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SYNTHESES OF 1,5-BENZOTHIAZEPINES: PART 45: SYNTHESES AND ANTIMICROBIAL STUDIES OF 8-SUBSTITUTED-2,5-DIHYDRO-4-(4-CHLOROPHENYL/PHENYL) -2-(3-NITROPHENYL)- 1, 5-BENZOTHIAZEPINES

SEEMA PANT* AND DEEPIKA SAXENA

P.G. Department of Chemistry, L.B.S. Government P.G. College, Kotputli-303108, Jaipur, Rajasthan

*Corresponding Author: drseemapant@yahoo.com/gemini.deepika@gmail.com

Abstract

Two series of 1,5-benzothiazepine derivatives, 8-substituted-2,5-dihydro-2-(3-nitrophenyl)-4-phenyl-1,5-benzothiazepines and 8-substituted-4-(4-chlorophenyl)-2,5-dihydro-2-(3-nitrophenyl)-1,5-benzothiazepines have been synthesized by the reactions of six 5-substituted-2-aminobenzenethiols, the substituents being fluoro, chloro, bromo, methyl, methoxy and ethoxy, with chalcones, 3-(3-nitrophenyl)-1-phenyl-2-propenone and 1-(4-chlorophenyl)-3-(3-nitrophenyl)-2-propenone , in acidic medium, using dry ethanol saturated with dry HCl. The products were characterized on the basis of elemental analyses for C, H, N and S and spectral analyses comprising IR, NMR and mass studies. The synthesized compounds have been screened for their antimicrobial activity against gram positive bacteria, *Staphylococcus aureus*, gram negative bacteria, *Escherichia coli* and fungus, *Candida krusei* with respective reference drugs. It was found that 8-ethoxy and 8-methyl substituted 2, 5-dihydro-2-(3-nitrophenyl)-4-phenyl-1,5-benzothiazepines displayed significant antibacterial activities against *Staphylococcus aureus*, higher than that of the reference standard, Vancomycin at the concentration of 100µg/disc. None of the compounds were found to show any activity against *Pseudomonas aeruginosa.*

Keywords: 1,5-Benzothiazepines, chalcones, *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Candida krusei*

