International Journal of Chemical Sciences and Applications
ISSN 0976-2590, Online ISSN 2278 – 6015
Vol 5, Issue 1, 2014, pp 7-12
http://www.bipublication.com

SYNTHESES AND ANTIMICROBIAL EVALUATION OF 8-SUBSTITUTED-2,5-DIHYDRO-2-(3,4-DIMETHOXYPHENYL-4-(4-METHYLPHENYL)-1,5-BENZOTHIAZEPINES

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[Received-16/01/2014, Accepted-12/02/2014]

ABSTRACT:

8-Substituted-2,5-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-methylphenyl)-1,5-benzothiazepines have been prepared by the reaction of 3-(3,4-dimethoxyphenyl)-1-(4-methylphenyl)-2-propenone with six 5-substitiuted-2-aminobenzenethiols, the substituents being fluoro, chloro, bromo, methyl, methoxyl and ethoxyl, in dry ethanol saturated with dry HCl gas. All the synthesized compounds are characterized by analytical and spectral data comprising IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and mass studies. All these compounds have been screened for antimicrobial activity against the gram-positive bacteria, *Staphylococcus aureus*withstandard gatifloxin and the gram-negative bacteria, *Pseudomonas aeruginosa*with standard natilmicinand the fungus, *Candida albicans*with standard fluconazole. The antifungal activity was found to be more pronounced than antibacterial.

Key words: methoxyl substituents, methyl substituent, diltiazem, 1,5-benzothiazepines, antimicrobial activity

INTRODUCTION

The 1,5-benzothiazepine moiety is a privileged class of pharmacophore, as compounds bearing this structural unit possess a broad spectrum of biological activities such as anticonvulsant[1], Ca^{2+} channel antagonist[2], antianginal[3], anti HIV[4], Equalenesynthetase inhibitor[5], V_2 arginine vasopressin in receptor antagonist[6], HIV-1 reverse transcriptase inhibitor[7] etc. The first molecule of 1,5-benzothiazepine used clinically was diltiazem followed by clentiazem, for their cardiovascular action. Diltiazem&clentiazem, both possess methoxyl as well as methyl group as a substituent in 1,5-benzothiazepine nucleus. Two methoxyl group with methyl substituent are also present in structurally related 1,5-benzodiazepine compound, zimet, interestingly, known for its antineoplastic activity against dreadful diseases[8] like leukemia, melanoma B_{16}, Lewis lung carcinoma, and tumor.

Some bicyclic &tetracyclic 1,5-benzothiazepines[9-18] having methoxyl groups in the nucleus in different proportionswere synthesized and reported, presuming that methoxyl & methyl group is functioning as pharmacophore.With this anticipation, the syntheses, spectral and antimicrobial studies of a series of 1,5-benzothiazepines, 8-substituted-2,5-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-methylphenyl-1,5-benzothazepines have been undertaken and reported in the present communication.

MATERIAL & METHODS

Equimolar quantities of 5-substituted-2-aminobenzenethiols,**1a-f** and 3-(3,4-dimethoxyphenyl)-1-(4-methylphenyl)-2-propenone,**4** were refluxed in dry ethanol saturated with dry HCl gas for 6-8 hrs to obtain the title products, 8-substituted-2,5-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-methylphenyl)-1,5-benzothiazepines,**5a-f** in one step in 60-70% yields. The purity of the final products was checked by TLC. The structural assignments are based on the results of elemental analysis for carbon, hydrogen and nitrogen (**Table 1**) and spectral investigations comprising IR, ^{1}H NMR (**Table 2**), ^{13}C NMR, ^{19}F NMR and mass studies. All the compounds,**5a-f** were evaluated for antimicrobial activity comprising antibacterial and antifungal.

EXPERIMENTAL

All the melting points are uncorrected. TLC was used for checking homogeneity of the compounds 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 Shimadzu 8201 PC spectrophotometer. NMR spectra were recorded on a Bruker DRX-300 (300 MHz FT NMR) instrument using $CDCl_3$ as solvent and TMS as internal standard. The FAB mass spectra were recorded on JEOL-SX 102/DA-6000 Mass spectrometer/Data system using Argon/Xenon (6kV, 10 mA) as the FAB gas at room temperature. The accelerating voltage was 10kV and *m*-nitrobenzyl alcohol (NBA) was used as the matrix. Microestimations for elements were carried out in Elemental Analyzer, Carlo Erba 1108. The spectral analysis and elemental analysis were carried out at the Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute, Lucknow. Antimicrobial activity was carried out at Microbiology Department, S.M.S. Medical College, Jaipur.

