Syntheses, Structural Evaluation and Antimicrobial Studies of Substituted -1,5-benzothiazepines

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***Abstract*: The enolizable ketone, 1-(4-nitrophenyl)-3-(4-chlorophenyl)-1-prop-2-enone was reacted with six 5-substituted-2-aminobenzenethiols in dry ethanol containing dry hydrogen chloride, to obtain the series of 8-substituted-2,5-dihydro-4-(4-chlorophenyl)-2-(4-nitrophenyl)-1,5-benzothiazepines in yields ranging from 40-49%. The structure of the final products has been elucidated by micro estimation for C, H and N; and IR, ^{1}H NMR, and mass spectroscopies. The low yield of the products may be due to the presence of deactivating nitro group. The compounds have the potential of showing some useful bioactivities so the synthesized compounds were studied for their relative antimicrobial activity by selecting gram-positive bacteria Staphylococcus aureus and gram-negative bacteria Pseudomonas aeruginosa against the reference compound Vancomycin and Amikacin respectively. The activity of these compounds against the fungi, Candida albicans and Aspergillus niger was also studied. A few compounds showed very good antifungal activity with respect to the reference standards, against Candida albicans and Aspergillus niger.**

Index Terms: Enolizable ketone, 1,5-benzothiazepines, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger

INTRODUCTION

Diltiazem, a cardiovascular medication from the 1,5-benzothiazepine family of chemicals, is effective in treating angina pectoris, coronary vasodilation [1], hypertension [2], Ca^{+2} concentration management [3], migraine therapy, cancer therapy, and other conditions. It's second-generation medication, clentiazem, has a chloro substituent at position 8. Our research showed that adding chlorine as a chlorophenyl substituent to the 1,5-benzothiazepine nucleus at various positions successfully produced compounds with pharmacological activity, including cardiovascular activity, anti-depressive activity, tranquillizer activity, anti-ulcerous, anti-cancerous, anti-cholinergic activity, etc. in addition to anti-bacterial and antifungal activity [4,5].

The finding of cardiovascular drug Clentiazem [6] showing better activity than diltiazem, suggests that the halogen present may have acted as a pharmacophore. These observations have encouraged the focus on the syntheses, characterization and the antimicrobial studies of such 1,5-benzothiazepines [7-10]. Thus, the syntheses of 1,5-benzothiazepines having 4-chlorophenyl group, in addition to a 4-nitrophenyl group, were chosen to study the effect of substituents on the antibacterial and antifungal activity.

MATERIAL AND METHODS

The α, β-unsaturated carbonyl compound, 1-(4-nitrophenyl)-3-(4-chlorophenyl)-1-prop-2-enone (1) was reacted with 5-substituted-2-aminobenzethiols (2a-f), prepared from p-substituted anilines [11], the substituents being halogeno as fluoro, chloro, bromo; alkyl as methyl; alkoxy as methoxy and ethoxy, in dry ethanol, saturated with hydrogen chloride gas to give the target compounds, 8-substituted- 2,5 - dihydro- 4 - (4-chlorophenyl) - 2 - (4-nitrophenyl) -1,5 - benzothiazepines (3a-f, Scheme I).

Experimental

General procedure for the syntheses of 8-substituted-2,5-dihydro-4-(4-chlorophenyl)-2-(4-nitrophenyl)-1,5-benzothiazepines (3a-f)

To the ethanolic solution of 3-(4-chlorophenyl)-1-(4-nitrophenyl)-2-propenone (1, 0.001 mol), the alcoholic solution of 5-substituted-2-amino benzenethiol (2, 0.001 mol) was added drop wise with stirring on magnetic stirrer. The reaction mixture was saturated with dry hydrogen chloride and refluxed for 3-4 hrs, till the color change became constant. Concentration and cooling of the resulting mixture afforded the crude solid; which was crystallized from ethanol to obtain the title compounds, 8-substituted-2,5-dihydro-4-(4-chlorophenyl)-2-(4-nitrophenyl)-1,5-benzothiazepines in 40-49% yields (Table 1).

All the melting points are uncorrected. Purity of the compounds was checked by TLC on silica gel 'G' coated glass plates, using benzene: ethanol: aq. ammonia (50%) in the ratio 7:2:1 as solvent system.

The IR spectra were taken in KBr pellets on a Perkin Elmer spectrum RX1 FT IR spectrophotometer (Table 2). ^{1}H NMR spectra was recorded on a Bruker DRX-300 (300 MH_2 FT NMR) instrument using $CDCl_3$ as solvent and TMS as internal standard (Table 3).

