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Synthesis and anti-anxiety activity of some 7-chloro-5-phenyl-1,3-dihydro-1*H*, 3*H*-1,4-benzodiazepine-2-one derivatives in mice

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Abstract

Some new various aromatic aldehyde derivatives of 7-chloro-5-phenyl-1,3-dihydro-1H,1,4-benzodiazepine-2-one were synthesized and evaluated for their anti-anxiety activity by using elevated plus-maze method. The activity of all synthesized compounds were compared with diazepam which was used as standard drug. Compounds **4a, 4b, 4e, 4j** and **4k** were found to have significant activiity .

Keywords: 1,4-benzodiazepin-2-one, diazepam, elevated plus-maze apparatus, anti-anxiety activity.

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Introduction

1,4-Benzodiazepine-2-one derivatives were found to have potential anti-anxiety, antidepressant, anticonvulsant, anticancer and anti-inflammatory actvitiy[1-3]. The title compounds were synthesized by reacting 7-chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one with different types of aromatic aldehyde. The structure activity parameters revealed that electron withdrawing substituents such as chloro, bromo and fluoro at C-7 conferred significant anti-anxiety activity. Replacement of phenyl group at C-2 by other substituent lowered the pharmacological activity but substitution at C-3 did not studied widely. The aim of the presented work was concentrated on synthesizing various 3-(substitutedbenzylidene)-7-Chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one derivatives and evaluation for their anti-anxiety activity[4-8].

The use of this class of bioactive derivatives with CNS therapeutic value is not only confined to anxiety and other neuronal disorders as mentioned above but the minor structural modifications in their structures have produced a host of biologically active products namely chlordiazepoxide, diazepam, and nitrazepam to mention a few acting on a number of CNS

related disorders including sleep induction. The benzodiazepines interact with macromolecular membrane complex that has recognition sites for GABA (γ-amino butyric acid) at synapses in which GABA is a neurotransmitter as a primary site of action and a chloride ionophore. The proposal by Haefely and Costa Sternbach and Childres that benzodiazepines may produce their effects by enhancing GABAergic transmission provided an explanation for various other secondary alterations induced by these drugs in other transmitter systems [9-11]. Consequently, several types of benzodiazepine receptors, over a dozen, have been identified. The GABA$_A$ receptor is a ligand-gated chloride ion channel and is proteinaceous in nature which is anchored in the cell membrane and is pentomeric (**Fig. 1**).
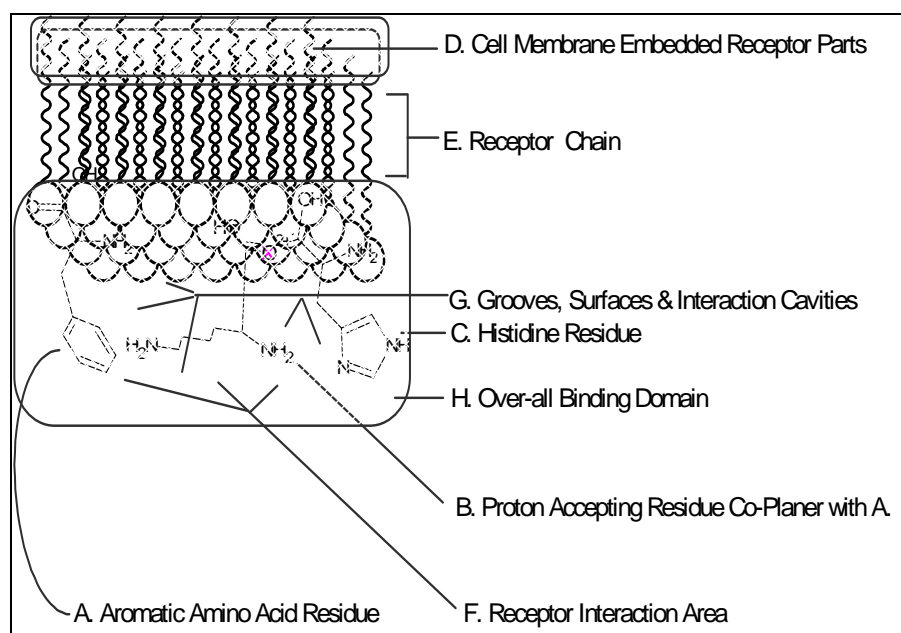

