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One Pot Synthesis and Antimicrobial Evaluation of 8-Substituted-2,5-dihydro-2-(2-nitrophenyl/4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines

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The reaction of an α,β-unsaturated heterocyclic ketone, 3-(2-nitrophenyl/4-nitrophenyl)-1-(2-thienyl)-2-propenone (**3a-b**) with 5-substituted-2-aminobenzenethiols (**4a-d**) with substituent -CH_3, -Cl, -F and -Br was carried out in dry methanol containing trifluoroacetic acid (TFA) in catalytic amount as well as by swirling for 20 min in diethyl ether at room temperature. The yields of the products, 8-substituted-2,5-dihydro-2-(2-nitrophenyl/4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (**5a-h**), ranged from 60% to 75% by first method and 65% to 85% by second method. The synthesized compounds have been characterized by micro-estimation of C, H, N and ^{1}H NMR, ^{13}C NMR as well as mass spectral studies. All the synthesized compounds are tested for biological efficacy against, Gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli* and fungi, *Candida albicans*. All of the compounds demonstrated effective antibacterial and antifungal activities.

Keywords: 1,5-Benzothiazepines, α,β-Unsaturated ketone, Heterocyclic moiety, Antimicrobial activity.

INTRODUCTION

1,5-Benzothiazepine derivatives have shown promising antifungal, antibacterial and anticancer efficacies [1,2]. It has been found that 1,5-benzothiazepines containing different heterocyclic groups at position 2,3,4 are physiologically active shield structures and has numerous therapeutic uses [3-5] as coronary vasodialation, Ca^{2+} channel antagonist, antidepressant, analgesic, antihypertensive, amnesia and antidementia and insecticidal activities.

A review of the relevant literature prompted our interest in synthesising some of the 1,5-benzothiazepine derivatives that are difficult to obtain. From the various reported synthetic methods, most common synthetic method for 1,5-benzothiazepine scaffold includes the reaction of substituted benzenethiol with variously substituted benzalacetophenone in dry ether and dry HCl gas [6,7] as well as through ultrasonic irradiation of precursors as improved synthetic process [8].

In the current study, it is thought, worthwhile, to synthesize, characterize and pharmacologically evaluate certain novel 1,5-benzothiazepine moieties by reacting one of the precursor, 5-substituted-2-aminobenzenethiols where substituent being -CH_3, -Cl, -F and -Br with other precursor having α,β-unsaturated carbonyl systems to give 8-substituted-2,5-dihydro-2-(2-nitrophenyl/4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (**5a-h**) in both acidic and basic medium *i.e.* (i) dry methanol containing trifluoroacetic acid and (ii) dry diethyl ether.

EXPERIMENTAL

Melting points were determined by NISCO melting point apparatus and in open capillaries in silicon bath and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G coated on aluminium sheet using benzene:ethanol:aq. ammonia (7:2:1 upper layer) as solvent system for first method and ethyl acetate:petroleum ether (20:80) as solvent system for second method and inspected in UV cabinet. Microestimations for carbon, hydrogen and nitrogen were carried out on Euro Vector E 3000 elemental analyzer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance 400/AvIII HD-300 (FT NMR) instrument using $CDCl_3$ as solvent and TMS as internal standard. The mass spectra were recorded on water alliance e2695/HPCL-TQD mass spectrometer. All the spectral and elemental analysis were

carried out at the Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute, Lucknow, India.

