1,5-Benzothiazepine-2-carboxylic Acids: Synthesis and Bioactivity Screening

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ABSTRACT

A set of 8-substituted-2,3-dihydro-1,5-benzothazepine-2-carboxylic acids were created through one-pot reactions involving six different 5-substituted-2-amino benzenethiols, with substituents including methoxy, ethoxy, methyl, fluoro, chloro, and bromo; and substituted β-benzoyl acrylic acid in ethanol, using trifluoroacetic acid as a catalyst, resulting in yields between 62-74%. The structures of the synthesized compounds were identified using FT-IR, NMR, and elemental analysis techniques. The antimicrobial properties of these compounds were assessed utilizing the Agar Well Diffusion Method against both gram-positive and gram-negative bacteria, especially *Staphylococcus aureus* and *Escherichia coli*, as well as *Pseudomonas aeruginosa*, and the fungus *Candida albicans*.

INDEX TERMS

β-(4-hydroxy benzoyl) acrylic acid, 1,5-benzothazepine-2-carboxylic acids, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

INTRODUCTION

The 1,5-benzothiazepines are heterocyclic compounds that contain nitrogen and sulfur comprising the seven membered ring, demonstrating significant pharmacological properties such as anti-HIV effects, calcium (II) channel antagonism [1], anticancer activity [2] and antimicrobial properties [3]. The research findings have sparked interest in developing innovative techniques for synthesizing novel 1,5-benzothiazepines. The initial 1,5-benzothiazepine compound, used chemotherapeutically for its cardiovascular properties, is diltiazem, followed by clentiazem, having a chloro substituent in the fused benzene ring, for their advantageous cardiovascular effects [4]. The existence of a carboxyl group in the nucleus of 1,5-benzothiazepines is significant, as it plays a crucial role in drug design and can be utilized to create further derivatives that exhibit beneficial bioactivity.

Several 1,5-benzothiazepine derivatives featuring an unbound carboxylic group have been documented to exhibit biological activities [5-6]. The earlier synthesis of 2-substituted -2,3-dihydro-4-[4-aryl] 1,5-benzothiazepine, which lacks substituents at position -8, has been documented [7]. Additionally, β-benzoyl acrylic acid has been noted [8-9] for its ability to inhibit phospholipase derived from snake venom and procaine pancreas; it also exhibits antibacterial properties [10] and anti-proliferative effects [11] against human cervix carcinoma. This research presents the synthesis of a novel series of 8-substituted -1,5-benzothiazepine-2-carboxylic acids, featuring a substituted aryl group at the 4-position, as well as their antimicrobial activity evaluations.

MATERIAL AND METHOD

The synthesis of 8-substituted -4-(4-hydroxy phenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids was achieved through the reaction of 5-substituted-2-aminobenzenethiols [12] with β-(4-hydroxy benzoyl) acrylic acid [13] in an acidic environment.

Standard procedure for synthesizing 8-substituted -4-(4-hydroxyphenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids, (8-13):

Equimolar amounts of β-keto acid, β-(4-hydroxy benzoyl) acrylic acid (**1**), and 5-substituted-2-aminobenzenethiols (**2-7**) were dissolved in anhydrous ethanol and combined. To this reaction mixture, 1 ml of trifluoroacetic acid was added, and the mixture was refluxed for a duration of 3-4 hours. The resulting mixture was then concentrated under reduced pressure. The crude solid obtained was purified by crystallization from dry ethanol, yielding six new compounds, namely, 8-substituted -4-(4-hydroxy phenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids (**8-13**).

The progress of reactions was monitored through TLC with a solvent system of benzene, ethanol, and 50% aqueous ammonia (7:2:1) applied on silica gel 'G' coated glass plates. The melting points reported are uncorrected. The IR spectra were recorded using KBr pellets on a Perkin Elmer Spectrum version 10.4.00 IR Spectrophotometer. NMR spectra were obtained using a JNM-ECS400 (DELTA2-NMR) spectrometer with DMSO-D6 as the solvent and TMS as the internal standard. Mass spectra were recorded with a JEOL -Accu TOF JMS-T100LC Mass spectrometer that featured a DART source. Dry helium was utilized at 4 LPM flow rate. Elemental analysis for carbon, hydrogen, nitrogen, and sulfur was performed in the Materials Research Centre at MNIT, Jaipur.