Figure 1. Receptor Mechanism model

Different receptor subtypes are located in different parts of the brain as well as scattered in spine [12]. The GABA$_A$ has profound bearings on the pharmacological response of the benzodiazepines. The 1,4-benzodiazepines are positive modulators or agonists and enhance the effect of GABA binding to GABA$_A$ sites to increase the chloride ion flux into the neuron. Thus, the diminishing receptor binding or inhibition of GABA synthesis can abolish benzodiazepine related side effects. The increased benzodiazepine binding caused by GABA also involves the chloride ion that act synergistically with GABA. The binding of drugs to GABA or chloride ionophore can allosterically modify the benzodiazepine receptors, classified as BZ$_1$ and BZ$_2$, and the drug may elicit their action by direct occupation of the benzodiazepine receptor BZ$_1$ or interaction with GABA-chloride ionophore by allosteric interaction complex. We approached to synthesize these compounds with modified structures for stereochemically flexible and interconvertible geometries with an aim for prolonged retention by increasing polarity, improved water solubility, lesser metabolic degradation, better brain impermeation with specified lipophilic endings and better pass-out abilities in metabolically-challenged subjects [13-16] .

The 3-substituted benzylidene derivative of 7-Chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one emerged as promising anti-anxiety agents which are analogous to chlordiazepoxide, oxazepam, diazepam, nitrazepam and lormetazepam in activity. The earlier structure-activity relationship studies on 1,4-benzodiazepin-5-phenyl-2-ones have revealed

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that compounds having electron withdrawing substituent like chloro, bromo, nitro, triflouromethyl and cyano groups at position C_7, small polar/hydrophilic substitutions at position C_3, and chloro, bromo or flouro substitutions at position $C_{2'}$ in the C_5 phenyl ring of the 1,4-benzodiazepine skeleton may enhance anti-anxiety activity whereas substitutions at all other positions except for position N_1 for methyl linked to the nitrogen atom (N-CH$_3$) will result in loss/decrease of activity along with the introduction of electron donors at any 1,4-benzodiazepine substitutions . Moreover, the replacement/removal of lipophilic phenyl group at position C_5 by other substituent or hydrogen also decreases the pharmacological activity. Interestingly, the other substitutable active methylene group containing position C_3 have not been studied widely in this class for their SAR which needed further elaboration with regard to the substituent effects on the pharmacological activity vis a vis the stereo-electronic behavior of the C_3 substituent's and the internal substitutional replacements with the group attached to C_3. Hence, we, concentrated on synthesizing some new 3-(substituted benzylidene) derivatives of 7-chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-N,N-benzodiazepine-2-one.

Materials and Methods

Chemistry
The Chemicals were supplied by E. Merck (Germany) and S.D Fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene-ethylacetate-formic acid (5:4:1) and benzene-methanol (8:2), the spots were located under iodine vapors and UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ^{1}H-NMR spectra were recorded or a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO-d$_6$/CDCl$_3$ and Mass spectra under electron impact conditions (EI) were recorded at 70 ev ionizing voltage with a VG Prospec instrument and are presented as m/z. Microanalysis of the compounds was performed on a Perkin-Elmer model 240 analyzer.

Synthesis of 2-chloro-N-(4-halophenyl) acetamide (1).
p-cloroaniline (25.5g, 0.2mole) was suspended in 250ml toluene used as a solvent and chloroacetylchloride (90.4g, 0.8 mole) was gradually added with stirring. The mixture was allowed for refluxing for 12 hours. The completion of reaction was indicated by TLC using silica gel as stationary phase and toluene: ethylacetate: formic acid (5:4:1) as a mobile phase. It was then cooled to room temperature. After the completion of the reaction, the mixture was added to crushed ice. The solid product obtained was separated and dried [17, 18].

