 (3H, s, –CO–CH_3), 2.18 (3H, s, $-CH_3$).

*4.1.3.14. 7-Chloro-2,5-dihydro-4-methyl-2-(2-chlorophenyl)-3-acetyl-1,5-benzothiazepine (**1n**).* Yield 29%; mp 134 °C; MS $[M+H^+]$: 364; $C_{18}H_{15}Cl_2NOS$: found: C, 59.31; H, 4.02; N, 3.90; Calcd: C, 59.34; H, 4.12; N, 3.85; IR KBr (ν cm^{-1}) 1716 (C═O), 1553 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.53–7.32 (7H, m, $-C_6H_4$, $-C_6H_3$), 6.47 (1H, s, NH), 5.82 (1H, s, SCH), 2.54 (3H, s, –CO–CH_3), 2.14 (3H, s, $-CH_3$).

*4.1.3.15. 7-Chloro-2,5-dihydro-4-methyl-2-phenyl-3-acetyl-1,5-benzothiazepine (**1o**).* Yield 32%; mp 171 °C; MS $[M+H^+]$: 330; $C_{18}H_{16}ClNOS$: found: C, 73.31; H, 5.70; N, 4.82; Calcd: C, 73.22; H, 5.76; N, 4.75; IR KBr (ν cm^{-1}) 1708 (C═O), 1543 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.53–7.32 (8H, m, $-C_6H_5$, $-C_6H_3$), 6.38 (1H, s, NH), 5.82 (1H, s, SCH), 2.54 (3H, s, $-COCH_3$), 2.14 (3H, s, $-CH_3$).

*4.1.3.16. 7-Chloro-2,5-dihydro-4-methyl-2-(4-flurorophenyl)-3-acetyl-1,5-benzothiazepine (**1p**).* Yield 35%; mp 158 °C; MS $[M+H^+]$: 349; $C_{18}H_{15}ClFNOS$: found: C, 62.12; H, 4.29; N, 4.07; Calcd: C, 62.07; H, 4.31; N, 4.02; IR KBr (ν cm^{-1}) 1695 (C═O), 1639 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.71–7.06 (7H, m, $-C_6H_4$, $-C_6H_3$), 6.15 (1H, s, NH), 5.61 (1H, s, SCH), 2.53 (3H, s, –CO–CH_3), 2.19 (3H, s, $-CH_3$).

*4.1.3.17. 7-Chloro-2,5-dihydro-4-methyl-2-(4-bromophenyl)-3-acetyl-1,5-benzothiazepine (**1q**).* Yield 33%; mp 156 °C; MS $[M+H^+]$: 410; $C_{18}H_{15}ClBrNOS$: found: C, 52.78; H, 3.60; N, 3.38; Calcd: C, 52.81; H, 3.67; N, 3.42; IR KBr (ν cm^{-1}) 1718 (C═O), 1639 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.74–7.07 (7H, m, $-C_6H_4$, $-C_6H_3$), 6.29 (1H, s, NH), 5.63 (1H, s, SCH), 2.56 (3H, s, –CO–CH_3), 2.18 (3H, s, $-CH_3$).

*4.1.3.18. 7-Chloro-2,5-dihydro-4-methyl-2-(2,4-dichlorophenyl)-3-acetyl-1,5-benzothiazepine (**1r**).* Yield 27%; mp 152 °C; MS $[M+H^+]$: 398; $C_{18}H_{14}Cl_3NOS$: found: C, 54.31; H, 3.56; N, 3.40; Calcd: C, 54.20; H, 3.51; N, 3.51; IR KBr (ν cm^{-1}) 1715 (C═O), 1543 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.47–7.33 (6H, m, $-C_6H_3$), 6.17 (1H, s, NH), 5.76 (1H, s, SCH), 2.53 (3H, s, –CO–CH_3), 2.13 (3H, s, $-CH_3$).

*4.2. Synthesis of 2,5/2,3-dihydro-4-methyl-2-aryl-3-ethoxycarbonyl-1,5-benzothiazepine (**2a**–**e**)*

The procedure for the synthesis of **2** was repeated in Section 4.1. Compounds **2** were obtained in a similar manner to that used for compounds **1**, by replacing reactant 2,4-pentandione with ethyl acetoacetate. The products were crystallized from methanol.

*4.2.1. 2,5-Dihydro-4-methyl-2-(4-methylphenyl)-3-ethoxycarbonyl-1,5-benzothiazepine (**2a**)*

Yield 33%; mp 155 °C; MS $[M+H^+]$: 340; $C_{20}H_{21}NO_2S$: found: C, 63.28; H, 5.16; N, 3.76; Calcd: C, 63.41; H, 5.04; N, 3.89; IR KBr (ν cm^{-1}) 1730 (C═O), 1632 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.71–7.26 (8H, m, $-C_6H_4$,), 6.19 (1H, s, NH), 5.89 (1H, s, SCH), 4.01–4.13 (2H, q, $J = 5.8$ Hz, $-COOCH_2$), 2.61 (3H, s, –C═C–CH_3), 2.16 (3H, s, –PhCH_3), 1.17–1.23 (3H, t, $J = 5.8$ Hz, $-CH_2CH_3$).