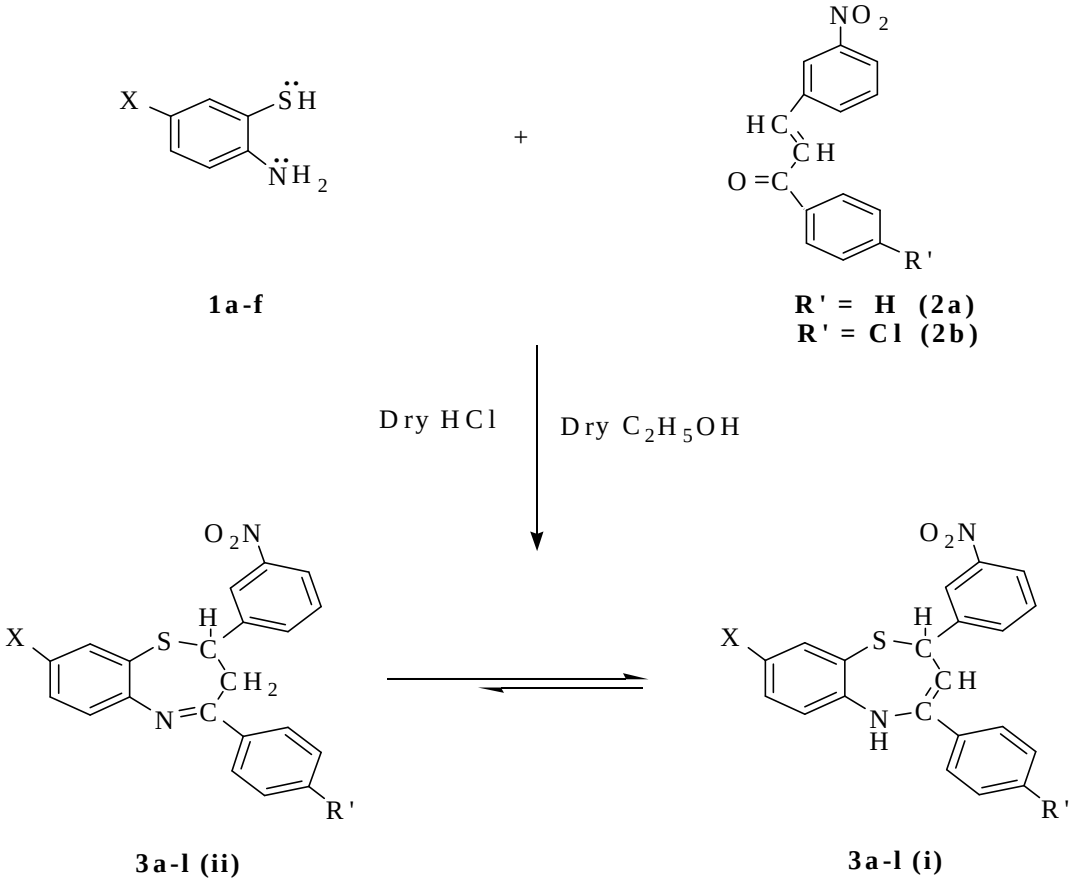
Introduction

The compounds having benzodiazepine/ benzothaizepine nucleus, possess interesting biological activities like antitumor (Hutchinson et al, 2003), antimicrobial (Garg et al, 2010), antimalarial (Burger et al, 1968), anticonvulsant (Chakole et al, 2005), and anti-inflammatory activity (Siddiqui et al, 2004). The first 1,4-benzodiazepine drug chlordiazepoxide (Librium) having chloro group, was reported (Liljequist et al 1979), as promising psychoactive drug. Other patented 1,4 benzodiazepine drugs, Diazepam(Valium) (Mandrioli et al, 2008), Nitrazepam (Yasui et al, 2005), Oxazepam, Nimetazepam (Fukinaga et al, 1988), Nordazepam (Ator et al, 1997) and Clonazepam (Marc et al, 2005), having nitro/chloro group as substituents, possess antidepressive, antipanic, anxiolytic and sedative activity. 1,5-benzodiazepine drug, Clobazam, having chloro group in the fused benzene ring, possess psychopharmacological properties (Shenoy et al, 1982); another drug, Clentiazem (Giasson et al, 1995), patented as TA-3090, is a more potent antihypertensive and cardiovascular drug. It may thus be hypothesized that the introduction of chloro/nitro group as substituents in the benzodiazepine/benzothiazepine nucleus may have led to the development of compounds having pharmacological/ biological activities (Barot et al, 2001). Analogous reactions of 5-substituted-2-aminobenzenethiols with chloro chalcones have been recently reported to give the respective 1,5-benzothiazepine compounds which have exhibited good antifungal activity against *Candida albicans* and *Aspergillus niger* (Pant et al, 2014). Considering the importance of the presence of these substituents, we herein report the syntheses of twelve new products, 8-substituted-2,5-dihydro-2-(3-nitrophenyl)-4-phenyl-1,5-benzothiazepines **(3a-f)** and 8-substituted-4-(4-chlorophenyl)-2,5-dihydro-2-(3- nitrophenyl)-1,5-benzothiazepines **(3g-l)** by the reactions of six 5-substituted - 2- aminobenzenethiols **(1a-f),** the substituent's being fluoro, chloro, bromo, methyl, methoxy and ethoxy, with 3-(3-nitrophenyl)-1-phenyl-2-

propenone **(2a)** and 1-(4-chlorophenyl)-3-(3-nitrophenyl)-2-propenone **(2b).** These compounds have been screened for their antibacterial and antifungal activity by using Paper Disc Method (Pant et al, 2014) against gram-positive bacteria, *Staphylococcus aureus*, gram-negative bacteria, *Escherichia coli* and fungus, *Candida krusei* with reference compounds Vancomycin, Gatifloxacin/Amikacin and Fluconazole respectively.