Synthesis of N-(2-benzoyl-4-chlorophenyl)-2-chloroacetamide (2).
2-Chloro-N-(4-chlorophenyl) acetamide was dissolved completely in carbon tetrachloride, then mixed with benzoyl chloride in equi molar quantity. Finely the powdered anhydrous aluminium chloride was added with frequent shaking, during 10 minutes to the contents of the flask fitted a reflux condenser and then refluxed for 9 hours. The completion of reaction was indicated by TLC using silica gel as stationary phase and toluene: ethylacetate: formic acid (5:4:1) as a mobile phase then poured the mixture in the crushed ice. The solid product so obtained washed with aq. NaOH then water [19].

Synthesis of 7-chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one (3).
N-(2-benzoyl-4-chlorophenyl)-2-chloroacetamide (18.48g, 0.06mole) was dissolved in 340 ml of ethanol after that 17gm of hexamine and 2gm of ammonium chloride was added. Then

the reaction mixture was refluxed for 14 hours. Progress of the reaction was monitored by TLC using silica gel as stationary phase and toluene: ethylacetate: formic acid (5:4:1) as a mobile phase. From the reaction mixture 240 ml of the ethanol was distilled out and then the reaction mixture was cooled at room temperature. After that the reaction mixture was filtered out and then the HCl gas was passed into the filtrate containing 30ml isopropyl alcohol. Stirred the reaction mixture for 2 hours then dissolved in water, filtered the solid product and washed with chilled water [20].
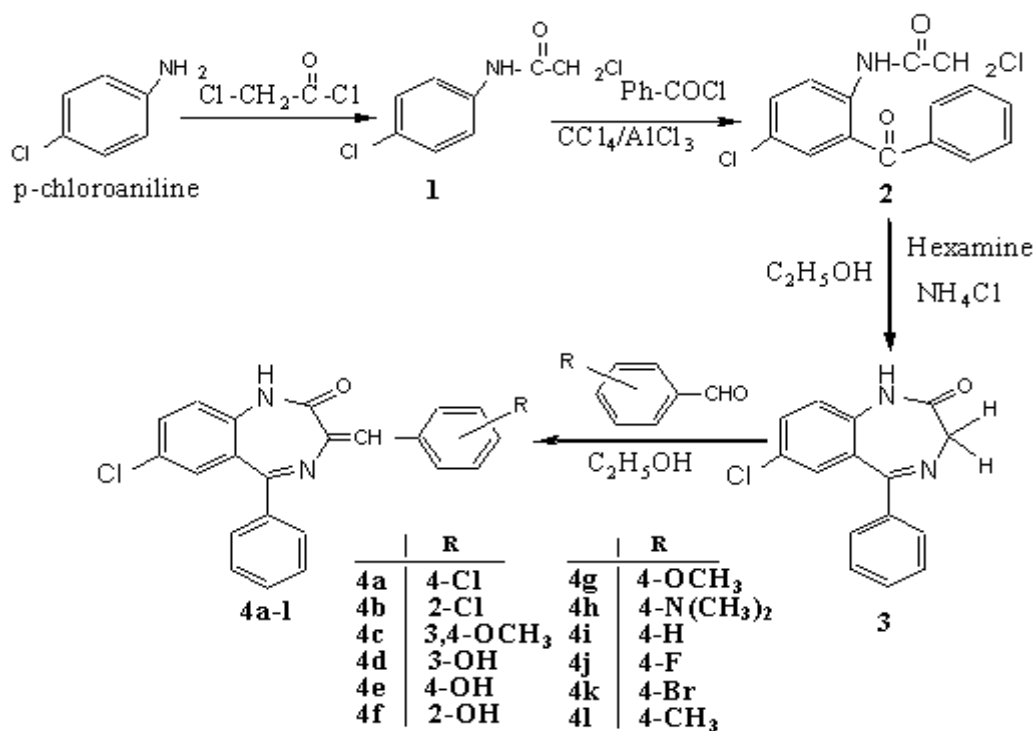

Figure 2: Scheme for synthesis of 7-chloro-5-phenyl-1, 3 -dihydro-1H, 3H- 1, 4-benzodia -zepine-2-one compounds (4a-l).