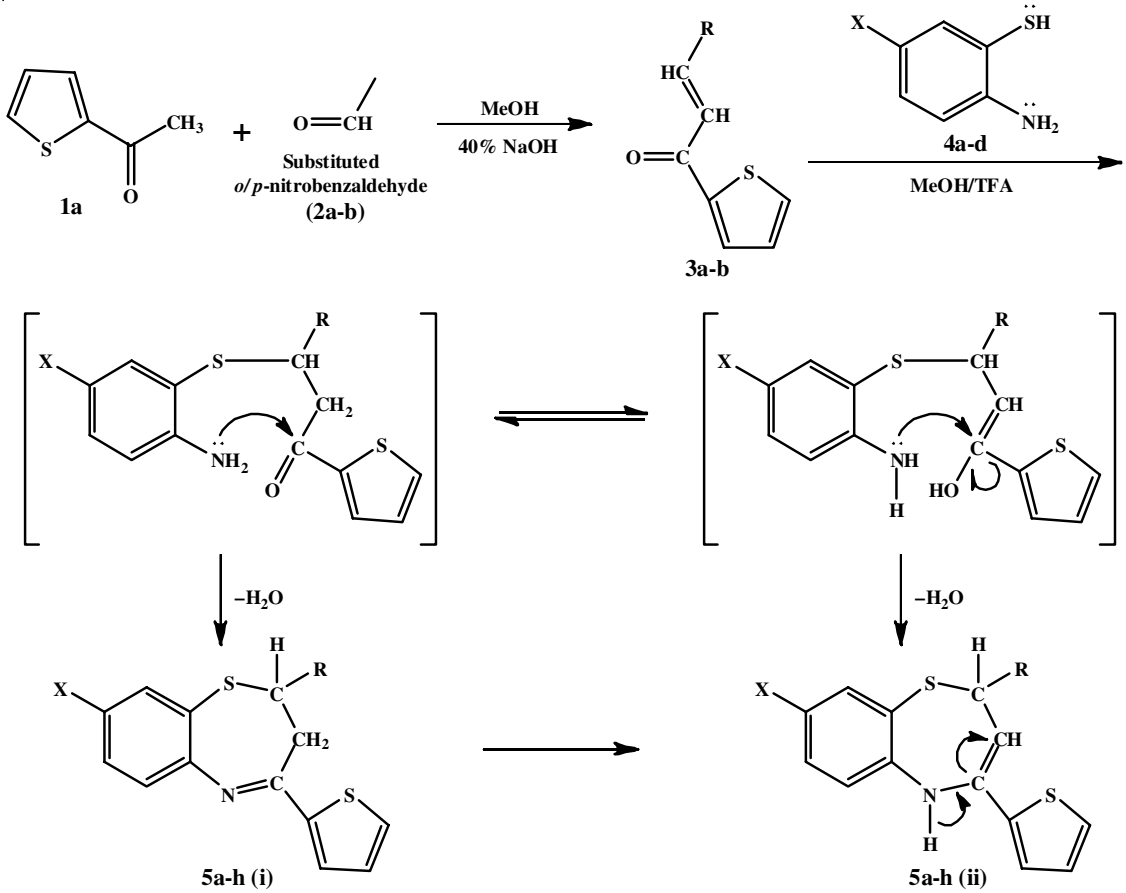
Synthesis of 3-(2-nitrophenyl)-1-(2-thienyl)-2-propenone (3a): The chalcone analogue, 3-(2-nitrophenyl)-1-(2-thienyl)-2-propenone (**3a**) was synthesized by reacting equimolar quantities of 2-acetylthiophene (**1a**) and *o*-nitrobenzaldehyde (**2a**) dissolved in methanol and stirred on magnetic stirrer at room temperature. On gradual addition of 40% NaOH on magnetic stirrer, an orange red solid product was precipitated out. This crude product was purified by recrystallization from methanol to obtain the yellow crystals of 3-(2-nitrophenyl)-1-(2-thienyl)-2-propenone (**3a**) (**Scheme-I**). Homogeneity of the compound was checked by TLC. Yield 87%, m.p.: 128-130 ºC.

Synthesis of 3-(4-nitrophenyl)-1-(2-thienyl)-2-propenone (3b): The chalcone analogue, 3-(4-nitrophenyl)-1-(2-thienyl)-2-propenone (**3b**) was synthesized by reacting equimolar quantities of 2-acetylthiophene (**1a**) and *p*-nitrobenzaldehyde (**2b**) dissolved in methanol and stirred on magnetic stirrer at room temperature. On gradual addition of 40% NaOH on magnetic stirrer, an orange red solid product was precipitated out. The crude product was purified by recrystallization from methanol to obtain the yellow crystals of 3-(4-nitrophenyl)-1-(2-thienyl)-2-propenone (**3b**) (**Scheme-I**). Homogeneity of the compound was checked by TLC. Yield 84%, m.p.: 122-124 ºC.

The 5-substituted-2-aminobenzenethiols were synthesized by literature methods [9,10].

