Synthesis Of Some Novel C_3 Substituted New Diazo-[1,4]-Benzodiazepine-2-One Derivatives As Potent Anticonvulsants

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Abstract

A number of new diazonium salt derivatives of 7-chloro-5-phenyl-3-(phenyl-n-substituted)-diazo-1,3-dihydro-benzo[e][1,4]-diazepin-2-one (IVa-l) from 7-chloro-5-phenyl-1,3-dihydro-benzo[e]-[1,4]-diazepin-2-one (III) with diazonium salts (phenyl diazo salt derivatives) were prepared and tested for anticonvulsant activity. The compounds provided significant protection against maximal electroshock induced seizures (MES) and seizures indicated by *sc* pentetrazole administration (*sc* PTZ) at 300 mg/kg after 0.5 h. The compounds IVb, IVd, and IVf were active in MES and *sc* PTZ indicated seizure. A conformational study of these derivatives was undertaken to explore the mechanistic details of the mode of action and their interlinked, interconvertible and energetically favored geometrical configurations acting on the receptor site.

Keywords: Diazo-1,4-benzodiazepin-2-one; anticonvulsant activity; geometrical configurations; phenytoin; pentylene tetrazole (PTZ).

1. Introduction

Benzodiazepines are bicyclic heterocyclic compounds possessing various types of biological activities over varied N,N-positioned skeletal types [1-3]. Among the large number of benzodiazepines that have been synthesized, the members of 1,4-N,N-benzodiazepine group have shown sufficient pharmacological activity to warrant introduction as new drugs or templates for tranquilizing, muscle-relaxant, anticonvulsant, and sedative effects while all other benzodiazepine derivatives have marginal or no CNS related bioactivity. The use of this class of bioactive derivatives with CNS therapeutic value is not only confined to anxiety and other neuronal disorders 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 [4, 5], Sternbach and Childress [6, 7] 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. Consequently, several types of benzodiazepine receptors, over a dozen, have been identified [8, 9]. The benzodiazepines synergistically increase the effects of ionotophoretically applied synaptic $GABA_A$ receptor subtype and, similarly, elevation of brain GABA transaminase inhibitors increases the electrophysiologic effects of benzodiazepines. 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. Different receptor subtypes are located in different parts of the brain as well as scattered in spine. 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. The metabolism of these classes of compounds has been extensively studied and methods for their analytical detection and blood levels determination have been reported while studying the neurotoxicity associated. 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 [10-13].

2. Methods

2.1 Chemistry

Chemicals were procured from E. Merck (India) and S. D. Fine Chemicals (India). Melting points were taken in open capillary tubes and are uncorrected. Microanalysis of the compounds was done on a Perkin-Elmer model 240 analyzer (Perkin-Elmer, USA) and the values were found within ± 0.4 % of the theoretical values. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (λ max in cm^{-1}) (Perkin-Elmer, USA) and 1H NMR spectra were recorded on a Varian E-360 MHz (Perkin-Elmer, USA) or Bruker spectrometer DPX-300MHz (Bruker, Germany) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 instrument (Jeol, Japan) fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with the assigned structures. The progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent. All solvents were distilled prior to use.

2.2 General method for the preparation of various diazonium salts

2.2.1 Preparation of 2-chloro-N-(4-halophenyl) acetamide (I)

p-Haloaniline (0.2 mole) was suspended in toluene used as a solvent and chloroacetylchloride (0.8 mole) was gradually added with stirring. The mixture was allowed for refluxing for 12 hours. After the completion of reaction, content was cooled to room temperature and poured over crushed ice. The solid product obtained was separated and dried.

2.2.2 Preparation of N-(2-benzoyl-4-chlorophenyl)-2-chloroacetamide (II)

2-Chloro-N-(4-chlorophenyl) acetamide **(I)** was dissolved in carbontetrachloride and benzoyl chloride was added in equimolar amounts. A finely powdered, fresh anhydrous aluminum chloride was added in small portions with continuous stirring during 10 minutes period and reaction mixture was refluxed for 10 hours. After the completion of reaction, content was poured over crushed ice. The solid obtained was washed with aqueous sodium hydroxide solution and with freshly cooled water repeatedly [20].