General procedure for Synthesis of various aromatic aldehyde derivatives of 7-chloro-5 phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one (4a-l)
7-chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one (8.12gm, 0.03mole) mixed with 4-chloro benzaldehyde (4.2g, 0.03mole) in 25 ml of absolute ethanol. After that few drops of piperidine (3-4 drops) was added and refluxed for 7 hr. The completion of reaction was indicated by TLC using silica gel as stationary phase and toluene: ethylacetate: formic acid (5:4:1) and benzene: methanol (9:1) as a mobile phase. After that the reaction mixture was cooled to room temperature during this process the solid product was obtained which was filtered and recrystallized from DMF and ethanol.

3-(4-chlorobenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4a).** %Yield 65, Solid; Mp. 225-226 ºC; R_f : 0.52 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3220-3070 (N-H$_{str}$), 3000-3100 (=C-H$_{str}$), 1700 (C=O$_{str}$), 1650 (C=N$_{str}$), 1610 (Ar C=C$_{str}$ Bz), 830(ArC-Cl $_{str}$), 720(Ar C-H$_{def}$ Bz); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm): 5.84 (s, 1H, ArN-H), 7.06-8.19 (7brm, 12H, Ar-H), 9.92(s, 1H, ArC-H); MS: m/z 392/393 (M$^+$/M$^+$+1); Anal. Calcd for $C_{22}H_{14}N_2O_1Cl_2$: C,67.19; H,3.59; N,7.12. Found: C,67.17; H,3.56; N,7.10.

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3-(2-chlorobenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4b).** %Yield 55, Solid; Mp. 231-232 ºC; R_f : 0.68 (toluene: ethylacetate: formic acid; 5: 4: 1); FTIR (KBr, cm^{-1}): 3300-3070 (N-H$_{str}$), 3000-3100 (=C-H$_{str}$), 1690 (C=O$_{str}$),1650 (C=N$_{str}$), 1610 (Ar C=C$_{str}$ Bz), 850 (ArC-Cl $_{str}$), 720(Ar C-H$_{def}$ Bz); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm): 5.82(s, 1H, ArN-H), 9.92(s, 1H, ArCH=), 7.10-8.19 (7brm, 12H, Ar-H); MS: m/z 392/393 (M$^+$/M$^+$+1); Anal. Calcd for $C_{22}H_{14}N_2O_1Cl_2$: C,67.19; H,3.59; N,7.12. Found: C,67.16; H,3.56; N,7.10.

*3-(3,4-dimethoxybenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one***(4c).** %Yield 70, Solid; Mp. 245-247ºC; R_f : 0.60 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3220-3080 (N-H$_{str}$) 3030-3110 (=C-H$_{str}$),1618 (Ar C=C$_{str}$ Bz),1440 (C=N$_{str}$), 1710 (C=O$_{str}$),1375 (C-N$_{str}$), 2850-2815(CH$_3$O-$_{str}$), 812(Ar C-Cl $_{str}$); ^{1}H-NMR (300 MHz, CDCl$_3$-d$_6$, TMS, δ ppm): 3.85 (s, 3H, Ar-OCH$_3$), 5.34 (s, 1H, Ar-NH), 6.88-7.85 (6brm, 11H, Ar-H), 9.88(s, 1H, Ar-CH); MS: m/z 418/419(M$^+$/M$^+$+1); Anal. Calcd for $C_{24}H_{19}N_2O_3Cl_1$: C,68.82; H,4.57; N,6.69. Found: C,68.80; H,4.56; N,6.68.

3-(3-hydroxybenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4d).** %Yield 72, Solid; Mp. 230-233ºC; R_f : 0.62 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}):3600 -3200 (ArO-H$_{str}$), 3220-3070 (N-H$_{str}$), 3010-3090 (=C-H$_{str}$), 720 (Ar C-H$_{def}$ Bz), 1688 (C=O$_{str}$), 1654 (C=N$_{str}$), 1603-1561 (Ar C=C$_{str}$ Bz), 1245(C-N$_{str}$), 764 (Ar C -Cl$_{st}$); ^{1}H-NMR (300 MHz, CDCl$_3$-d$_6$, TMS, δ ppm): 5.33(s,1H, Ar-NH), 7.01-7.98(12 H, 5brm, Ar-H), 9.92 (s, H, Ar-CH=), 10.98(s, 1H, Ar-OH); MS: m/z 374/375(M$^+$/ M$^+$+1); Anal. Calcd for $C_{22}H_{15}N_2O_2Cl_1$: C,70.50; H,4.03; N,7.47. Found: C,70.48; H, 4.02; N,7.45.