The DART-MS was recorded on a JEOL-AccuTOF JMS-T100LC Mass spectrometer. Dry Helium was used with 4 LPM flow rate for ionization at 350°C. Micro estimations for carbon, hydrogen and nitrogen were carried out on Elemental Analyzer, Carlo Erba 1108. The spectral and elemental analyses of selected compounds were carried out at the Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute, Lucknow.

Antimicrobial Activity

All the synthesized compounds were screened for antibacterial activity against the gram-positive bacteria *Staphylococcus aureus* and the gram-negative bacteria *Pseudomonas aeruginosa* with Vancomycin and Amikacin as reference drugs; and antifungal activity against the fungi, *Candida albicans* and *Aspergillus niger* using Itriconazole as the reference drugs respectively. The Paper Disc Method [12] was used to evaluate activity in the form of activity index, i.e., as the ratio of zone of inhibition exhibited by the test compounds to that of the reference compounds.

RESULT AND DISCUSSION

The reactions are initiated by nucleophilic attack of the sulfhydryl electrons of 5-substituted-2-aminobenzenethiols at the activated β-carbon atom of the α,β-unsaturated carbonyl compounds (enolizable ketone), to give Michael adduct type intermediates, which simultaneously undergo dehydrative cyclisation to give seven membered heterocyclic products.

Literature studies [13-15] revealed that such reactions have been carried out under varying reaction conditions, such as in methanol containing piperidine, in anhydrous toluene, methanol containing glacial acetic acid, methanol saturated with hydrochloric acid etc. It has also been found that cyclized products were obtained in a single step in maximum yield in the acidic medium. Hence, the reactions of 3-(4-chlorophenyl)-1-(4-nitrophenyl)-2-propenone with 5-substituted-2-aminobenzenethiols were carried out in dry ethanol saturated with dry hydrogen chloride to obtain the series of six new title compounds, the 8-susbtituted-2,5-dihydro- 4 - (4-chlorophenyl) - 2 - (4-nitrophenyl) - 1,5-benzothiazepines (3a-f, Scheme-I).

IR Spectral Analyses

The IR spectra of the final products did not show characteristic absorptions for υ C=O and υ NH_2 in the region 1685-1650 cm^{-1} and 3500-3400 cm^{-1} respectively. However, a broad absorption in the region 3300-3108 cm^{-1} was obtained which may be assigned to the secondary amino group. The final products also showed strong absorption band in the region, 1110-1050 cm^{-1} due to C-Cl stretching**.** Weak absorptions in the region around 1540 and 1200 cm^{-1} may be assigned to vibrations due to the nitro group (Table 2).

^{1}H NMR Spectral Analyses

The ^{1}H NMR spectra of all the final products showed one proton doublet at δ 6.88-7.30 (d, 1H, J= 8Hz) which may be assigned to C_2-H, and another doublet between δ 7.10-8.00 (d, 1H, J= 8Hz) integrating for one proton, may be assigned to the proton present at C_3-H; the downfield absorptions may be due to the protons lying in the deshielding zone of aryl rings. A broad absorption signal shown in the region, δ 4.08-4.14 (br, 1H) may be assigned to N-H. Multiplets at around δ 6.93-7.99 may be assigned to the 11 aromatic protons. The singlet at δ 3.90 (s, 3H) of three protons may be assigned to methoxy group protons in compound 3e at position-8. Compound 3f showed a triplet at 1.46 (t, 3H, J=7Hz) and quartet at 4.09 (q, 2H, J = 7Hz), due to methyl and methylene protons of the ethoxy group (Table 3).

Mass Spectral Analyses

The mass spectra of 3b showed cluster of the molecular ion peak, m/z, $[M]^+$, $[M+2]^+$ and $[M+4]^+$ at 428, 430 and 432 respectively, corresponding to the molecular mass of the product. The intensity of the $[M+2]^+$ peak was found nearly one third of the M^+ peak, which ascertained the presence of chlorine in the compound. The mass spectra of 3c showed molecular ion peaks, m/z, $[M]^+$ and $[M+2]^+$ at 474 and 476; the intensity of $[M+2]^+$ peak was found to be nearly equal to the M^+ peak which confirmed the presence of bromine. The results of elemental analyses were found to be satisfactory being within the permissible limits of error.

Antibacterial Activity

Most of compounds 3b, 3d, and 3e exhibited antibacterial activity nearly equivalent to the reference standard Vancomycin against *Staphylococcus aureus*.

Relatively low antibacterial activity against the Gram-negative bacterium *Pseudomonas aeruginosa* was demonstrated compared to the reference compound Amikacin.

Antifungal Activity

All compounds showed excellent antifungal activity against *Candida albicans* and *Aspergillus niger*, using Itriconazole as a reference standard. Compound 3b was found to exhibit the highest relative activity against Aspergillus niger (activity index = 1.50).

