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Molecular Docking, Synthesis and Evaluation of Antianxiety and Anticonvulsant potential of some Novel 3-(substituted benzylidene)-5-phenyl-7-nitro-1, 3-dihydro-1*H*, 3*H*-1,4-Benzodiazepine-2-one

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ABSTRACT

The given title proposed with the aim to synthesize some novel benzylidene substituted benzodiazepine analogue to evaluate $GABA_A$ receptor inhibitory activity. Molecular docking simulation studies were also performed on life molecular suite software. All compounds found to possess good dock score, but varied in formation of intermolecular complexes at the receptor site. Bonding interactions like hydrogen bonds, hydrophilic bonds and *pi*-interactions of the ligand library with receptor binding site were considered as parameter for synthesis of selected and potent molecules. Diazepam to be selected as the reference compound and included in ligand library to ran simultaneously. Diazepam found to develop two hydrogen bonds with TYR 106A amino acid residue whereas π-π stacking interaction with TYR 106A and PHE 3A protein moiety. It is capable to form hydrophobic interactions with PHE 104A, PHE 3A, VAL 4A, ILE 32A, TYR 6A, TYR 5A, LYS 6A, and ARG 14A. Most of the proposed compounds interacted in similar manner as diazepam. Dock score of diazepam and tested compounds were also comparable. All the synthesized molecules were characterized by IR, ^1H-NMR and Mass spectrometric data. Elevated plus maze test and Maximal electroshock methods were selected to evaluate antianxiety and anticonvulsant activity respectively. Substitution of methoxy, ethoxy and dimethyl amino groups on benzylidene benzene found to have much therapeutic value but halogen and nitro substitution were not active even unable to show interaction forces with target receptor. Isopropyl group also found to possess potential effect on CNS.

Keywords: Benzodizepines. $GABA_A$, Molecular docking, Antianxiety, Antiepileptic.

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INTRODUCTION

Preliminary docking simulation computational method that permits all the aspects of drug discovery today, have been performed to get better understanding of peculiar chemical structural that influence affinity to interact specific receptor of the interest and gaining importance to be an initial step better than direct weight lab synthetic plan[1,2]. As per the drug receptor theories, drug forms intermolecular complexes with the receptor and exerts its biological activity by binding to the pocket of receptor molecule (usually protein). Thus docking is a tool to evaluate the drug receptor interactions and reveals an idea about utility of proposed chemical entity for human benefit without exposure of any kind of animals and chemicals for experimental studied and laboratory synthesis respectively[3,4]. Thus docking studies, discloses information about a ligand whether is able to fit both geometrically and energetically into the binding site of a protein or not[5]. Most widely useful psychoactive heterocyclic nucleus, benzodiazepines, is superior to the other alternative despite the important unwanted side effects[6]. The benzodiazepines swiftly gained an enormous market as antianxiety agents and anticonvulsant drug therapy[7]. They exert their significant therapeutic effects via binding to the specific benzodiazepine binding site of gamma-aminobutyric acid (GABA) type A receptors, and allosterically modulating the chloride flux through the ion channel complex[8]. The present communication is an attempt to consider the structural variations on benzylidene moiety of 5-phenyl-7-nitro-1,3-dihydro-1*H*, 3*H*-1,4-benzodiazepine-2-one to attract towards $GABA_A$ receptor on the basis of docking simulations result and synthesis of promising analogues and assessment of the antianxiety and anticonvulsant potential.

MATERIALS AND METHOD

VLife MDS Software (version 4.3.1) is utilized for docking study and library was drawn on Chemsketch software. The chemicals were procured (AR and LR grade) from Merck, Hi-Media and Sigma-Aldrich. The melting points of the synthesized compounds were determined by Thiele's melting point apparatus (open capillary tube method) and all the compounds gave sharp melting points and were uncorrected. The synthesized compounds were purified by recrystallization and purity of the compounds was ascertained by single spot on thin layer chromatography. Confirmed of synthesized compounds were ascertained by IR, ^{1}HNMR and Mass spectral studies. EPM (Elevated Plus Maze) test and MES (Maximal Electroshock) method were employed to evaluate antianxiety and anticonvulsant potential.