3-(4-hydroxybenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4e).** %Yield 66, Solid; Mp. 240-242ºC; R_f : 0.58 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3560-3200 (ArO-H$_{str}$), 3220-3070 (N-H$_{str}$), 3010-3100 (=C-H$_{str}$), 1680 (C=O$_{str}$), 1601 (C=N$_{str}$ 1514 (Ar C=C$_{str}$ Bz), 1241(C-N$_{str}$), 831 (ArC-Cl $_{str}$); ^{1}H-NMR (300 MHz, CDCl$_3$-d$_6$, TMS, δ ppm): 5.34 (s,1H, ArNH), 6.95-7.81(4brm,12H, Ar-H), 9.87(s, 1H, BzCH) 10.50(s, 1H, Ar-OH); MS: m/z 374/375(M$^+$/ M$^+$+1); Anal. Calcd for $C_{22}H_{15}N_2O_2Cl_1$: C,70.50; H,4.03; N,7.47. Found: C,70.48; H, 4.02; N,7.45.

3-(2-hydroxybenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4f).** %Yield 62, Solid; Mp. 235-237ºC; R_f : 0.58 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3580-3300(ArO-H$_{str}$), 3220-3070 (N-H$_{str}$), 3010-3050 (=C-H$_{str}$), 1662 (C=N$_{str}$),1650 (C=O$_{str}$), 1600 (Ar C=C$_{str}$ Bz), 1231(C-N$_{str}$) , 812 (ArC-Cl $_{str}$), 720 (Ar C-H$_{def}$ Bz); ^{1}H-NMR (300 MHz, CDCl$_3$-d$_6$,TMS, δ ppm): 5.33 (s, 1H, Ar-NH), 6.98-7.85 (7brm, 12H, Ar-H), 9.90 (s, 1H, Ar-CH=), 11.02(s, 1H, Ar-OH); MS: m/z 374/375(M$^+$/ M$^+$+1); Anal. Calcd for $C_{22}H_{15}N_2O_2Cl_1$: C,70.50; H,4.03; N,7.47. Found: C,70.48; H, 4.02; N,7.45.

3-(4-methoxybenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4g).** %Yield 67, Solid; Mp. 241-243ºC; R_f : 0.58 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3220-3080 (N-H$_{str}$), 3030-3110 (=C-H$_{str}$), 1618 (Ar C=C$_{str}$ Bz), 1540 (C=N$_{str}$), 1690 (C=O$_{str}$), 1375 (C-N$_{str}$), 2850-2815(CH$_3$O-$_{str}$), 812(Ar C-Cl $_{str}$); ^{1}H-NMR (300 MHz, CDCl$_3$-d$_6$, TMS, δ ppm): 3.85 (s, 3H, Ar-OCH$_3$), 5.34 (s, 1H, Ar-NH), 6.88-7.85 (6brm, 12H, Ar-H), 9.88(s, 1H, Ar-CH); MS: m/z 388/389(M$^+$/M$^+$+1); Anal.Calcd for $C_{23}H_{17}N_2O_2Cl_1$: C,71.04; H,4.41; N,7.20. Found: C,71.02; H,4.40; N,7.18.