Synthesis of 8-methyl-2,5-dihydro-2-(2-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5a): The prepared chalcone 3-(2-nitrophenyl)-1-(2-thienyl)-2-propenone (**3a**) (0.01 mol) was treated with freshly prepared 5-methyl-2-aminobenzenethiol (**4a**) (0.01 mol) in methanol containing TFA in catalytic amount under reflux for 5 h as well as in diethyl ether by swirling for 20 min at room temperature. A crude solid product was obtained which on purification by recrystallization from methanol gave 8-methyl-2,5-dihydro-2-(2-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (**5a**) (**Scheme-I**). Homogeneity of the compound was checked by TLC. Yield: 70%, R_f: 0.7, m.p.: 101-103 ºC. ^{1}H NMR ($CDCl_3$): δ 2.38 (s, 3H), 4.32 (br, 1H), 6.54 (d, 1H, *J* = 8 Hz), 7.13 (d, 1H, *J* = 8 Hz), 6.82-8.85 (m, 10H). Anal. calcd. (found) % of $C_{20}H_{16}O_2S_2N_2$ (*m.w.* 380.3): C, 62.75 (62.16); H, 4.25 (4.20); N, 7.12 (7.36). MS: *m/z* 380 $[M]^+$.

8-Chloro-2,5-dihydro-2-(2-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5b): Yield: 67% (methanol), 74% (Et_2O), R_f: 0.7, m.p.: 98-99 ºC. ^{1}H NMR ($CDCl_3$): δ 4.39 (br, 1H), 6.62 (d, 1H, *J* = 8 Hz), 6.54 (d, 1H, *J* = 8 Hz), 7.25-8.83 (m, 10H). Anal. calcd. (found) % of $C_{19}H_{13}O_2S_2N_2Cl$ (*m.w.* 400.8): C, 57.32 (56.93) ; H, 4.10 (3.24); N, 7.02 (6.98). MS: *m/z* 400 $[M]^+$.



Compd.	5a	5b	5c	5d	5e	5f	5g	5h
X	CH_3	Cl	F	Br	CH_3	Cl	F	Br
R	2-Nitrophenyl	2-Nitrophenyl	2-Nitrophenyl	2-Nitrophenyl	4-Nitrophenyl	4-Nitrophenyl	4-Nitrophenyl	4-Nitrophenyl

Scheme-I

8-Fluoro-2,5-dihydro-2-(2-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5c): Yield: 63% (methanol), 75% (Et_2O), R_f: 0.72, m.p.: 80-81 ºC. ^{1}H NMR ($CDCl_3$): δ 4.38 (br, 1H), 6.57 (d, 1H, *J* = 8 Hz), 7.11 (d, 1H, *J* = 8 Hz), 6.63-8.29 (m, 10H). Anal. calcd. (found) % of $C_{19}H_{13}O_2S_2N_2F$ (*m.w.* 384.3): C, 61.99 (62.16); H, 4.18 (4.20); N, 7.33 (7.36). MS: *m/z* 384 $[M]^+$.

8-Bromo-2,5-dihydro-2-(2-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5d): Yield: 69% (methanol), 72% (Et_2O), R_f: 0.81, m.p.: 83-84 ºC. ^{1}H NMR ($CDCl_3$): δ 4.33 (br, 1H), 6.64 (d, 1H, *J* = 8 Hz), 7.10 (d, 1H, *J* = 8 Hz), 7.13-8.22 (m, 10H). Anal. calcd. (found) % of $C_{19}H_{13}O_2S_2N_2Br$ (*m.w.* 445.2): C, 52.12 (51.25); H, 2.89 (2.92); N, 6.12 (6.29). MS: *m/z* 445 $[M]^+$.

8-Methyl-2,5-dihydro-2-(4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5e): Yield: 66% (methanol), 68% (Et_2O), R_f: 0.88, m.p.: 104-106 ºC. ^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H), 3.59 (br, 1H), 6.72 (d, 1H, *J* = 8 Hz), 7.34 (d, 1H, *J* = 8 Hz), 7.08-8.40 (m, 10H). Anal. calcd. (found) % of $C_{20}H_{16}O_2S_2N_2$ (*m.w.* 380.3): C, 62.22 (63.16); H, 4.22 (4.20); N, 7.29 (7.36). MS: *m/z* 380 $[M]^+$.

8-Chloro-2,5-dihydro-2-(4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5f): Yield: 66% (methanol), 84% (Et_2O), R_f: 0.9, m.p.: 128-130 ºC. ^{1}H NMR ($CDCl_3$): 4.39 (br, 1H), 7.14 (d, 1H, *J* = 8 Hz), 7.26 (d, 1H, *J* = 8 Hz), 7.12-8.29 (m, 10H). Anal. calcd. (found) % of $C_{19}H_{13}O_2S_2N_2Cl$ (*m.w.* 400.8): C, 56.33 (56.93); H, 4.09 (3.24); N, 6.75 (6.98). MS: *m/z* 400 $[M]^+$.