Compound, 3-(3,4-dimethoxyphenyl)-1-(4-methylphenyl)-2-propenone was synthesized and six 5-substituted-2-amino-benzenethiols[9-17], the substituents being fluoro, chloro, bromo, methyl, methoxyl and ethoxyl**1a-f**, were prepared by literature reported methods.

3-(3,4-dimethoxyphenyl)-1-(4-methylphenyl)-2-propenone, 3:

Equimolar quantity of 3,4-dimethoxybenzaldehyde (0.01 mole) and 4-methyl acetophenone (0.01 mole) were taken in dry ethanol. Aqueous NaOH was added dropwise with continuous stirring. The colour of the reaction mixture first turned yellow, finally yellow coloured solid obtained. The crude solid thus separated recrystallizedfrom dry ethanol to afford 3,4-dimethoxybenzal-4-methyl acetophenone, **3**. m.p. 90^0C; yield 86%; R_f 0.76; ^{1}H NMR ($CDCl_3$): δ 2.4 (s, 3H), 3.81 (s, 3H), 3.93 (s, 3H), 6.88-6.91 (d, 1H), 7.92-7.94 (d, 1H), 7.16-7.82 (Ar, 8H). Anal. Found: C, 76.48; H, 4.81; Calcd for $C_{22}H_{18}OSNF$ (363): C, 76.59; H, 6.38%.

8-methoxy-2,5-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-methylphenyl)-1,5-benzothiazepine, 5e:

2-Amino-5-methoxybenzenethiol (**4e**, 0.001 mole, 0.17g) and 3-(3,4-dimethoxyphenyl)-1-(4-methylphenyl)-2-propenone (**3**, 0.001 mole, 0.268 g) were taken in dry ethanol saturated with dry HCl gas. Reaction mixture was refluxed for 6 hrs when colour changed from pale yellow to

deep red. The reaction mixture was cooled and solvent is removed by distillation under reduced pressure. The residue obtained after concentration was crystallized from benzene to give crystals of 8-methoxy-2,5-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-methylphenyl)-1,5-benzothiazepine **5e.**m.p. 132-3^{0}C; yield 62%; R_f 0.71; ^{1}H NMR ($CDCl_3$): δ 3.9 (s, 3H), 4.08 (br, 1H), 5.92 (d, 1H, *J*=7 Hz), 6.01 (d, 1H, *J*=7 Hz), 6.90-7.90 (m, 12H). Anal. Found: C, 71.55; H, 5.92; N, 3.37; S, 7.67; Calcd for $C_{25}H_{25}O_3SN$ (419): C, 71.59; H, 5.96; N, 3.34; S, 7.63%.

On similar lines **5a-d** and **f**were prepared. Their physical and analytical constants and spectral data are given in **Tables 1** and **2** respectively.

RESULT AND DISCUSSION

The reactions were catalysed by acid and hence are understood[9-16] to be initiated by the protonation of the carbonyl group of the propenone, **4**. On protonation, the methine carbon becomes more electrophilic and hence susceptible to nucleophilic attack by the sulphhydryl electrons of the 5-substituted-2-aminobenzenethiols,**1a-f,** to give Michael adduct intermediates, which simultaneously undergo dehydrativecyclization to give final products, 8-substituted-2,5-dihydro-2-(3,4-dimethoxyphenyl)-4-(4-methylphenyl)-1,5-benzothiazepines,**5a-f,** in a single step in a 60-70% yields (**Figure1**).

In the IR spectra of the final products (**5a-f**), a broad absorption band was observed at around 3140-3135 cm^{-1},which may be due to secondary amino group ν (N-H). However, characteristic absorptions in the range 3450-3350 cm^{-1} and 1700-1640 cm^{-1} were found to be absent which indicated the absence of primary amino group and carbonyl group respectively. All the products showed strong and medium intensity absorptions in the range, 1270-1260 cm^{-1} and weak absorptions at around 1030-1020 cm^{-1} indicating the presence of aralkyl linkage vibrations ν (C-O-C), which may be assigned to methoxyl group.