3-(4-N, N-dimethyl benzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4h).** %Yield 58, Solid; Mp. 250-252ºC; R_f : 0.45 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr,

cm^{-1}): 3220-3070 (N-H$_{str}$), 3010-3100 (=C-H$_{str}$), 2950 (N-CH$_3$ $_{str}$), 1690 (C=O$_{str}$), 1650 (Ar C=C$_{str}$ Bz), 1540 (C=N$_{str}$), 1250 (C-N$_{str}$),780 (Ar C-Cl $_{str}$); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm): 2.95 (s, 6H, Bz-N-2×CH$_3$), 4.24 (bs,1H, N-H), 6.68-7.61 (4brm, 12H, Ar-H), 9.58 (s, 1H, Ar-CH); MS: m/z 401/402(M$^+$/M$^+$+1); Anal. Calcd for C$_{24}$H$_{20}$N$_3$O$_1$Cl$_1$: C,71.73; H,5.02; N,10.46. Found: C,71.72; H,5.01; N,10.43.

3-(benzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4i).** %Yield 55, Solid; Mp. 220-222ºC; R$_f$: 0.50 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3200-3070 (N-H$_{str}$), 3000-3100 (=C-H$_{str}$), 1720 (C=O$_{str}$), 1650 (C=N$_{str}$), 1620   (Ar C=C$_{str}$ Bz), 800 (ArC-Cl $_{str}$), 720(Ar C-H$_{def}$ Bz); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm): 4.97 (s, 1H, ArN-H), 7.06-8.19 (7brm,13H, Ar-H), 8.90 (s, 1H, ArCH); MS: m/z 358/359(M$^+$/M$^+$+1) ;Anal. Calcd for C$_{22}$H$_{15}$N$_2$O$_1$Cl$_1$: C,73.64; H,4.21; N,7.81. Found: C,73.6; H,4.20; N,7.80.

3-(4-floro benzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one **(4j).** %Yield 53, Solid; Mp. 210-212ºC; R$_f$: 0.40 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3220-3070 (N-H$_{str}$), 3000-3100 (=C-H$_{str}$), 1790 (C=O$_{str}$), 1650 (C=N$_{str}$), 1610   (Ar C=C$_{str}$ Bz), 850(ArC-Cl $_{str}$), 720(Ar C-H$_{def}$ Bz); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm): 5.84(s, 1H, ArN-H), 7.06-8.19 (6brm, 12H, Ar-H), 9.92(s, 1H, ArCH); MS: m/z 376/377 (M$^+$/M$^+$+1); Anal. Calcd for C$_{22}$H$_{14}$N$_2$O$_1$Cl$_1$F: C,70.12; H,3.74; N,7.43. Found: C,70.10; H,3.72; N,7.42.

3-(4-bromobenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one   **(4k).** %Yield 74, Solid; Mp. 214-215ºC; R$_f$: 0.44 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3310-3090 (N-H$_{str}$), 3000-3100 (=C-H$_{str}$), 1690 (C=O$_{str}$), 1650   (C=N$_{str}$), 1650   (Ar C=C$_{str}$ Bz), 750(ArC-Br$_{str}$), 720(Ar C-H$_{def}$ Bz); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm) : 4.95(s, 1H, ArN-H), 7.06-8.19 (7brm, 12H, Ar-H), 9.50 (s, 1H, ArCH); MS: m/z 437/438(M$^+$/ M$^+$+1); Anal. Calcd for C$_{22}$H$_{14}$N$_2$Cl$_1$Br$_1$O$_1$: C,60.37; H,3.22; N,6.40. Found: C,60.36; H,3.20; N,6.38.

*3-(4-methylbenzylidene)-7-chloro-5-phenyl-1,4-benzodiazepine-2-one***(4l).** %Yield 65, Soid; Mp. 221-223ºC; R$_f$: 0.56 (toluene: ethylacetate: formic acid ; 5: 4: 1); FTIR (KBr, cm^{-1}): 3250-3070 (N-H$_{str}$), 3050-3120 (=C-H$_{str}$), 1710 (C=O$_{str}$), 1640 (Ar C=C$_{str}$ Bz), 1540 (C=N$_{str}$), 1250 (C-N$_{str}$), 780 (Ar C-Cl $_{str}$); ^{1}H-NMR (300 MHz, DMSO-d$_6$, TMS, δ ppm): 1.75 (b, 3H, Bz-CH$_3$), 4.24 (bs, 1H, N-H), 6.68-7.61 (4brm, 12H, Ar-H), 9.50 (s, 1H, Ar-CH); MS: m/z 372/373(M$^+$/ M$^+$+1); Anal. Calcd for C$_{23}$H$_{17}$N$_2$O$_1$Cl$_1$: C,74.09; H,4.60; N,7.51. Found: C,74.08; H,4.58; N,7.50.