In-silico **Molecular docking study:**

Molecular docking study was carried on VLife MDS Software (version 4.3.1) [9]. A library of compounds for proposed ligands were constructed with help of Chemsketch software. Ligands were converted to 3D structures followed by energy optimization with MMFF slant in Vlife software tools. The receptor, GABA$_A$ (PDB code: 1KJT)[10] available on RCSB protein data bank, was collected and selected for the interaction studies. Pre-docking process such as removal of water molecules and cofactors, addition of hydrogen atoms, regulating atom type and finally minimization process were carried out. Ligands **(4a-x)** and receptor were subjected to genetic algorithm based docking method employing the 'Biopredicta' module of Vlife software. The compounds with comparatively good dock score, hydrophilic, hydrophobic and charged interactions are shown in **Table 1**; the library was screened to limit the compounds to thirteen numbers for purpose of synthesis and subsequent pharmacological screening.

Table 1. Showing various substituents in the proposed pharmacophore (4) and molecular docking results

Comps	**R^1**	**R^2**	**R^3**	**R^4**	**Hydro-gen Bonds**	**Hydroph-obic bonds**	**Charge Interactions**	**Dock Score**
4a	-H	-H	-H	-H	00	00	00	-4.87
4b	-OCH$_3$	-H	-H	-H	04	15	04	-4.22
4c	-H	-H	-OCH$_3$	-H	03	11	04	-4.44
4d	-H	-OH	-OCH$_3$	-H	03	10	04	-4.41
4e	-H	-OCH$_3$	-OH	-H	04	12	04	-3.98
4f	-H	-OCH$_3$	-OH	-OCH$_3$	06	04	21	-3.97
4g	-H	-H	-CH$_3$	-H	01	11	04	-5.04
4h	-OCH$_3$	-H	-H	-Br	03	16	04	-4.23
4i	-H	-OCH$_3$	-OCH$_3$	-OCH$_3$	05	20	04	-4.10
4j	-H	-H	-CH(CH$_3$)$_2$	-H	03	23	03	-4.88
4k	-H	-H	-OCH$_2$C$_6$H$_5$	-H	00	07	03	-4.22
4l	-N(CH$_3$)$_2$	-H	-N(CH$_3$)$_2$	-H	04	46	03	-4.20
4m	-NO$_2$	-H	-H	-H	00	00	00	-5.33
4n	-H	-NO$_2$	-H	-H	00	00	00	-4.91
4o	-H	-H	-OH	-H	00	00	00	-4.85
4p	-OH	-OH	-H	-H	00	00	00	-4.71
4q	-H	-H	-Cl	-H	00	00	00	-5.01
4r	-Cl	-H	-Cl	-H	00	00	00	-4.96
4s	-H	-H	-F	-H	01	00	00	-4.25
4t	-Cl	-H	-H	-H	00	00	00	-4.83
4u	-Cl	-H	-H	-NO$_2$	00	00	00	-4.56
4v	-H	-OC$_2$H$_5$	-OH	-H	03	25	02	-4.45
4w	-OH	-H	-H	-NO$_2$	01	00	00	-4.96
4x	-H	-H	-F$_3$C	-H	00	00	00	-5.07
Diazepam					02	22	03	-4.56

Synthetic Scheme:

Synthetic scheme for the synthesis of proposed library is schemed out in Figure 1.