CONCLUSION

Most of the compounds were found to have good to moderate activity against *Staphylococcus aureus*. The newly synthesized compound 3b, containing a higher percentage of chlorine, showed 1.5-fold greater antifungal activity against *Aspergillus niger* and *Candida albicans*, and also showed good antibacterial activity against all microorganisms tested.

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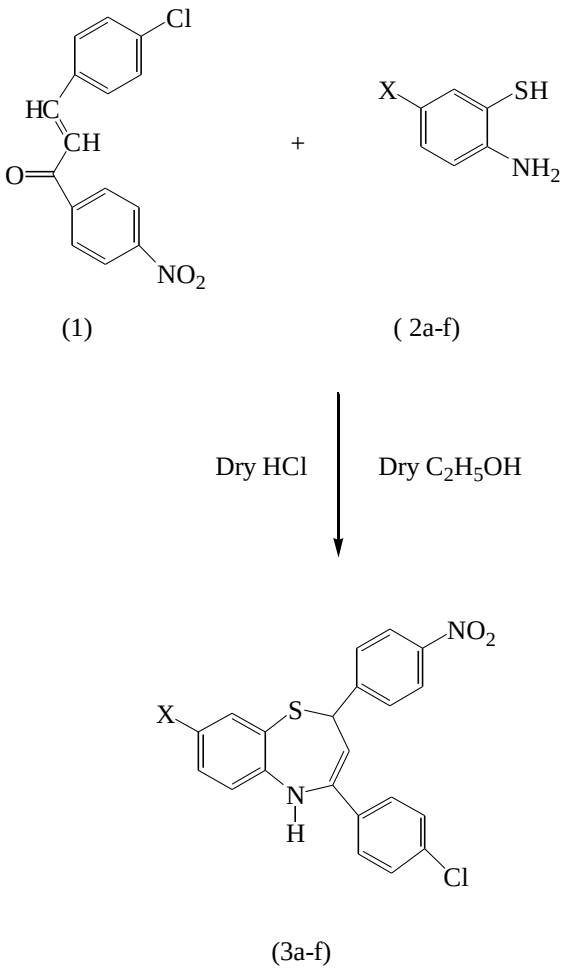

Scheme 1

Table. 1 Physical and antimicrobial data of 3a-f

Comp. No.	M.P. (°C)	R_f	Yield (%)	Bacteria		Fungi	
				S. aureus	***P. aeruginosa***	***C. albicans***	***A. niger***
3a	96	0.70	40.47	17 (1.30)	6 (0.37)	17 (1.21)	19 (1.35)
3b	160	0.76	46.78	12 (0.92)	10 (0.62)	18 (1.28)	21 (1.50)
3c	155	0.58	48.75	--	6 (0.37)	11 (0.78)	--
3d	130	0.71	41.19	11 (0.84)	8 (0.50)	18 (1.28)	18 (1.28)
3e	55	0.86	43.62	11 (0.84)	14 (0.87)	20 (1.42)	--
3f	140	0.65	44.29	8 (0.61)	--	-	13 (0.92)

Zones of inhibition are given in mm; Values in parentheses represent activity index

Zone of inhibition of Vancomycin for Staphylococcus aureus is 13 mm

Zone of inhibition of Amikacin for Pseudomonas aeruginosa is 16 mm

Zone of inhibition of Itriconazole for Candida albicans is 14 mm

Zone of inhibition of Itriconazole for Aspergillus niger is 14 mm

Concentration of test and reference compounds were 100µg/disc.

Table.2 Characteristic IR absorptions bands (λ in cm^{-1}) of 3a-f

Compd. No.	N-H	O-N-O	C-Cl	C-O-C	C-N
3a	3250-3110	1530	1100	-	1220
3b	3240-3105	1540	1050	-	1190
3c	3300-3108	1545	1080	-	1180
3d	3260-3100	1530	1010	-	1185
3e	3250-3150	1535	1110	1255 1020	1210
3f	3280-3100	1550	1050	1260 1020	1195

Table. 3 Characteristic ^1H NMR data of 3a-f

Compd. No.	N-H (br, 1H)	C_2-H (d,1H, J8)	C_3-H (d,1H, J8)	C_8-XH	Ar Protons (m,11H)
3a	4.10	6.88	7.10	-	6.93-7.99
3b	4.14	7.04	7.99	-	7.18-7.92
3c	4.08	7.08	8.00	-	7.11-7.92
3d	4.12	7.30	7.95	2.39 (s,3H)	7.37-7.75
3e	4.10	6.97	7.92	3.90 (s,3H)	7.24-7.81
3f	4.10	7.05	7.93	1.46 (t,3H, J7) 4.09 (q,2H, J7)	7.26-7.75