Anti-anxiety activity
Animals

Male mice weighing 25-30 g were housed in a cage with controlled room temperature at 22-25ºC. Food and water were available ad libitum. Tests were performed only after the mice had been acclimatized to the above environment for at least 7 days. Each mouse rece4ed a single i.p. injection of drug or vehicle and was tested once in the elevated plus-maze (EPM).

Elevated plus-maze apparatus

The apparatus comprised of two open arms (35× 5 cm) and two closed arms (30× 5× 15cm) that extended from a common central platform (5 × 5 cm). The floor and the walls of each arm were wooden and painted black. The entire maze was elevated to a height of 50 cm above floor level as validated and described by Lister [21, 22]. Testing was conducted in a

quiet room that was illuminated only by a dim light. Mice were given a single i.p. dose of various test compounds or diazepam 30 min before their placement on the EPM. To begin a test session, mice were placed on the open arm facing the center of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed arms were recorded during a 5-min test period. The percentage of open arm entries ($100 \times$ open/total entries) was calculated for each animal. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels.

Table 1. Anti-anxiety activity of synthesized compounds (4a-l)

Compd.	Dose (mg/kg;ip)	% preference of open arm	No. of entries in open arm	Average time spent in open arm
[y]Control	0.1ml/10g	20	2 ± 0.31	7.5 ± 1.14
4a	25	55.33	7 ± 0.10	11.1 ± 0.23
4b	25	48.00	5 ± 0.45	9.2 ± 0.12
4c	25	30.66	3 ± 0.19	7.6 ± 1.11
4d	25	33.33	3 ± 0.30	7.7 ± 1.23
4e	25	41.66	4 ± 0.39	8.7 ± 0.45
4f	25	40.12	4 ± 0.65	8.2 ± 0.10
4g	25	38.00	3 ± 0.19	7.6 ± 0.65
4h	25	40.32	4 ± 0.31	7.8 ± 0.22
4i	25	37.32	3 ± 0.30	7.7 ± 1.23
4j	25	64.95	7 ± 0.79	12.0 ± 0.45
4k	25	54.62	6 ± 0.85	10.1 ± 1.12
4l	25	38.42	4 ± 0.42	7.9 ± 0.82
Diazepam	25	66.66	8 ± 0.75	13.6 ± 0.54

[y] 1% w/v gum acacia solution was used as vehicle; Six animals were used in each group;
Standard drug: Diazepam

Statistics
Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Tukey test. $P<0.05$ was considered significant. All data are expressed as mean $\pm$ standard error of mean (S.E.M.).

Results and Discussion

Chemistry
The protocol of synthesized compounds (**4a-4l**) was given in Fig. 2.

Anti-anxiety activity
Synthesized compounds (**4a-l**) were screened for anti-anxiety activity by elevated plus-maze apparatus using diazepam as a standard drug. The parameters used to assess anti-anxiety activity were given first preference of mouse to open or enclosed arm, number of entries in open and enclosed arms and average time spent by animals in each arm (Average time=total duration in the arm/number of entries) was calculated by elevated plus-maze apparatus in albino mice. All the synthesized compounds and standard drug were given at the dose of 25mg/kg intraperitoneally. The compounds **4a, 4b, 4e, 4j and4k** showed the good % preference to open arms as compared to standard drug (diazepam) and the compound **4c, 4d,**

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4f, 4g, 4h and **4l** were found to have less% preference to open arms as compared to standard drug.

Conclusion

Compound **4a, 4b, 4h** and **4j** were exhibited significant anti-anxiety activity as compared to diazepam. Anti-anxiety data of synthesized compounds revealed that compound possessing electron withdrawing group were found to good activity in comparison with standard drug (diazepam).The data were shown in Table 1.

Acknowledgement

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