Materials and Methods

To have the substituents in the fused benzene ring of 1,5-benzothiazepine nucleus, the first precursors required were 5-substituted-2-aminobenzenethiols **(1a-f),** which were prepared from readily available p-substituted anilines (Mittal R.L. et al, 1971). In order to have nitro and chloro substituents in the thiazepine nucleus, the second precursors required were chalcones, 3-(3-nitrophenyl)-1-phenyl-2-propenone **(2a)** and 1-(4-chlorophenyl)-3-(3-nitrophenyl)-2-propenone **(2b)**, which were prepared by reported methods (Kanthi R. B.et al, 1957), by reacting equimolar quantities of 3-nitrobenzaldehyde with acetophenone / 4-chloroacetophenone in ethanol containing 10% NaOH. The two categories of precursors were reacted to obtain twelve new products, 8-substituted-2,5-dihydro-2-(3-nitrophenyl)-4-phenyl-1,5-benzothiazepines **(3a-f)** and 8-substituted- 4- (4-chlorophenyl) -2, 5-dihydro-2-(3-nitrophenyl)-1,5-benzothiazepines **(3g-l)** in acidic medium, i.e. dry ethanol saturated with dry HCl. The reaction mixtures were refluxed for 7-10 hrs to obtain the products in single step in 50-69% yields; the purity of final products was determined by thin layer chromatography on silica gel 'G' coated glass plates, using benzene: ethanol: aq. ammonia (50%) (7:2:1) and R_f values were noted.



C. No.	X	R'	C. No.	X	R'
3a	F	H	3g	F	Cl
3b	Cl	H	3h	Cl	Cl
3c	Br	H	3i	Br	Cl
3d	CH_3	H	3j	CH_3	Cl
3e	OCH_3	H	3k	OCH_3	Cl
3f	OC_2H_5	H	3l	OC_2H_5	Cl

Scheme-1

2,5-Dihydro-8-methyl-2-(3-nitrophenyl)-4-phenyl-1,5-benzothiazepine (3d) :

The solution of 2-amino-5-methylbenzenethiol (**1d**, 0.001 mol, 0.139 gm) and 3-(3-nitrophenyl)-1-phenyl-2-propenone (**2a,** 0.001 mol, 0.253 gm) in dry ethanol (10 ml) were mixed drop wise with stirring. Dry hydrogen chloride was passed into this reaction mixture, which was then refluxed for 10 hrs. The reaction mixture was concentrated and cooled to obtain yellowish orange crude. The crude on crystallization from dry ethanol afforded orange crystals of the target compound [**3d**, m.p. 104˚C; yield 0.196 gm; 52.46 %,; R_f 0.70; IR (KBr) 3143 cm^{-1}(NH); ^1H NMR ($CDCl_3$) 2.50(s,3H), 4.02(br, 1H), 7.35(d,1H,J=7Hz),8.05(d,1H,J=7Hz), 6.90-7.24(m,12H)].

4-(4-Chlorophenyl)-8-ethoxy-2,5-dihydro-2-(3-nitrophenyl)-1,5-benzothiazepine (3l):

2-Amino-5-ethoxybenzenethiol (**1f**, 0.001 mol, 0.169 gm) was dissolved in dry ethanol (5ml), saturated with dry HCl. To this 1-(4-chlorophenyl)-3-(3-nitrophenyl)-2-propenone (**2b**, 0.001 mol, 0.287 gm) dissolved in dry ethanol (15ml) was added dropwise with continuous stirring. The reaction mixture was saturated with dry HCl. The color of the reaction mixture changed to light yellow. It was refluxed for 8.5 hrs, till color of the reaction mixture changed to brown and then concentrated, cooled to give reddish brown crude, which was crystallized from dry ethanol to afford **3l** [m.p. 180˚C; yield 0.295 gm; 67.32%,; R_f 0.62; IR (KBr) 3145 cm^{-1}(NH); ^1H NMR ($CDCl_3$)1.49 (t,3H,J=7 Hz), 4.12 (q,2H,J=7 Hz), 4.02 (br, 1H), 7.12 (d,1H,J=7Hz), 8.27 (d,1H,J=7Hz), 6.92-7.96 (m,11H)]. Employing similar methods, rest of the target compounds were prepared **(Table 1)**.