8-Fluoro2,5-dihydro-2-(4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5g): Yield: 71% (methanol), 78% (Et_2O), R_f: 0.88, m.p.: 103-105 ºC. ^{1}H NMR ($CDCl_3$): δ 4.32 (br, 1H), 6.58 (d, 1H, *J* = 8 Hz), 7.96 (d, 1H, *J* = 8 Hz), 6.99-8.40 (m, 10H). Anal. calcd. (found) % of $C_{19}H_{13}O_2S_2N_2F$ (*m.w.* 384.3): C, 58.86 (59.37); H, 3.45 (3.38); N, 7.13 (7.28). MS: *m/z* 384 $[M]^+$.

8-Bromo-2,5-dihydro-2-(4-nitrophenyl)-4-(2-thienyl)-1,5-benzothiazepines (5h): Yield: 74% (methanol), 68% (Et_2O), R_f: 0.91, m.p.: 120-122 ºC. ^{1}H NMR ($CDCl_3$): δ 4.33 (br, 1H), 6.64 (d, 1H, *J* = 8 Hz), 7.51 (d, 1H, *J* = 8 Hz), 6.54-8.29 (m, 10H). Anal. calcd. (found) % of $C_{19}H_{13}O_2S_2N_2Br$ (*m.w.* 445.2): C, 52.20 (51.25); H, 2.98 (2.92); N, 6.14 (6.29). MS: *m/z* 445 $[M]^+$.

Similar methods were employed to synthesize compounds **5b-h**. However, the reaction that needed to take place in the case of compound **5d** required more than 7 h of reflux.

Antimicrobial activity: The disc diffusion method [11] was used to assess the relative antibacterial and antifungal activity of all the synthesized compounds **5a-h** against the Gram-positive bacteria *Staphylococcus aureus*, the Gram-negative bacteria *Escherichia coli* and fungi, *Candida albicans* at concentration of 100 μg/disc. Erythromycin, amikacin and fluconazole were employed as reference compounds for Gram-positive, Gram-negative and fungus, respectively to assess the relative activity. The relative activities of the test compounds **5a-h** were calculated as an activity index and zones of inhibition displayed by the reference and test compounds were assessed.

$$\text{Activity index} = \frac{\text{Zone of inhibition exhibited by test compound}}{\text{Zone of inhibition exhibited by the reference compound}}$$

RESULTS AND DISCUSSION

The chalcone analogue, unsaturated heterocyclic ketone, 3-(2-nitrophenyl/4-nitrophenyl)-1-(2-thienyl)-2-propenone (**3a-b**) was synthesized by reaction of 2-acetylthiophene (**1a**) with 2-nitro/4-nitrobenzaldehyde (**2a-b**). Equimolar quantities of 3-(2-nitrophenyl/4-nitrophenyl)-1-(2-thienyl)-2-propenone (**3a-b**) and 5-substituted-2-aminobenzenethiols reacted in acidic and basic conditions. According to existing knowledge [12,13], the mechanistic pathway of the reactions of compounds **3a-b** and **4a-d** were started by the nucleophilic attack of sulphydryl electrons on the β-carbon atom of α,β-unsaturated carbonyl system. The vinyl-carbonyl conjugation provided the β-carbon atom of α,β-unsaturated carbonyl system electrophilic, so that when substituents are present in an α,β-unsaturated ketone, only the nucleophilic addition of a mercapto group to the β-carbon atom occurs, followed by condensation of the carbonyl group with the aromatic primary amine to give a seven-membered ring system. This results in the generation of the Michael adduct intermediate and the cyclized products **5a-h**.

By microestimating C, H and N and conducting ^{1}H and ^{13}C NMR spectral analyses, the final product's structure was determined. The predicted molecular masses based on the mass spectra of **5b-h** for the molecular ion peaks $[M]^+$, $[M+2]^+$, corresponds to the calculated molecular mass: 400, 402; 384, 386; 445, 447; 380, 382; 400, 402; 384, 386 and 445, 447, respectively (Table-1).

The ^{1}H NMR spectra showed a broad one proton absorption in the region of δ 3.59-4.39 ppm due to NH. Additionally, the presence of two doublets, integrating for one proton each, at δ 6.54-7.14 and δ 6.54-7.96 ppm support the formation of 2,5-dihydro derivatives, in preference to 2,3-dihydro tautomers.