2.2.3 Preparation of 7-chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one (III)

The N-(2-benzoyl-4-chlorophenyl)-2-chloroacetamide **(II)** (18.48gm, 0.06 mole) was dissolved in 340 ml of anhydrous ethanol, 17.0 g of hexamine and 2.0 g of ammonium chloride was added [24] and reaction mixture was refluxed for 14 hrs. After the completion of reaction, ethanol was distilled off. The rest of the content was allowed to cool at room temperature, filtered and hydrochloric acid gas was passed into filtrate containing 30 ml isopropyl alcohol for diluting. The content was further stirred for 2 more hrs and poured into cold water; solid filtered out and washed again repeatedly with prechilled water.

2.3 General method for the preparation of diazonium salt derivative of 7-chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one (IVa-l)

The primary aromatic amine was dissolved in 150 ml of water containing 2.5 equivalents of hydrochloric acid by application of heat, if necessary. The mixture was cooled over crushed ice-water and maintained at $0^{\circ}C$. An aqueous solution of sodium nitrite was added portion wise under gentle stirring and after allowing 3-4 minutes for reaction to complete which was indicated by potassium iodide-starch paper as also the excess of nitrous acid in the reaction mixture [28] the resulting diazonium salt (phenyl diazo salt derivatives) was added slowly under moderate stirring to a pre-cooled solution of 7-chloro-5-phenyl 1,4-benzodiazepine-2-one **(IV)** (8.12gm, 0.03 mole) in 25 ml ethanol containing sodium acetate (2 g) and stirred for 2 hr. The reaction mixture was poured into ice cold water and stirred again for 2 more hrs. The final product was obtained as colored azo-dye which was separated, filtered and washed with cold water, dried under vacuum, recrystallized in aqueous ethanol and acetone to give the crystals.

7-Chloro-3-[(phenyl) diazenyl]-5-phenyl-1,3-dihydro-1H, 3H-1,4-benzodiazepin-2-one **IVa**

Yield: 60%; m.p.: 240-243; R_f value: 0.66; Log P: 6.16; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3070 (N-H_{str}), 2345-2290 (N=N-$_{str}$), 2090 (-COCH N=N_{str}), 2020 (-CH N=N_{str}), 1655-1710 (C=O_{str}), 1600 (Ar C=C_{str} Bz), 1490 (C=N_{str}), 1250 (C-N_{str}), 755 (Ar C-H_{def} Bz), 691 (Ar C-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 3.72 (s, 1H, CH), 4.95 (s, 1H, Ar-NH),7.25-8.37 (6 brm, 13H, Ar-H); MS [m/z]: 374/376 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{15}ClN_4O$: C, 67.29; H, 4.03; N, 14.95;. Found: C, 67.25; H, 4.02 N, 14.93.

7-Chloro-3-[(4-chloro-phenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-enzodiazepin-2-one **IVb**

Yield: 65%; m.p.: 244-246; R_f value 0.60; Log P: 6.72; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3070 (N-H_{str}), 2270 (N=N-$_{str}$), 2050 (-CH-N=N_{str}), 1700 (C=O_{str}), 1640 (Ar C=C_{str} Bz), 1550 (C=N_{str}), 1156 (C-N_{str}), 834 (ArC-H_{def}Bz), 750 (ArC-Cl $_{str}$), 650 (ArC-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 3.71 (s, 1H, CH), 5.23 (s, 1H, Ar-NH), 7.11-8.01 (6 brm, 12H, Ar-H); MS [m/z]: 409/411 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{14}Cl_2N_4O$: C, 61.63; H, 3.45; N, 13.69. Found: C, 61.63; H,3.43; N, 13.67.