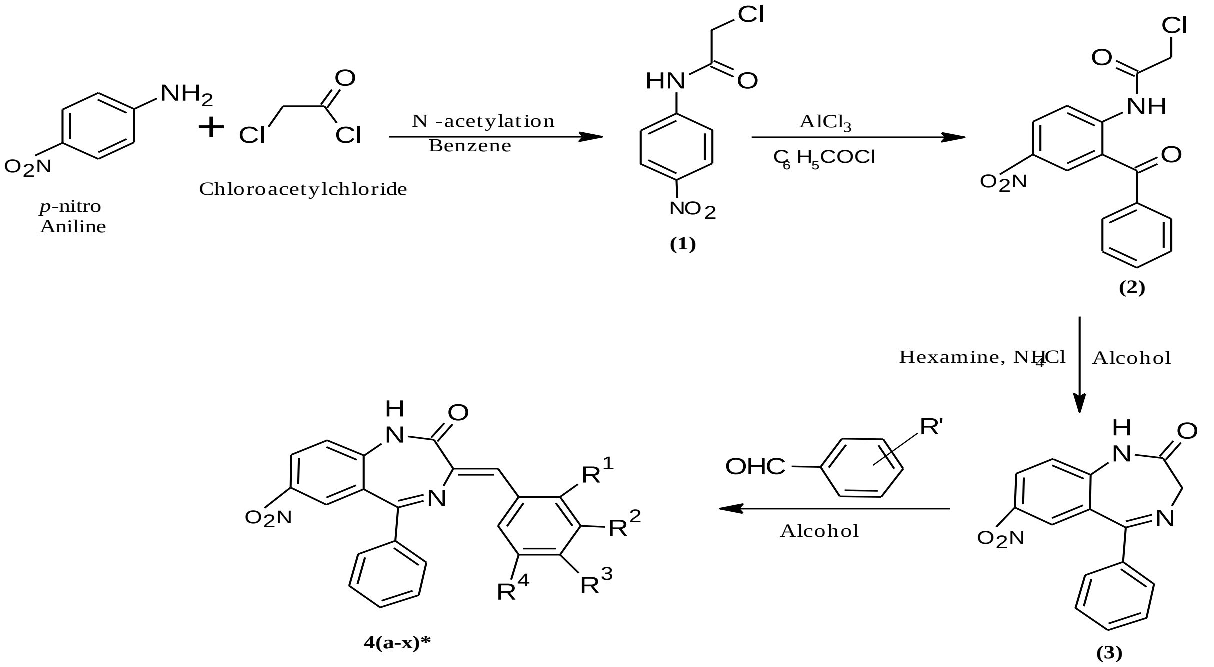


Figure 1. Proposed synthetic pathway for the proposed pharmacophore (4). * indicates synthesis of selected compound represented in table 2.

Synthesis of 2-chloro-*N*-(4-nitrophenyl)acetamide (1)[11]

In 250 ml round bottom flask 13.81 g (0.1 mol) of *p*-nitroaniline was dissolved in 100 ml benzene with two to three drops of pyridine and 11.29 ml (0.1 mol) of chloroacetyl chloride was added in dry benzene under cold condition and refluxed for 4 hr. After completion of reaction the reaction mixture was poured into crushed ice to obtain crystals. The solid product so obtained was filtered under suction and dried and purified by recrystallization from rectified spirit. Yield: 68%. mp: 134-136^0 C IR (KBr) cm^{-1}: N-H (amide) stretching- 3298, C-H stretching-3013, C=O stretching-1692, C=C stretching- 1597, 1481,C-N stretching- 1249, N=O stretching- 1530, 1350, C-Cl stretching- 885.

Synthesis of 2-(2-Chloroacetyl)amino-4-nitro benzophenone (2)[12]

Compound **1** (21.46 g, 0.1 mol) was dissolved completely in benzene and mixed with (14.05 ml) benzoyl chloride in equimolar quantity. Finely the small quantity of powdered anhydrous aluminium chloride was added with frequent shaking and the mixture was refluxed for 10 h. After completion of reaction the reaction mixture was poured into crushed ice. The solid product so obtained was filtered under suction, dried and purified by recrystallization from rectified spirit. Yield: 60%. mp: 82-84^0 C. IR (KBr) cm^{-1}: N-H (amide) stretching- 3302, C-H stretching-3086, C=O stretching- 1681, C=C stretching- 1597, C-N stretching- 1170, N=O stretching- 1520, 1355.C-Cl stretching- 886, C=O bending- 1251.