Table 1: Physical constants and microanalytical data of 3

C. No.	M.P °C	R_f	Yield (%)	Mol. Formula (Mol. Mass)	Elemental Analyses Found (Calculated) (%)			
					C	H	N	S
3a	120-122	0.79	50.32	$C_{21}H_{15}FN_2O_2S$ (378)	66.38 (66.65)	3.88 (4.00)	—	—
3b	130	0.76	50.53	$C_{21}H_{15}ClN_2O_2S$ (394.5)	63.99 (63.87)	3.80 (3.93)	7.34 (7.09)	8.17 (8.31)
3c	130-132	0.74	52.30	$C_{21}H_{15}BrN_2O_2S$ (439)	—	—	6.49 (6.38)	7.12 (7.30)
3e	102	0.81	56.41	$C_{22}H_{18}N_2O_3S$ (390)	67.43 (67.67)	4.81 (4.65)	6.98 (7.17)	8.09 (8.21)
3f	94	0.83	54.41	$C_{23}H_{20}N_2O_3S$ (404)	68.51 (68.30)	5.12 (4.98)	—	—
3g	160	0.62	67.38	$C_{21}H_{14}N_2O_2SFCl$ (412.5)	60.78 (61.09)	3.15 (3.39)	6.56 (6.78)	7.40 (7.75)
3h	96	0.52	69.43	$C_{21}H_{14}N_2O_2SCl_2$ (429)	59.14 (58.74)	3.38 (3.26)	6.38 (6.52)	7.27 (7.45)
3i	104	0.60	68.88	$C_{21}H_{14}N_2O_2SClBr$ (473.5)	—	—	5.76 (5.91)	6.38 (6.75)
3j	112	0.42	67.18	$C_{22}H_{17}N_2O_2SCl$ (408.5)	64.28 (64.62)	3.96 (4.16)	—	—
3k	110	0.65	68.56	$C_{22}H_{17}N_2O_3SCl$ (424.5)	—	—	6.36 (6.59)	7.32 (7.53)

Melting points of all the reported compounds are uncorrected. Homogeneity 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 Perkin Elmer RX1 FT IR spectrophotometer. ^1H NMR spectra were recorded on Bruker DRX-300 (300 MHz FT NMR with low and high temperature facility) using TMS as internal standard and $CDCl_3$ as solvent.

The DART-MS were recorded on JEOL-Accu-TOF JMS-T100LC mass spectrometer having a DART source. Dry Helium was used with 4LPM flow rate for ionization at 350 0C. Microestimations for carbon, hydrogen, nitrogen and sulphur were carried out in elemental analyzer, Carlo Erba 1108. The spectral and elemental analyses were carried out at the Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute, Lucknow.

All the synthesized compounds **3a-l** were assessed for their relative antibacterial activity against the bacteria, *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* and antifungal activity against the fungus, *Candida krusei* at the concentration of 100µg/disc, using reference drugs, Vancomycin, Gatifloxacin/Amikacin, Meropenem and Fluconazole, respectively.

Results and Discussion

The acid catalyzed reaction of 5-substituted-2-aminobenzenethiols **(1a-f)** is initiated by the nucleophilic attack of the lone pair of sulphhydryl electrons at the activated β-carbon atom of the enolisable ketones **(2).** The adduct thus formed cyclises simultaneously to give the target compounds in a single step.