7-chloro-3-[(4-bromophenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepi-2-one **IVc**

Yield: 70%; m.p.: 241-243; R_f value 0.55; Log P: 6.99; FTIR (KBr) [cm^{-1}]: 3220-3070 (N-H_{str}), 2270-2280 (N=N-$_{str}$), 2250 (-CH-N=N_{str}), 2090-2100 (-CO-CHN=N_{str}), 1690 (C=O_{str}), 1580 (Ar C=C_{str} Bz), 1540 (C=N_{str}), 1250 (C-N_{str}), 807 (ArC-Cl $_{str}$), 720 (Ar C-H_{def} Bz), 640 (Ar C-Br_{str});^{1}H-NMR (DMSO-d6) d [ppm]: 2.90 (s, 1H, CH), 5.58 (s, 1H, Ar-NH), 6.51-8.20(6 brm, 12H, Ar-H); MS [m/z]: 453/455 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{14}BrClN_4O$: C, 55.59; H, 3.11; N, 12.35. Found: C, 55.57; H,3.11; N, 12.33.

7-chloro-3-[(4-florophenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepi-2-one **IVd**

Yield: 58%; m.p.: 250-252; R_f value 0.45; Log P: 6.32; FTIR (KBr) ν_{max} [cm^{-1}]: 3270-3050 (N-H_{str}), 2170-2280 (N=N-$_{str}$), 2250 (-CH-N=N_{str}), 2090-2100 (-CO-CHN=N_{str}), 1690 (C=O_{str}), 1580 (Ar C=C_{str} Bz), 1540 (C=N_{str}), 1250 (C-N_{str}), 950 (ArC-F $_{str}$), 730 (Ar C-H_{def} Bz), 650 (Ar C-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 2.50 (s, 1H, CH), 5.45 (s, 1H, Ar-NH), 7.01-8.03 (6 brm, 12H, Ar-H); MS [m/z]: 392/394 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{14}ClFN_4O$: C, 64.21; H, 3.59; N, 14.26. Found: C, 64.20; H,3.58; N, 14.23.

7-chloro-3-[(3-chlorophenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepi-2-one **IVe**

Yield: 60%; m.p.: 230-232; R_f value 0.50; Log P: 6.72; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3070 (N-H_{str}), 2250 (N=N-$_{str}$), 2090-2100 (-CO-CHN=N_{str}), 2020 (-CH-N=N_{str}), 1690 (C=O_{str}), 1590 (Ar C=C_{str} Bz), 1500 (C=N_{str}), 1290 (C-N_{str}), 750 (ArC-Cl $_{str}$), 705 (Ar C-H_{def} Bz), 650 (ArC-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 2.04 (s, 1H, CH), 5.10 (s, 1H, Ar-NH), 6.05-7.99 (6 brm, 12H, Ar-H); MS [m/z]: 409/411 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{14}C_{l2}N_4O$: C, 61.63; H, 3.45; N, 13.69. Found: C, 61.60; H,3.43; N, 13.67.

7-chloro-3-[(4-nitrophenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepi-2-one **IVf**

Yield: 55%; m.p.: 255-256; R_f value 0.40; Log P: 6.02; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3050 (N-H_{str}), 2400 (-CH-N=N_{str}), 2270-2280 (N=N-$_{str}$), 1590- 1670 (C=O_{str}), 1620 (Ar C=C_{str} Bz),1540 (C=N $_{str}$), 1386 (N-$CH_{3\ def}$), 1119 (C-N_{str}), 750 (ArC-Cl_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 2.30 (s, 1H, CH), 4.99 (s, 1H, Ar-NH), 6.02-8.06 (6 brm, 12H, Ar-H); MS [m/z]: 419/421 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{14}ClN_5O_3$: C, 60.08; H, 3.36; N, 16.68. Found: C, 60.06; H,3.34; N, 16.67.