The ^{1}H NMR spectra of all the final products (**3a-f**) showed a doublet at δ 5.84-5.94 (d, 1H, *J*=7Hz) integrating for one proton, which may be assigned to C-2-H. Another doublet at δ6.01-6.38 (d, 1H, *J*=7Hz) may be assigned due to C-3 proton. The downfield absorption of C-2 proton may be accounted due to deshielding zone of aryl ring and attachment of it with electronegative sulphur atom whereas downfield absorption of C-3-H may be due to vinylic proton. Multiplets at around δ 6.76-7.94 (m, 10H) may be assigned to aromatic protons. Singlets at around δ 3.80-3.95 (s, 3H) and δ 2.40-2.44 (s, 3H) of three protons each may be assigned to methoxyl and methyl group protons respectively, in all the synthesized products. A broad singlet at around δ 4.08-4.13 (b, 1H) may be assigned to secondary amino proton. The pattern of the spectra indicated the formation of 2, 5-dihydro form (**5a-f(i)**) instead of 2, 3-dihydro form (**5a-f (ii)**). The ^{1}H NMR spectra of 2,3-dihydro form would show significantly different pattern, the ABX pattern, in which distinctive signals of methylene protons, H_A and H_B and the methine proton H_X were observed[19]. The presence of 2, 5-dihydro form over 2,3-dihydro form may be accounted due to the p-π conjugation resulting into more of resonance stabilization of the 2,5-dihydro form. Thus, these observations rule out the formation of 2,3-dihydro forms and explain the existence of 2,5-dihydro form of 1,5-benzothiazepine nucleus.

Absorption signals as quartet at around δ 3.44-3.46 (q, 2H, *J*=7Hz) may be assigned due to two methylene protons and a triplet at around δ 1.24-1.26 (t, 3H, *J*=7Hz), which may be due to three methyl protons, confirmed the presence of ethoxyl group (CH_3-CH_2-O-) protons in the compounds **5f** (**Table 2**).

In the ^{13}C NMR studies of the compound **5e**, the absorption signals were found at δ 56.1 and 56.5, which may be assigned to methoxyl carbons present as 3,4-dimethoxyphenyl group at position-2. C-2 was observed at δ 55.9 and a signal at δ

110.1 may be assigned to C-3. All other carbons of the molecule were observed in the aromatic region of the spectra i.e. at δ 111.1, 112.2, 119.4, 121.1, 121.3, 123.3, 126.3, 127, 127.6, 127.9, 128.8, 129.8, 138.9, 145.5, 149.2, 151.6 and 152.7.

In the ^{19}F NMR spectra of the compounds **5a** absorption signals were found at δ -114.00 and -114.4 respectively, which confirmed the presence of fluorine in the molecules.

In the mass spectra of **5b**, the presence of molecular ion peaks, m/z, $[M]^+$ and $[M+2]^+$ at 420 and 422 correspond 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 atom in compound **5b**. The mass spectra of **5c** showed molecular ion peaks, m/z, $[M]^+$ and $[M+2]^+$ at 464 and 466. The intensity of $[M+2]^+$ peak was found to be nearly equal to the M^+ peak which confirmed the presence of bromine in compound **5c** .

The results of elemental analysis were found to be satisfactory being within the permissible limit of error (**Table 1**).

Antimicrobial activity

All the reported compounds were evaluated for their relative antibacterial activity against the gram-positive bacteria, *Staphylococcus aureus* and the gram-negative bacteria, *Pseudomonas aeruginosa* and relative antifungal activity against the fungus, *Candida albicans* by using reference compounds gatifloxin, natilmicin and fluconazole respectively. The Paper Disc method[20] was used at the concentration of 100μg/disc. The zone of inhibitions for the test and reference compounds were measured in millimeters in 40 hr incubation period and compared to get the results in the form of activity index.

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

All the synthesized compounds of the series showed significant antifungal activity except **5c**. Compound **5e** showed highest relative activity as compared to reference compound, against the fungus, C. *albicans.* Compound **5a** showed higher activity but **5b** showed equal activity. Compounds **5d**&**5f** were found to be less active. None of the compounds showed significant activity against both the bacteria. However, methoxyl derivative, **5e**, showed moderate activity. (**Table 3**).

CONCLUSION

As per the results of the present study, it could be concluded that, the increased number of methoxyl substituents plays an important role in antifungal activity but has not much role in antibacterial activity. However, by increasing methyl proportion don't affect significantly antibacterial as well as antifungal activity.

ACKNOWLEDGEMENT

The author is gratefully acknowledging the Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute, Lucknow for providing the elemental analysis and spectral data.