ANTIMICROBIAL ACTIVITY

All synthesized compounds (8-13) were evaluated for their antibacterial effectiveness against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, as well as against the fungus *Candida albicans* using the Agar Well Method [14]. The findings were compared to those of reference compounds Streptomycin (for *S. aureus*, *E. coli*, and *P. aeruginosa*) to assess antibacterial efficacy and Ketoconazole (for C. albicans) to determine antifungal effectiveness. The zones of inhibition produced by both the reference and test compounds were recorded.

RESULT AND DISCUSSION

In research concerning the interaction of substituted 2-amino-benzenethiol with α, β-unsaturated acids, it has been documented [15-16] that the formation of 8-substituted-4-(4-hydroxyphenyl)-2,3-dihydro-1,5-benzothiazepine-2-carboxylic acids was achieved through a one-step reaction. The initiation of this reaction is thought to occur via the nucleophilic attack of sulfur-containing electrons from 5-substituted-2-aminobenzenethiols on the activated β-carbon of the α, β-unsaturated β-keto acids, resulting in the formation of Michael adduct-type intermediates in the initial step, which subsequently undergo dehydration and cyclization to yield cyclized seven-membered heterocyclic compounds in the following step. A flowchart illustrating the progression of this reaction is presented in **Scheme-I**.

IR SPECTRAL STUDIES

The infrared spectra of 8-substituted -4-(4-hydroxy phenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids (**8-13**) displayed significant absorptions between 1695 and 1675 cm^{-1}, likely corresponding to the carbonyl stretching frequency. The observation of this band at a lower frequency suggests that the carbonyl group is involved in conjugation with unsaturation. A broad absorption noted in the range of 3450 to 3390 cm^{-1} indicates O-H stretching vibrations, along with a broad, intense absorption observed between 1620 and 1610 cm^{-1} in all spectra, confirms the presence of C=N, as previously identified [17] in 1,5-benzothiazepines. Compounds **11-13** exhibited absorptions corresponding to halogens within their specific regions.

^{1}HNMR SPECTRA

The ^{1}H NMR spectra for all the compounds exhibited a singlet at δ 9.01-9.98 ppm, which can be attributed to the protons of carboxylic acids. A broad singlet observed in the region δ 8.18-8.38 may be assigned to the hydroxy proton attached to the aromatic ring. The spectra also revealed a series of three doublets following the ABX pattern. Each of the three double doublets observed in the ranges δ 3.19-3.24 ppm, δ 3.27-3.54 ppm, and δ 4.19-4.26 ppm were noted to integrate for one proton each. In the region δ 6.80-7.98 ppm, a multiplicity (m, 7H) was detected as multiplets related to the aromatic protons.

The signals for methyl, methoxy, and ethoxy protons were also detected in their respective regions, as shown in Table-2.

MASS SPECTRAL ANALYSES

The mass spectra of compound 12 displayed a cluster of molecular ion peaks at m/z $[M]^+$, $[M+2]^+$ and $[M+4]^+$, specifically at 333, 335, and 337, which corresponds to the molecular weight of the product. The intensity of the $[M+2]^+$ peak was observed to be approximately one-third that of the $[M]^+$ peak, indicating the presence of chlorine in the compound. The mass spectra of compound 13 revealed molecular ion peaks at m/z $[M]^+$ and $[M+2]^+$ at 378 and 380, respectively. The intensity of the $[M+2]^+$ peak was found to be nearly equal to that of the $[M]^+$ peak, confirming the presence of bromine. The results from the elemental analyses were deemed satisfactory, falling within acceptable limits of error.

ANTIMICROBIAL ACTIVITY

The majority of the synthesized compounds demonstrated effectiveness against the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The compounds having methoxy, ethoxy or ethyl substituents in the fused benzene ring showed better antimicrobial activity.

In comparison to the reference compound, most of the test compounds demonstrated reduced effectiveness against the fungus *Candida albicans*.

CONCLUSION

The desired compounds were synthesized through single pot reactions in an acidic environment. A number of the compounds exhibited antibacterial and antifungal properties against all tested microorganisms. Compound 10, which contains an 8-methyl group, demonstrated significant activity against the antifungal pathogen *Candida albicans*.