7-chloro-3-[(4-N,N-dimethylphenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiaze pin -2-one **IVg**

Yield: 58%; m.p.: 260-260; R_f value 0.66; LogP: 6.02; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3070 (N-H_{str}), 2950 (N-$CH_{3\ str}$), 2500 (-CH-N=N_{str}), 2270-2280 (N=N-$_{str}$), 1690- 1670 (C=O_{str}), 1610 (Ar C=C_{str} Bz),1540 (C=N $_{str}$), 1386 (N-$CH_{3\ def}$), 1119 (C-N_{str}), 821 (ArC-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 2.08-2.50 (6H,2s, N-2XCH_3), 3.01 (s, 1H, CH), 4.95 (s, 1H, Ar-NH), 6.77-8.02 (6 brm, 12H, Ar-H); MS [m/z]: 417/419 (M^+/ M^++2). Anal. Calcd. for $C_{23}H_{20}ClN_5O$: C, 66.10; H, 4.82; N, 16.76. Found: C, 66.08; H, 4.80; N, 16.73.

7-chloro-3-[(2,4-dinitrophenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one **IVh**

Yield: 50%; m.p.: 262-261; R_f value 0.38; Log P: 6.45; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3050 (N-H_{str}), 2350 (-CH-N=N_{str}), 2270-2280 (N=N-$_{str}$), 1590- 1670 (C=O_{str}), 1620 (Ar C=C_{str} Bz),1540 (C=N_{str}), 1386 (N-$CH_{3\ def}$), 1119 (C-N_{str}), 750 (ArC-Cl_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 3.01 (s, 1H, CH), 4.95 (s, 1H, Ar-NH), 6.77-8.02 (6 brm, 12H, Ar-H); MS [m/z]: 464/466 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{13}ClN_6O_5$: C, 54.26; H, 2.82; N, 18.08. Found: C, 54.24; H, 2.80; N, 18.06.

7-Chloro-3-[(2-methylphenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one **IVi**

Yield: 65%; m.p.: 245-247; R_f value 0.52; Log P: 6.65; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3070 (N-H_{str}), 2930 (Ar-CH_3), 2190-2150 (N=N-$_{str}$), 2050-2010 (-CH N=N_{str}), 1700 (C=O_{str}), 1650 (Ar C=C_{str} Bz), 1550 (C=N $_{str}$), 1375 (C-N_{str}), 630 (ArC-Br_{str}). ^{1}H-NMR (DMSO-d6) d [ppm]: 1.72 (3H, brs, Bz,), 2.58 (1H, Ar-CH_3), 5.05 (s, 1H, Ar-NH), 7.12-8.01 (6 brm, 12H, Ar-H); MS [m/z]: 388/390 (M^+/ M^++2). Anal. Calcd. for $C_{22}H_{17}ClN_4O$: C, 67.95; H, 4.41; N, 14.41. Found: C, 67.93; H, 4.40; N, 14.40.

7-Chloro-3-[(2-chlorophenyl)diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one **IVj**

Yield: 62%; m.p.: 240-242; R_f value 0.48; Log P: 6.72; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3070 (N-H_{str}), 2290 (N=N-$_{str}$), 1650 (Ar C=C_{str} Bz), 1540 (C=N_{str}), 1250 (C-N_{str}), 766 (ArC-Cl_{str}), 600 (ArC-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 3.17 (s, 1H, Ar-CH_3), 5.06 (s, 1H, Ar-NH), 7.01-8.30 (6 brm, 12H, Ar-H); MS [m/z]: 409/411 (M^+/ M^++2). Anal. Calcd. for $C_{21}H_{14}C_{l2}N_4O$: C, 61.63; H, 3.45; N, 13.69. Found: C, 61.62; H, 3.42; N, 13.66.

7-Chloro-3-[(3-methylphenyl) diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one **IVk**

Yield: 58%; m.p.: 234-236; R_f value 0.55; Log P: 6.65; FTIR (KBr) ν_{max} [cm^{-1}]: 3150-3090 (N-H_{str}), 2930 (Ar-CH_3), 2190-2150 (N=N-$_{str}$), 2050-2010 (-CH N=N_{str}), 1700 (C=O_{str}), 1650 (Ar C=C_{str} Bz), 1550 (C=N_{str}), 1375 (C-N_{str}), 630 (ArC-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 1.72 (brs, 3H, Bz,), 2.60 (s, 1H, Ar-CH_3), 5.02 (s, 1H, Ar-NH), 7.14-8.10 (6 brm, 12H, Ar-H); MS [m/z]: 388/390 (M^+/ M^++2). Anal. Calcd. for $C_{22}H_{17}ClN_4O$: C, 67.95; H, 4.41; N, 14.41. Found: C, 67.93; H, 4.40; N, 14.40.