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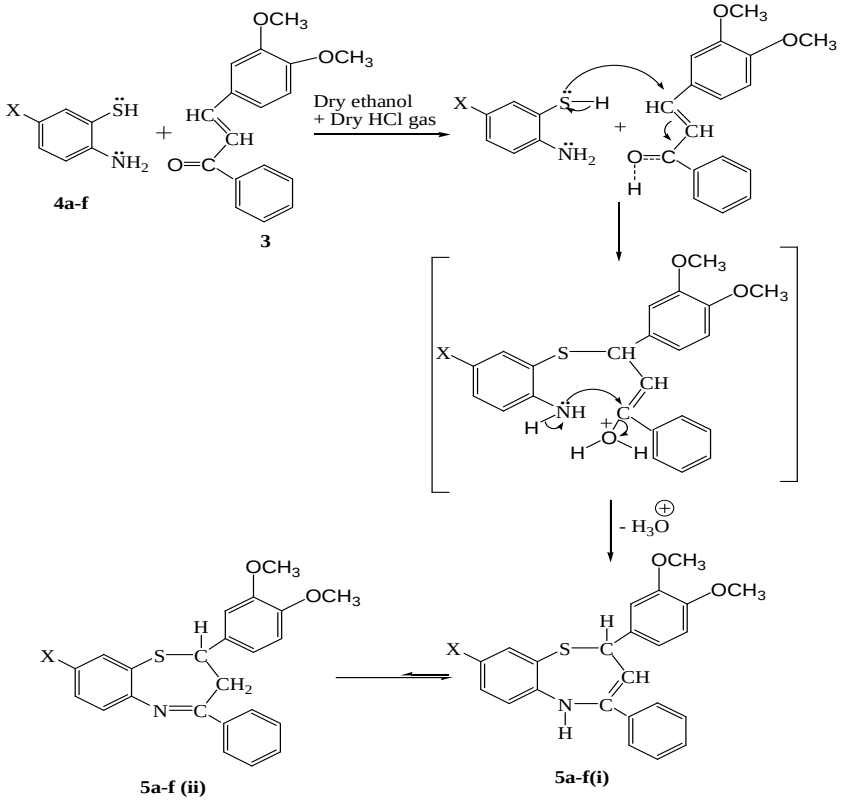

Figure 1

Compd No.	X	R
5a	F	3,4-$(OCH_3)_2$
5b	Cl	3,4-$(OCH_3)_2$
5c	Br	3,4-$(OCH_3)_2$
5d	CH_3	3,4-$(OCH_3)_2$
5e	OCH_3	3,4-$(OCH_3)_2$
5f	OC_2H_5	3,4-$(OCH_3)_2$

Table No. 1 Physical constants and analytical data of 5a-d & f

Compd No.	m.p. (^oC)	R_f	Yield (%)	Mol. formula (Mol. mass)	Found (Calcd) (%)			
					C	H	N	S
5a	160-161	0.72	60	$C_{24}H_{22}O_2SNF$ (407)	-	-	3.45 (3.68)	7.90 (7.86)
5b	171-172	0.76	67	$C_{24}H_{22}O_2SNCl$ (420.5)	-	-	3.12 (3.30)	7.61 (7.60)
5c	156-157	0.74	62	$C_{24}H_{22}O_2SNBr$ (465)	-	-	3.02 (3.89)	6.82 (6.88)
5d	125-126	0.72	70	$C_{25}H_{25}O_2SN$ (403)	74.32 (74.44)	6.22 (6.20)	3.50 (3.47)	7.92 (7.94)
5f	148-143	0.75	64	$C_{26}H_{27}O_3SN$ (433)	72.00 (72.05)	6.25 (6.23)	3.20 (3.23)	7.41 (7.39)

Table No. 2 Spectral data of 5a-d, f

Compd No.	OCH_3 (s, 3H)	C-2-H (1H, d, *J*=7)	C-3-H (1H, d, *J*=7)	C_8-XH	Aromatic protons (m)
5a	3.85	5.94	6.04	-	6.8-7.94
5b	3.85	5.92	6.12	-	6.82-7.90
5c	3.82	5.90	6.38	-	6.80-7.76
5d	3.87	5.84	6.24	1.92 (s, 3H)	6.76-7.74
5f	3.91	5.89	6.14	1.24 (t, J= 7Hz, 3H) 3.44 (q, J=7Hz, 2H)	6.82-7.82

Table No. 3Antimicrobial activity of 5a-f

Compd No.	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candidaalbicans*
5a	20 (0.90)	16 (0.67)	15 (1.07)
5b	14 (0.63)	16 (0.67)	14 (1.00)
5c	15 (0.68)	-	-
5d	10 (0.45)	-	12 (0.85)
5e	18 (0.81)	18 (0.75)	16 (1.14)
5f	-	15 (0.62)	10 (0.71)

Zone of Inhibitions are given in mm

Values in parentheses represent activity index

Zone of Inhibition of Gatifloxin for *Staphylococcus aureus* is 22 mm.

Zone of Inhibition of Natilmicin for *Pseudomonas aeruginosa* is 24 mm.

Zone of Inhibition of Fluconazole for *Candida albicans* is 14 mm.

Concentration of test and reference compounds were 100µg/disc