The IR spectra of the final products **3a-l,** showed a broad absorption in the region 3270-3100 cm^{-1}, indicating the presence of a secondary amino group. Absorptions at 1675-1665cm^{-1}, 2600-2500cm^{-1} and 3550cm^{-1}, were found to be absent, confirming the absence of carbonyl group, -SH group and primary amino group. The aralkoxyl linkage vibrations for νC-O-C of methoxy and ethoxy group in the compounds **3e,3f,3k,3l** were indicated by absorption in the region 1350-1150 cm^{-1}. All the products showed strong absorption bands in the range of 1570-1500 cm^{-1} and 1370-1300 cm^{-1} indicating the presence of Ar-NO_2; Other absorptions observed at around 600-800 cm^{-1} may be assigned to C-Cl stretching absorptions.

The ^{1}HNMR spectra of all the final products **3a-l** showed a doublet at δ 6.91-7.47 (d, 1H, J=7Hz) which may be due to C_2-H. The downfield absorption of C_2-H may be due to its presence in the deshielding zone of aryl ring and its attachment to electronegative sulphur atom. Another doublet at δ7.82-8.27 (d, 1H, J= 7Hz) may be assigned to C-3 vinylic proton. Absorption in the region δ 6.76-8.10 appeared as multiplets, corresponding to the aromatic protons. All the synthesized compounds showed a broad singlet at around δ 4.00-4.12 (s, 1H) which may be assigned to secondary amino proton. This indicated the preferential formation of 2,5-dihydro form over 2,3-dihydro form. The ^{1}HNMR spectra of compounds **3d** and **3j** showed a singlet at 2.50 (s,3H) and 2.53 (s,3H) due to three protons of methyl group while the presence of methoxyl group in compounds **3e** and **3k** was indicated by a singlet at around 3.73 (s, 3H) and 3.76 (s, 3H) respectively. Absorption signal, as quartet at around 4.03 to 4.05 (q, 2H, J= 7Hz) may be due to two methylene protons and a triplet at around 1.40 to 1.49 (t, 3H, J= 7Hz) which may be due to three methyl protons, confirming the presence of ethoxyl group in the compounds **3f** and **3l (Table 2).**

Table 2: Characteristic ^{1}H NMR data ($CDCl_3$, ¢ values in ppm, J in Hz) of 3

C. No.	NH(br,1 H)	C_2–H (1H,d,J=7Hz)	C_3–H (1H,d,J=7Hz)	C_8–XH	Aromatic Protons (12H,m)
3a	4.08	7.38	7.96	-	7.30-8.00
3b	4.10	7.32	7.88	-	7.03-7.86
3c	4.12	7.30	7.93	-	6.96-7.50
3d	4.02	7.35	8.05	2.50 (s,3H)	6.90-7.24
3e	4.05	7.36	7.82	3.73 (s,3H)	7.10-7.98
3f	4.06	6.96	7.84	1.43(t,3H,J=7), 4.05(q,2H,J=7)	6.92-7.96
3g	4.09	7.13	7.97		7.20-8.10
3h	4.11	6.91	8.02	-	7.05-8.66
3i	4.07	7.03	8.13	-	6.76-7.35
3j	4.03	7.37	8.25	2.53 (s,3H)	6.80-7.26
3k	4.05	7.18	8.23	3.76 (s,3H)	7.30-8.05
3l	4.02	7.12	8.27	1.49(t,3H,J=7), 4.12 (q,2H,J=7)	6.92-7.96

In the mass spectra of compound **3b**, the presence of cluster of molecular ion peaks, m/z $[M^+]$, $[M+2]^+$ and $[M+4]^+$at 395, 397 and 399 corresponded to the molecular mass of the products. 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 characteristic intensities of the $[M]^+$ and $[M+2]^+$ peaks at 439 and 441 in the spectrum of **3c** and at 473 and 474 in the spectrum of **3i** indicated the presence of bromine in the compounds.

Antimicrobial Activity

Most of the compounds were found to show promising antibacterial activity against *Staphylococcus aureus* and moderate activity against the fungus *Candida krusei*. against *Staphylococcus aureus,* compounds **3d** and **3f,** showed maximum relative activity (activity index = 1.06), higher than that of the reference standard; while **c**ompound **3a** showed relative activity equal to that of Vancomycin while rest of the compounds showed some activity (activity index = 0.73-0.93, **Table 3)**.