7-Chloro-3-[(4-methylphenyl) diazenyl]-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepin-2-one **IVl**

Yield: 60%; m.p.: 233-235; R_f value 0.54; Log P: 6.65; FTIR (KBr) ν_{max} [cm^{-1}]: 3220-3050 (N-H_{str}), 2950 (Ar-CH_3), 2250-2150 (N=N_{str}), 2050-2010 (-CH N=N_{str}), 1700 (C=O_{str}), 1650 (Ar C=C_{str} Bz), 1550 (C=N_{str}), 1375 (C-N_{str}), 630 (ArC-Br_{str}); ^{1}H-NMR (DMSO-d6) d [ppm]: 1.52 (brs, 3H, Bz,), 2.58 (s, 1H, Ar-CH_3), 5.02 (s, 1H, Ar-NH), 7.14-8.10 (6 brm, 12H, Ar-H); MS [m/z]: 388/390 (M^+/ M^++2). Anal. Calcd. for $C_{22}H_{17}ClN_4O$: C, 67.95; H, 4.41; N, 14.41. Found: C, 67.93; H, 4.40; N, 14.40.

2.4 Anticonvulsant Activity

Male albino mice weighing between 25-30 g were used for grandmal type of epileptic conditions [21]. They were housed in groups of three or four mice per cage under standard laboratory conditions maintained at 25^{o}C and humidity at 45-50% for one week before anti-convulsant activity was carried out by using both Maximal Electroshock (MES) and PTZ animal models**.** Food and water were withdrawn prior to the experiment. All the result which obtained in both the method was statistically analyzed and results expressed as mean ± S.E.M.

2.4.1 Maximal Electro Shock (MES) Model

The MES induced convulsions were divided into five phases as: Tonic flexion, Tonic extension, clonic convulsion, Stupor, Recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase. The standard drug phenytoin and test compounds were dissolved (20 mg/kg) in propylene glycol and administered intraperitoneally 30 min. before the test using electroconvulsometer for the observation by electroshock seizure method. Animals were subjected to supra maximal electroshock of 50 mA, 60Hz alternating current from a convulsometer for 0.2 sec through a pair of electrodes attached to each ear. The duration of the tonic hind limb extensor phase, clonic phase and the number of animals protected from convulsions were noted. The Results were shown in Table 1.

All the tested compounds showed a reduction in the duration of tonic hind limb extensor phase. A complete abolition of hind limb tonic extensor was considered as 100% protection while the abolition of the hind limb tonic extensor spasm was recorded as an increased anticonvulsant activity [22, 23].

2.4.2 PTZ Animal Model

Pentylene tetrazole (PTZ) produced clonic convulsions prevented by drugs effective in absences of seizures in this model represented action on seizures focus. PTZ (Sigma-Aldrich, Milwaukee WI, USA) was used to produce convulsion and Diazepam (Ranbaxy Laboratories, New Delhi, India) was used as standard drug dissolved in 2% gum acacia suspension [24, 25]. The animals were divided into fourteen groups of six mice in each group. The convulsions were induced 1 hr after administration of the standard drug or the test compounds by i.p. injection of (80 mg/kg) which was dissolved in saline at a volume of 0.1 ml/10gm body weight ratios. The time needed for the development of unequivocal sustained clonic seizure activity involving the limbs (isolated myoclonic jerks or other preconvulsion chewing behavior were not counted) was carefully noted. The duration of seizure was also recorded. The seizures free duration for a period of 1 hour was taken as protection. The number of animals protected in each group was also recorded and percent protection was calculated. The data were summarized in Table 2.