*4.2.2. 2,5-Dihydro-4-methyl-2-(4-flurorophenyl)-3-ethoxycarbonyl-1,5-benzothiazepine (**2b**)*

Yield 23%; mp 136 °C; MS $[M+H^+]$: 430; $C_{19}H_{18}FNO_2S$: found: C, 66.32; H, 5.40; N, 3.97; Calcd: C, 66.45; H, 5.28; N, 3.4.08; IR KBr (ν cm^{-1}) 1735 (C═O), 1620 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.68–7.05 (8H, m, $-C_6H_4$,), 6.25 (1H, s, NH), 5.89 (1H, s, SCH), 4.01–4.13 (2H, q, $J = 5.8$ Hz, $-COOCH_2$), 2.60 (3H, s, –C═C–CH_3), 1.16–1.19 (3H, t, $J = 5.8$ Hz, $-CH_2CH_3$).

*4.2.3. 2,5-Dihydro-4-methyl-2-(4-nitrophenyl)-3-ethoxycarbonyl-1,5-benzothiazepine (**2c**)*

Yield 20%; mp 152 °C; MS $[M+H^+]$: 371; $C_{19}H_{18}N_2O_4S$: found: C, 61.48; H, 4.79; N, 7.53; Calcd: C, 61.62; H, 4.87; N, 7.57; IR KBr (ν cm^{-1}) 1715 (C═O), 1635 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.72–7.89 (8H, m, $-C_6H_4$), 6.33 (1H, s, NH), 5.96 (1H, s, SCH) , 4.04–4.12 (2H, q, $J = 5.6$ Hz, $-COOCH_2$), 2.65 (3H, s, –C═C–CH_3), 1.16–1.19 (3H, t, $J = 5.6$ Hz, $-CH_2CH_3$).

*4.2.4. 2,5-Dihydro-4-methyl-2-(2-chlorophenyl)-ethoxycarbonyl-1,5-benzothiazepine (**2d**)*

Yield 23%; mp 135 °C; MS $[M+H^+]$: 361; $C_{19}H_{18}ClNO_2S$: found: C, 63.24; H, 5.06; N, 3.82; Calcd: C, 63.33; H, 5.00; N, 3.89; IR KBr (ν cm^{-1}) 1715 (C═O), 1635 (C═C); ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 6.50–7.28 (8H, m, $-C_6H_4$), 6.29 (1H, s, NH), 6.14 (1H, s, SCH), 3.96–4.08 (2H, q, $J = 5.6$ Hz, $-COOCH_2$), 2.63 (3H, s, –C═C–CH_3), 1.13–1.16 (3H, t, $J = 5.6$ Hz, $-CH_2CH_3$).

*4.2.5. 2,3-Dihydro-4-methyl-2-(4-hydroxylpheny)-3-ethoxycarbonyl-1,5-benzothiazepine (**2e**)*

Yield 20%; mp 161 °C; MS $[M+H^+]$: 342; $C_{19}H_{19}NO_3S$: found: C, 66.72; H, 5.75; N, 3.98; Calcd: C, 66.86; H, 5.57; N, 4.10; IR KBr (ν cm^{-1}) 1718 (C═O), 1637 (C═N); ^1H NMR (400 MHz, DMSO-d_6), δ (ppm): 6.63–7.52 (8H, m, $-C_6H_4$), 5.20–5.23 (1H, d, $J = 9.2$ Hz SCH), 4.04 (1H, s, –OH), 3.90–3.92 (2H, q, $J = 5.8$ Hz, $-COOCH_2$), 3.70–3.72 (1H, d, $J = 9.2$ Hz, CH–COO), 2.29 (3H, s, –N═C–CH_3), 0.96–0.99 (3H, t, $J = 5.8$ Hz, $-CH_2CH_3$).

4.3. Biological evaluation

Assay of antimicrobial activity *in vitro*. The synthesized compounds were tested for their antimicrobial (antibacterial and antifungal) activities by standardized disk diffusion methods. The assayed collection included the following microorganisms: *C. albicans* (ATCC 10231), *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 26069) and *E. coli*. (ATCC 44752).

In the disc-diffusion method, sterile paper discs (Φ 6 mm) impregnated with compounds dissolved in DMSO at concentrations of 12.5, 50, 100 and 200 μg/disc were used. Discs containing DMSO were used as controls. Paper discs impregnated with a solution of the compound tested were placed on the surface of the media inoculated with the microorganisms. The plates were incubated at 37 °C for 24 h for culture of microorganisms. After incubation, the growth inhibition zones around the discs were observed, which indicated that the examined compound inhibited the growth of microorganisms. Each assay in this experiment was repeated three times.

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