Table 3: Antimicrobial activity of 3a-l

Compd. No.	Bacteria			Fungus
	Gram-positive *Staphylococcus aureus*	Gram-negative *Escherichia coli*	Gram-negative *Pseudomonas aeruginosa*	*Candida krusei*
3a	15 (1.00)	11 (0.36)	----	**----**
3b	14 (0.93)	----	----	----
3c	-----	----	----	9 (0.36)
3d	16 (1.06)	----	----	12 (0.48)
3e	-----	----	----	----
3f	16 (1.06)	12 (0.40)	----	----
3g	-----	----	----	----
3h	12 (0.80)	----	----	14 (0.56)
3i	12 (0.80)	----	----	7 (0.28)
3j	13 (0.86)	----	----	12 (0.48)
3k	----	----	----	----
3l	11 (0.73)	**----**	**----**	13 (0.52)

Values in parentheses represent activity index.

Zone of inhibition of Vancomycin for *Staphylococcus aureus* is 15 mm, of Gatifloxacin for *Escherichia coli* is 30 mm, of Amikacin for *Escherichia coli* is 22 mm, of Fluconazole for *Candida krusei* is 25 mm.

Conclusion

Majority of the newly synthesized compounds exhibited good to higher antibacterial activity against *Staphylococcus aureus* whereas moderate antifungal activity was shown against *Candida krusei* by most of the compounds.

Thus it is expected that these findings will lead to the discovery of novel compounds of biological importance which may be especially developed later for chemotherapeutic utilization.

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References

Ator N. A., Griffiths R. R., 1997, J. Pharmacol. Exp. Ther. 282(3): 1442–1457.

Barot V. M., Patel M. R. and Naik H. B., 2001, Asian J. Chem., 13(1): 347; 1983, Chem. Abstr., 99: 22439w.

Burger A., Sawhey S. N., 1968, J. Med. Chem., 11: 270-273..

Chakole R. D., Amnerkar N. D., Khedekar P. B., Bhusari K. P., 2005, Indian J. Heterocycl. Chem., 15: 27-30.

Fukinaga M., Ishizawa K., Kamei C., 1988, Pharmacol., 5(57): 233–241.

Garg N., Chandra T., Kumar A., 2010, Eur. J. Med. Chem., 45: 1529.

Giasson S., Garceau D., Homsy W., Dumont L., 1995, Cardiovasc. Drugs Ther., 9(5): 685-92.

Hutchinson I., Bradshaw T. D., Matthews C. S., Westwell A. D., 2003, Bioorg. & Med. Chem. Letters, 13: 471-474.

Kanthi R. B. and Nargund K. S., 1957, J. Karnatak Univ., 2(1): 8; 1959, Chem. Abstr., 53: 8067b.

Liljequist R., Palva E., Linnoila M., 1979, Int. Pharmacopsychiatry, 14(4): 190–198.

Mandrioli R., Mercolini L., Raggi M. A., 2008, Curr. Drug. Metab, 9(8): 827–844.

Marc C. J., 2005, Curr Opin Psychiatry, 18(1): 45-50.

Mittal R. L. and Taunk P. C., 1971, Montshefte fur Chemie, 102: 760.

Pant S., Avinash, Jadon R. and Yadav M., 2014, Int. J. Curr. Res. Chem. Pharma Sci., 1(7): 25-30.

Shenoy K. A., Miyahara T. J., Swinyard A. E. and Kupferbery J. H., 1982, Chem. Abstr., 97: 156391s.

Siddiqui N., Alam M., 2004, Asian J. Chem., 16: 1005-1008.

Yasui M., Kato A., Kanemasa T., Murata S., Nishitomi K., Koike K., Tai N., Shinohara S., Tokomura M., Horiuchi M., 2005, Nihon Shinkei Seishin Yakurigaku Zasshi, 25(3): 143–151.