3. Results and Discussion

3.1 Chemistry

The novel 3-diazo derivative of 7-Chloro-5-phenyl-1,3-dihydro-1H,3H-1,4-benzodiazepine-2-one emerged as promising anticonvulsant agents with CNS anti-depressant activity which are analogous to oxazepam, diazepam and lormetazepam in activity. The earlier structure-activity relationship studies [14-16] on 5-phenyl-1,4-benzodiazepin-2-ones have revealed that the 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-convulsant 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 donators at any 1,4-benzodiazepine substitutions. The various derivatives of substituted new Diazo-[1,4]-benzodiazepine-2-one were synthesized as shown in Figure 1.

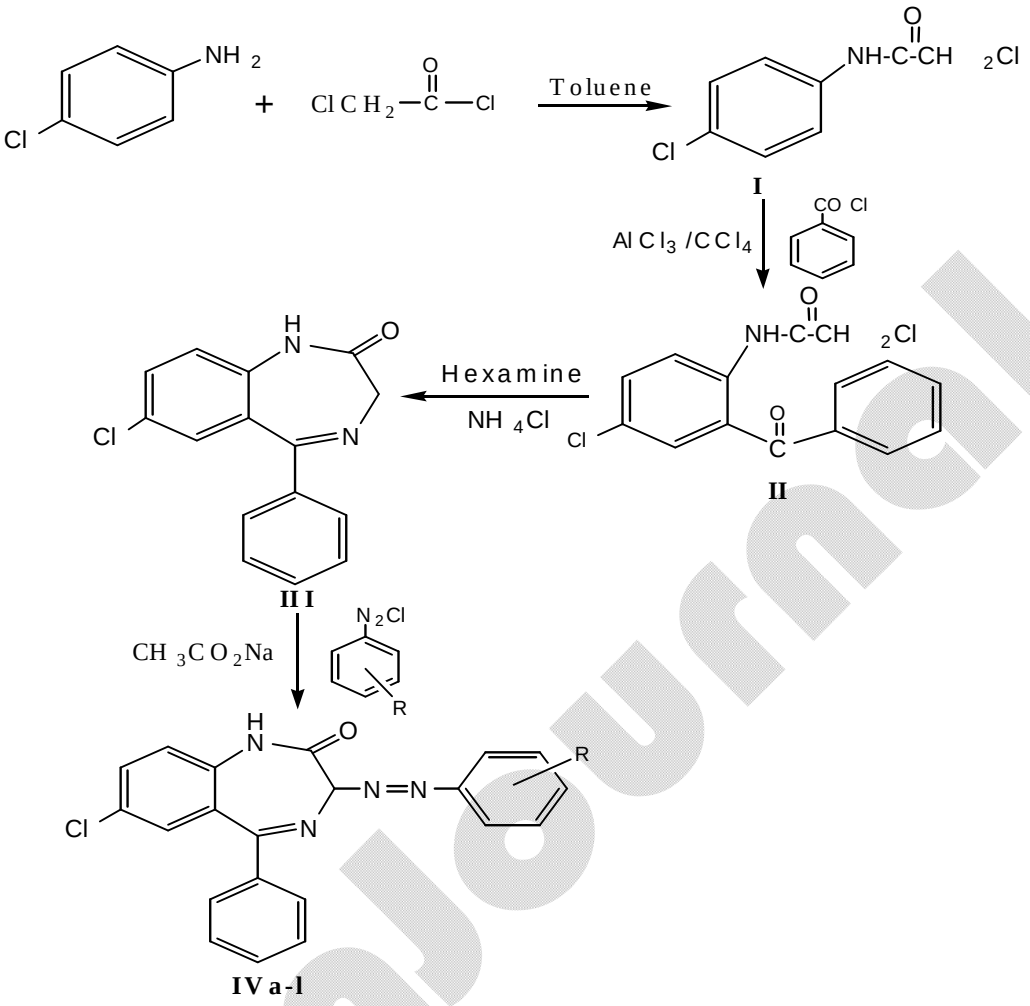

Figure 1. Synthesis protocol for novel C_3 substituted new Diazo-[1,4]-benzodiazepine-2-one derivatives.