Synthesis of 7- Nitro, 5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (3)[13]

Compound **2** (14.06, 0.05 mol) was dissolved in 400 ml of ethanol and 14.00g of hexamine and 2g of ammonium chloride was added and refluxed for 4h. Progress of reaction was monitored by TLC. About 300 ml of ethanol was removed by distillation and cooled to room temperature. To filtrate, 30 ml of isopropyl alcohol were added and adjusted the pH of solution in the range of 1.0 to 1.5 by passing appropriate amount of HCL gas through the solution and maintaining the temperature of solution below 25^{o}C. Reaction mixture was stirred for 1h and filtered. The hydrochloride was then dissolved in water and simultaneously neutralized with ammonia to precipitate the solid. Filtered the solid and washed with chilled water twice to remove chloride. R_f : 0.52 (benzene: methanol), 45% yield, mp: 106-108^{0c}. IR (KBr) cm^{-1}: N-H (amide) stretching- 3354, =C-H stretching-3113, C=O stretching- 1700, C=N stretching- 1658, Ar C=C stretching- 1593, N=O stretching- 1543, 1360.

General Procedure for synthesis of 3-benzylidene derivatives[14]

Compound **4a** was dissolved in 30 ml of absolute alcohol and corresponding substituted benzaldehyde was added to it. Three to four drops of piperidine were added to mixture and refluxed for 9 h. The mixture is poured to crushed ice while hot to precipitate out solid, filtered and recrystallized from rectified spirit.

Synthesis of 3-(2-methoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4b)

5.66 g (0.02 mol) of compound **3** was dissolved in 30 ml of absolute alcohol and 2.72 g (0.02 mol) of 2-methoxy benzaldehyde was added to it. Three to four drops of piperidine were added to mixture and refluxed for 9 h at water bath. The mixture is poured to crushed ice while hot to precipitate out solid, filterd and recrystallized from rectified spirit. R_f : 0.56, 60% yield, mp: 224-226^{0c}. IR (KBr) cm^{-1}: N-H (amide) stretching- 3245, =C-H stretching-3142, C=O stretching- 1670, C=N stretching- 1608, N=O stretching- 1560, 1344, Ar C=C stretching- 1500, C-O stretching- 1292, 1030, CH_3 bending- 1250. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.32 (s, 1H, NH), 8.70-7.12 (m, 12H-ArH, 1H-CH=), 4.25 (s, 3H, O-CH_3). Anal. calc. for ($C_{23}H_{17}N_3O_4$): C, 69.16; H, 4.29; N, 10.52; O, 16.02. Found: C, 69.19; H, 4.31; N, 10.48; O, 16.00. ESI-MS:[M^+] 400.39.

Synthesis of 3-(4-methoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4c)

5.66 g (0.02 mol) of compound **3** and 0.02 mol (2.72 g) of p-methoxy benzaldehyde and 3-4 drops of piperidine were dissolved in 30 ml of absolute alcohol. The mixture after refluxing for 9h, were poured to crushed ice. The solid precipitate out was collected, dried and recrystallized from rectified spirit. R_f : 0.53, 64% yield, mp: 228-230 ^{0}C. IR (KBr) cm^{-1}: N-H (amide) stretching-3246, =C-H stretching-3138, C=O stretching- 1677, C=N stretching- 1608, N=O stretching- 1562, 1340, Ar C=C stretching- 1500, C-O stretching- 1292, 1044, CH_3 bending- 1250.^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.32 (s, 1H, NH), 8.70-7.12 (m, 12H-ArH, 1H-CH=), 4.25 (s, 3H, O-CH_3). Anal. calc. for ($C_{23}H_{17}N_3O_4$): C, 69.16; H, 4.29; N, 10.52; O, 16.02. Found: C, 69.18; H, 4.33; N, 10.48; O, 16.02. ESI-MS:[M$^+$] 400.39.