TABLE-1
CHARACTERISTIC ^{1}H NMR ($CDCl_3$, δ VALUES IN ppm, *J* IN Hz) SIGNALS OF COMPOUNDS

Compound	N-H	C-8-XH	C-3-H	C-2-H	Ar-H
5a	4.32 (br, 1H)	2.38 (s, 3H)	7.13 (d, 1H, *J* = 8)	6.54 (d, 1H, *J* = 8)	6.82-8.85 (m, 10H)
5b	4.39 (br, 1H)	–	6.54 (d, 1H, *J* = 8)	6.62 (d, 1H, *J* = 8)	7.25-8.83 (m, 10H)
5c	4.38 (br, 1H)	–	7.11 (d, 1H, *J* = 8)	6.57 (d, 1H, *J* = 8)	6.63-8.29 (m, 10H)
5d	4.33 (br, 1H)	–	7.10 (d, 1H, *J* = 8)	6.64 (d, 1H, *J* = 8)	7.13-8.22 (m, 10H)
5e	3.59 (br, 1H)	2.50 (s, 3H)	7.34 (d, 1H, *J* = 8)	6.72 (d, 1H, *J* = 8)	7.08-8.40 (m, 10H)
5f	4.39 (br, 1H)	–	7.26 (d, 1H, *J* = 8)	7.14 (d, 1H, *J* = 8)	7.12-8.29 (m, 10H)
5g	4.32 (br, 1H)	–	7.96 (d, 1H, *J* = 8)	6.58 (d, 1H, *J* = 8)	6.99-8.40 (m, 10H)
5h	4.33 (br, 1H)	–	7.51 (d, 1H, *J* = 8)	6.64 (d, 1H, *J* = 8)	6.54-8.29 (m, 10H)

TABLE-2
ANTIMICROBIAL ACTIVITY OF COMPOUNDS

Compound	Zone of inhibition (mm)								
	Bacteria						Fungi		
	Gram-positive			Gram-negative					
	Staphylococcus aureus			*Escherichia coli*			*Candida albicans*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
5a	11 (0.91)	11 (0.91)	10 (0.83)	10 (0.83)	10 (0.83)	9 (0.75)	16 (1.14)	16 (1.14)	14 (1.00)
5b	13 (1.08)	13 (1.08)	12 (1.00)	11 (0.91)	11 (0.91)	10 (0.83)	17 (1.21)	17 (1.21)	16 (1.14)
5c	12 (1.00)	12 (1.00)	10 (0.83)	10 (0.83)	10 (0.83)	8 (0.66)	11 (0.78)	10 (0.71)	10 (0.71)
5d	11 (0.91)	11 (0.91)	10 (0.83)	9 (0.75)	9 (0.75)	8 (0.66)	9 (0.64)	8 (0.57)	8 (0.57)
5e	10 (0.83)	11 (0.91)	11 (0.91)	9 (0.75)	11 (0.91)	9 (0.75)	15 (1.07)	16 (1.14)	16 (1.14)
5f	12 (1.00)	13 (1.08)	13 (1.08)	10 (0.83)	11 (0.91)	11 (0.91)	16 (1.14)	17 (1.21)	17 (1.21)
5g	10 (0.83)	11 (0.91)	11 (0.91)	9 (0.75)	10 (0.83)	9 (0.75)	11 (0.78)	10 (0.71)	9 (0.64)
5h	10 (0.83)	11 (0.91)	11 (0.91)	8 (0.66)	9 (0.75)	9 (0.75)	9 (0.64)	9 (0.64)	8 (0.57)
Erythromycin	12			–			–		
Amikacin	–			12			–		
Fluconazole	–			–			14		

Activity index is represented by values in parenthesis.

Antimicrobial activity: On incubation for a period of 24, 48 and 72 h, all the synthesized compounds **5a-h** have shown moderate to good activity indexes, ranging from 0.83 to 1.08 mm, against Gram-positive bacteria, *S. aureus* and 0.66 to 0.91 mm against Gram-negative bacteria, *E. coli*. It was found that compounds containing chloro and methyl substituents were slightly more effective against *S. aureus* and moderately effective against *E. coli*. On incubation for 24 to 72 h, all the compounds have shown good effectiveness against the fungus *C. albicans*. Chloro compound has demonstrated reasonably effective antifungal action with activity index 1.21. At 24 h and 48 h of incubation period, the biological activities were found comparable while at 72 h, both antibacterial and antifungal activity index were decreased (Table-2).

Conclusion

The chemistry of benzothiazepines has undergone significant changes in the last decade, leading to numerous novel and fascinating syntheses for the construction of this scaffold. It could be concluded that synthesis of this scaffold in diethyl ether is better approach as it required lesser time with better yield as well as it was also found that chloro substituted benzothiazepines showed more pronounced antimicrobial activity than other derivatives.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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