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 7-chloro-3-diazo derivatives of 5-phenyl-1,3-dihydro-[1H,3H]-1,4-N,N-benzodiazepine-2-one compounds with C_3 substitutions also displaying the internal substitution patterns at positions $C_{2''}$, $C_{3''}$ and $C_{4''}$ and their stereo-electronic effects on their anticonvulsant activities.

3.2 Anticonvulsant activity

The products with diazonium moiety as 3-(4-chlorodiazonium salt) **(IVb)**, 3-(4-floro diazonium salt) **(IVd)**, 3-(4-nitrodiazonium salt) **(IVf)** and 3-(2-chlorodiazonium salt) of 7-Chloro-1,4-benzodiazepine-2-one-5-phenyl **(IVj)** had shown excellent anticonvulsant activity in comparison with standard drug diazepam, used in the PTZ animal model. The substitution of aniline containing moiety at position C_4 with flouro, chloro, nitro group and 2-chloro was employed. The substitution of aniline moiety at C_4 in the synthesis of diazonium salt enhanced the activity in case of corresponding compounds **(IVb**, **IVd**, **IVf)**. Since these compounds **(IVb**, **IVd**, **IVf**) contained electron withdrawing group at position C_4, showed the higher order of activity as predicted earlier. The higher-end biological activity of the compound **(IVj**) is due to presence of chlorine substitution at position C_2. So the compounds **(IVb, IVd, IVf, IVj)** were more effective than compounds **(IVc, IVe, IVg, IVi**). The compounds **(IVb, IVd, IVf, IVj**) were further studied and have shown anti-anxiety, anti-depressant activities and neurotoxicity elevation effects. Some of the

compounds possessed better biological activity profile in comparison to standard drugs on pharmacological testing. Other compounds have also shown moderate activity.

3.3 Structure activity relationship and molecular modeling

At the benzodiazepine receptor after primary recognition of the pharmacophore, the receptor undergoes a shift in conformation either to an agonist or an inverse agonist state based on electronic, hydrophobic and steric characteristics of the incoming ligand. This conformational change allosterically modulates the binding of GABA to the receptor. An agonist facilitates the change for binding of GABA which is observed as anticonvulsant in biological activity measures whereas the conformation that inhibits the binding of GABA to receptor behaves pro convulsant. An antagonist binds to benzodiazepine receptor but does not undergo any conformational change. The conformational changes are key factor in defining the benzodiazepine actions. The minimal requirements for exhibiting anticonvulsant activity is the presence of an aromatic ring-A undergoing π/π stacking within the receptor with an aromatic amino acid residue and a proton accepting group existing in the plane of this aromatic ring interacting with histidine residue on the receptor. The unsubstituted C_5 phenyl contributes to the hydrophobic interaction and is necessary for minimal activity. An electron-rich substitution favors an increased activity.