Synthesis of 3-(3-hydroxy-4-methoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4 benzodiazepin-2-one (4d)

To 5.66 g (0.02 mol) of compound **3**, an equimolar quantity (3.04g, 0.02 mol) of 3-hydroxy-4-methoxy benzaldehyde was refluxed in 30 ml of absolute alcohol in presence of piperidine (3-4 drops) for 9 h. After completion of reaction, the mixture is poured to crushed ice while hot to precipitate solid. Solid was filtered and purified by recrystallization from rectified spirit. R_f : 0.66, 55% yield, mp: 230-232^{0c}. IR (KBr) cm^{-1}: O-H stretching- 3659, N-H (amide) stretching- 3280, C-H stretching-3040, C=O stretching- 1690, C=N stretching- 1600, N=O stretching- 1556, 1354, Ar C=C stretching- 1494, C-O stretching- 1255, 1044, CH_3 bending- 1409. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.27 (s, 1H, NH), 5.82 (s, 1H, OH), 8.13-7.17 (m, 11H-ArH, 1H-CH=), 4.08 (s, 3H, O-CH_3). Anal. calc. for ($C_{23}H_{17}N_3O_5$): C, 66.50; H, 4.12; N, 10.11; O, 19.25. Found: C, 66.56; H, 4.19; N, 10.04; O, 19.28. ESI-MS:[M$^+$] 416.39.

Synthesis of 3-(4-hydroxy-3-methoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4e)

5.66 g (0.02 mol) of compound **3** was dissolved in 30 ml of absolute alcohol and 3.04g, 0.02 mol of 4-hydroxy-3-methoxy benzaldehyde was added to it. Three to four drops of piperidine were added to mixture and refluxed for 9 h at water bath. The mixture is poured to crushed ice while hot to precipitate out solid, filterd and recrystallized from rectified spirit. R_f : 0.63, 54% yield, mp: 254-256^{0c}. IR (KBr) cm^{-1}: O-H stretching- 3639, N-H (amide) stretching- 3281, =C-H stretching-3015, C=O stretching- 1697, C=N stretching- 1600, N=O stretching- 1560, 1340, Ar C=C stretching- 1496, C-O stretching- 1252, 1038, CH_3 bending- 1402. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.42 (s, 1H, NH), 5.89 (s, 1H, OH), 8.65-7.17 (m, 11H-ArH, 1H-CH=), 4.08 (s, 3H, O-CH_3). Anal. calc. for ($C_{23}H_{17}N_3O_5$): C, 66.50; H, 4.12; N, 10.11; O, 19.25. Found: C, 66.52; H, 4.17; N, 10.04; O, 19.25. ESI-MS:[M$^+$] 416.39.

Synthesis of 3-(4-hydroxy-3,5-dimethoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4f)

To 5.66 g (0.02 mol) of compound **3,** an equimolar quantity (3.68g 0.02 mol) of 4-hydroxy-3,5-dimethoxy benzaldehyde were reacted in 30 ml of absolute alcohol in presence of piperidine (3-4 drops) for 9 h. After completion of reaction, the mixture is poured to crushed ice while hot to precipitate solid. Solid was filtered and purified by recrystallization from rectified spirit. R_f : 0.57, 59% yield, mp: 256-258^{0c}. IR (KBr) cm^{-1}: O-H stretching- 3650, N-H (amide) stretching- 3282, =C-H stretching-3026, C=O stretching- 1685, C=N stretching- 1603, N=O stretching- 1560, 1350, Ar C=C stretching- 1500, C-O stretching- 1256, 1050, CH_3 bending- 1350. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.11 (s, 1H, NH), 5.85 (s, 1H, OH), 8.16-6.84 (m, 10 H-ArH, 1H-CH=), 4.41 (s, 6H, O-CH_3). Anal. calc. for ($C_{24}H_{19}N_3O_8$): C, 64.71; H, 4.29; N, 9.43; O, 28.73. Found: C, 64.71; H, 4.26; N, 9.44; O, 28.58. ESI-MS:[M$^+$] 446.42.