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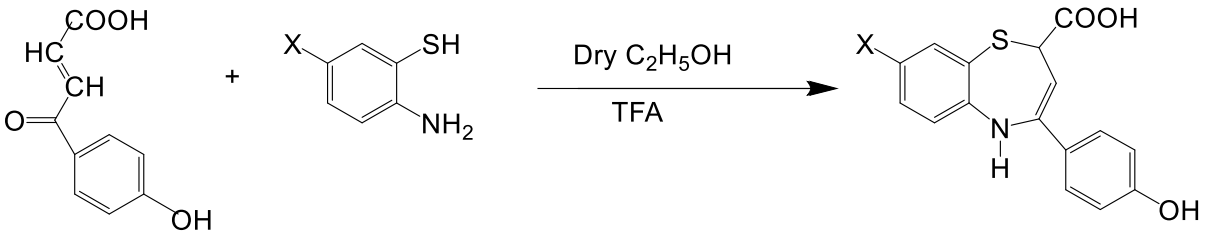
Scheme-1



1 2-7 **8-13**

Comp. No.	2,8	3,9	4,10	5,11	6,12	7,13
X	OCH_3	OC_2H_5	CH_3	F	Cl	Br

Table 1. Physical constant data of 8-substituted -4-(4-hydroxy phenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids (8-13):

C. No.	M.P. (°C)	Yield (%)	R_F	M.F. (M.W.)	Elemental Analyses (%) Found (Calculated)			
					C	H	N	S
8	90-92	65	0.68	$C_{17}H_{15}SNO_4$ (329)	61.96	4.55	4.22	9.71
9	130-132	62	0.66	$C_{18}H_{17}SNO_4$ (343)	62.94	4.96	4.04	9.33
10	78-80	72	0.70	$C_{17}H_{15}SNO_3$ (313)	65.13	4.79	4.43	10.20
11	95-98	66	0.62	$C_{16}H_{12}SNO_3F$ (317)	60.52	3.78	4.38	10.07
12	235-240	74	0.72	$C_{16}H_{12}SNO_3Cl$ (333)	57.55	3.58	4.18	9.58
13	165-170	68	0.65	$C_{16}H_{12}SNO_3Br$ (378)	50.78	3.17	3.67	8.45

Table 2. ^{1}H-NMR data of 8-substituted -4-(4-hydroxy phenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids (8-13):

C. No,	C_3-H_A (dd; J_{AB} 15, J_{AX} 8; 1H) (δ, ppm)	C_3-H_B (dd; J_{AB} 15, J_{BX} 8; 1H) (δ, ppm)	C_2-H_X (dd; J_{AX} 15, J_{BX} 8; 1H) (δ, ppm)	C_8-X (δ, ppm)	-OH (brs, 1H) (δ, ppm)	COOH (s, 1H) (δ, ppm)	Aromatic Protons (7H, m) (δ, ppm)
8	3.22	3.52	4.22	3.78 (s, 3H)	8.26	9.82	6.80-7.94
9	3.24	3.54	4.26	1.29 (t, 3H) 4.13 (q, 2H)	8.22	9.85	6.82-7.98
10	3.19	3.27	4.19	2.26 (s, 3H)	8.18	9.88	6.83-7.92
11	3.20	3.33	4.24	-	8.28	9.94	6.86-7.90
12	3.22	3.40	4.20	-	8.32	9.01	6.89-7.96
13	3.18	3.46	4.26	-	8.37	9.98	6.82-7.91

Table 3. Antimicrobial activity data of 8-substituted -4-(4-hydroxy phenyl) -2,3-dihydro -1,5-benzothiazepine -2-carboxylic acids (8-13):

Comp./ Conc.	Zone of Inhibition (in mm)/ conc. in (µg/ml)															
	Antibacterial activity												Antifungal activity			
	E. coli				*P. aeruginosa*				*S. aureus*				*C. albicans*			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
8	9	11	13	15	10	11	12	14	8	10	12	14	9	10	11	13
9	8	10	11	13	9	12	13	15	10	11	13	15	8	9	11	14
10	7	9	12	14	12	13	14	15	9	12	13	14	8	12	13	16
11	9	10	11	13	9	10	12	14	10	11	12	13	9	10	12	14
12	9	13	15	16	7	8	10	11	7	9	10	12	7	8	10	12
13	10	11	12	14	8	9	11	13	8	10	11	13	8	9	11	12

Zone of Inhibition of Streptomycin against *S. aureus* is 31, 35, 36, 38.

Zone of Inhibition of Streptomycin against *E. coli* is 24, 30, 31, 32.

Zone of Inhibition of Streptomycin against *P. aeruginosa* is 22, 23, 27, 27.

Zone of Inhibition of Ketoconazole against *C. albicans* is 18, 20, 21, 23.