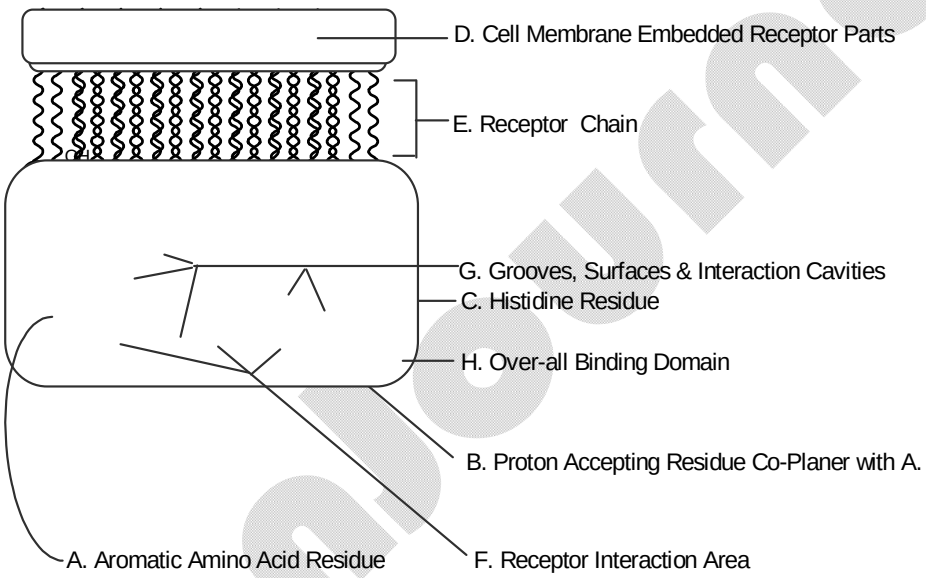

Figure 2: Receptor Model.

The substitution of the active methylene at position C_3 is reported sterically unfavorable for agonist activity but has no effects on the antagonistic action. The seven-membered ring B may adopt one of the two 9 or allowed more possible, energetically favorable boat conformations which are enantiomeric in nature and are interconvertible in solution at room temerature. In the sterically defined C_3 product with S conformation wherein the C_3 carbon positions itself opposite and away from the C_7 substitution of ring A seemed to be the active conformer [17]. Any introduction of the substitution at C_3 will stabilize this geometry in S conformer as studied in detail for diazepam [18, 19]. Thus, it has been suggested that the ring B conformation is important in activity elicitation rather than the absolute configuration of the molecule. In our series of compounds **(IVa-l**), a conformational study of the compounds showed three tautomeric forms of the products along with rigid conformation wherein C_3 carbon is placed away from the flat ring-A and its substitution at position C_7 for the most active derivatives **(IVb, IVd**, **IVf**) while the internal substitution on the diazonium group attached to the C_3 carbon seems to be following over all benzodiazepin-2-one pattern of electron withdrawing groups exhibiting increased activity. The derivatives **(IVc, IVe, IVg, IVh**) showed reduced activities while products with electron rich/donor groups showed no or highly diminished biological activity.

Table 1: Anticonvulsant activity of compound **(IVa-l**) by MES model.

Group (n=6)	Compound	Dose (mg/kg)	Protection (%)	Recovery
1	C^{+}	0.1	0	-
2	IVa	20	50	Late
3	IVb	20	100	Very soon
4	IVc	20	67	Late
5	IVd	20	100	Very soon
6	IVe	20	60	Late
7	IVf	20	100	Very soon
8	IVg	20	83	Soon
9	IVh	20	83	Soon
10	IVi	20	62	Late
11	IVj	20	65	Late
12	IVk	20	83	Soon
13	IVl	20	80	Soon
14	P^{++}	20	100	Very soon

C^{+} means Control (vehicle unit) (in ml/10gm) and P^{++} means Phenytoin (Reference drug); n is the number of animals in each group

Table 2: Anticonvulsant activity of **(IVa –IVl**) by PTZ animal model.

Group (n=6)	Compound	Dose (mg/kg)	Protection (%)	Duration of seizure (sec)
1	C^{+}	0.1	0	311±0.577
2	IVa	20	60	11±0.477
3	IVb	20	100	0
4	IVc	20	80	8±0.576
5	IVd	20	100	0
6	IVe	20	60	11±0.577
7	IVf	20	100	0
8	IVg	20	50	14±0.842
9	IVh	20	0	12±0.842
10	IVi	20	80	9±0.577
11	IVj	20	60	11±0.477
12	IVk	20	85	8±0.200
13	IVl	20	80	8±0.576
14	$D^{\ddagger}$	20	100	0

C^{+} means Control (vehicle unit) (in, ml/10gm), $D^{\ddagger}$ means Diazepam (Reference drug); n is number of animals in each group.

4. Competing Interests

The authors declare that they have no competing interests.

5. Authors' Contributions

MR and BA developed the project and supervised preparation of the manuscript; RM assisted with preparation of the manuscript, sample collection and data analysis; AH assisted with spectral analysis and biological activity.

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