Synthesis of 3-(4-methylbenzylidene)-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4g)

To 5.66 g (0.02 mol) of compound **3,**an equimolar quantity (3.68g 0.02 mol) of 4-methyl benzaldehyde were reacted in 30 ml of absolute alcohol in presence of piperidine (3-4 drops) for 9 h. After completion of reaction, the mixture is poured to crushed ice while hot to precipitate solid. Solid was filtered and purified by recrystallization from rectified spirit. R_f : 0.55, 60% yield, mp: 252-254^{0c}.IR (KBr) cm^{-1}: N-H (amide) stretching- 3276, =C-H stretching-3078, C=O stretching- 1698, C=N stretching- 1600, N=O stretching- 1570, 1344, Ar C=C stretching- 1570. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.40 (s, 1H, NH), 8.42-7.00 (m, 12H-ArH, 1H-CH=), 3.48 (s, 3H, CH_3). Anal. calc. for ($C_{23}H_{17}N_3O_3$): C, 72.05; H, 4.46; N, 10.96; O, 16.69. Found: C, 72.06; H, 4.39; N, 10.94; O, 19.62. ESI-MS:[M$^+$] 384.39.

3-(5-bromo-2-methoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4h)

5.66 g (0.02 mol) of compound **3,** was dissolved in 30 ml of absolute alcohol and 4.30 g (0.02 mol) of 5-bromo-2-methoxy benzaldehyde was added to it. Three to four drops of piperidine were added to mixture and refluxed for 9 h at water bath. The mixture is poured to crushed ice while hot to precipitate out solid, filterd and recrystallized from rectified spirit. R_f : 0.52, 60% yield, mp: 256-258^{0c}. IR (KBr) cm^{-1}: N-H (amide) stretching- 3279, =C-H stretching-3035, C=O stretching- 1680, C=N stretching- 1603, N=O stretching- 1566, 1354, Ar C=C stretching- 1462, C-O stretching- 1257, 1040, -CH_3 bending- 1350, C-Br stretching- 501. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.87 (s, 1H, NH), 8.60-6.90 (m, 11H-ArH, 1H-CH=), 4.42 (s, 3H, -OCH_3). Anal. calc. for

($C_{23}H_{16}BrN_3O_4$): C, 57.75; H, 3.37; Br, 16.70; N, 8.78; O, 13.38. Found: C, 57.75; H, 3.39; Br, 16.75; N, 8.74; O, 13.30. ESI-MS:[M^{+1}] 479.29.

Synthesis of 3-(3,4,5-trimethoxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4i)

To 5.66 g (0.02 mol) of compound **3,** an equimolar quantity 0.02 mol (3.92 g) of 3,4,5-trimethoxy benzaldehyde was refluxed in 30 ml of absolute alcohol in presence of piperidine (3-4 drops) for 9 h. After completion of reaction, the mixture is poured to crushed ice while hot to precipitate solid. Solid was filtered and purified by recrystallization from rectified spirit. R_f : 0.66, 54% yield, mp: 250-252^{0c}.IR (KBr) cm^{-1}: N-H (amide) stretching- 3254, =C-H stretching-3030, C=O stretching-1665, C=N stretching- 1607, N=O stretching- 1564, 1334, Ar C=C stretching- 1500, C-O stretching- 1252, 1046, CH_3 bending- 1356. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.25 (s, 1H, NH), 7.98-7.06 (m, 12H-ArH, 1H-CH=), 4.31 (s, 9H, -OCH_3). Anal. calc. for ($C_{25}H_{21}N_3O_6$): C, 65.35; H, 4.60; N, 9.14; O, 20.89. Found: C, 65.39; H, 4.69; N, 9.14; O, 20.88. ESI-MS:[M^+] 460.45.

Synthesis of 3-(4-(propan-2-yl)-benzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4j)

5.66 g (0.02 mol) of compound **3** and 0.02 mol (2.96 g) of 4-(propan-2-yl) benzaldehyde and 3-4 drops of piperidine were dissolved in 30 ml of absolute alcohol. The mixture after refluxing for 9h, were poured to crushed ice. The solid precipitate out was collected, dried and recrystallized from rectified spirit. R_f : 0.52, 47% yield, mp: 230-232^{0c}. IR (KBr) cm^{-1}: N-H (amide) stretching-3280, =C-H stretching-3100, C=O stretching- 1683, C=N stretching- 1604, N=O stretching- 1567, 1340, Ar C=C stretching- 1500. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.26 (s, 1H, NH), 8.34-7.16 (m, 10H-ArH, 1H-CH=), 4.75 (s, 1H-CH), 2.37-2.53 (m, 3H, CH_3), 1.24-1.28 (m, 3H, CH_3). Anal. calc. for ($C_{25}H_{21}N_3O_3$): C, 72.97; H, 5.14; N, 10.21; O, 11.66. Found: C, 73.01; H, 5.19; N, 10.15; O, 11.68. ESI-MS:[M^+] 412.54.

Synthesis of 3-(4-benzyloxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4k)

5.66 g (0.02 mol) of compound **3** was dissolved in 30 ml of absolute alcohol and 4.24 g (0.02 mol) of 4-benzyloxy benzaldehyde was added to it. Three to four drops of piperidine were added to mixture and refluxed for 9 h at water bath. The mixture is poured to crushed ice while hot to precipitate out solid, filterd and recrystallized from rectified spirit. R_f : 0.53, 47% yield, mp: 214-216^{0c}.IR (KBr) cm^{-1}: N-H (amide) stretching- 3260, =C-H stretching-3056, C=O stretching- 1700, C=N stretching- 1600, N=O stretching- 1560, 1344, Ar C=C stretching- 1570, C-O stretching-

1266, 1035, -CH_2 bending- 1465. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.22 (s, 1H, NH), 8.80-7.12 (m, 17H-ArH, 1H-CH=), 3.40 (s, 2H, -OCH_2-). Anal. calc. for ($C_{29}H_{21}N_3O_4$): C, 73.25; H, 4.45; N, 8.83; O, 10.09. Found: C, 73.30; H, 4.49; N, 8.74; O, 10.13. ESI-MS:[M^+] 476.49.

Synthesis of 3-(2,4-dimethylamino benzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4l)

To 5.66 g (0.02 mol) compound **3**, an equimolar quantity (3.84 g, 0.02 mol) of 2,4-dimethylamino benzaldehyde was refluxed in 30 ml of absolute alcohol in presence of piperidine (3-4 drops) for 9 h. After completion of reaction, the mixture is poured to crushed ice while hot to precipitate solid. Solid was filtered and purified by recrystallization from rectified spirit. R_f : 0.55, 46% yield, mp: 270-272^{0c}. IR (KBr) cm^{-1}: N-H (amide) stretching- 3248, =C-H stretching-3065, C=O stretching-1687, C=N stretching- 1600, N=O stretching- 1550, 1340, Ar C=C stretching- 1560. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.79 (s, 1H, NH), 8.82-6.87 (m, 11H-ArH, 1H-CH=), 3.91 (s, 6H, -NCH_3-), 3.78 (s, 6H, -NCH_3-). Anal. calc. for ($C_{26}H_{25}N_5O_3$): C, 68.55; H, 5.53; N, 9.22; O, 10.53. Found: C, 68.56; H, 5.49; N, 9.24; O, 10.58. ESI-MS: [M^+] 456.50.

Synthesis of 3-(4-fluorobenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4s)

5.66 g (0.02 mol) of compound **3** and 0.02 mol (2.40 g) of 4-fluoro benzaldehyde and 3-4 drops of piperidine were dissolved in 30 ml of absolute alcohol. The mixture after refluxing for 9h, were poured to crushed ice. The solid precipitate out was collected, dried and recrystallized from rectified spirit. R_f : 0.60, 58% yield, mp: 220-222^{0c}. IR (KBr) cm^{-1}: N-H (amide) stretching- 3266, =C-H stretching-3123, C=O stretching- 1696, C=N stretching- 1602, N=O stretching- 1560, 1344, Ar C=C stretching- 1520, C-F stretching- 520. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.21 (s, 1H, NH), 8.51-6.97 (m, 12H-ArH, 1H-CH=). Anal. calc. for ($C_{22}H_{14}FN_3O_3$): C, 68.21; H, 3.64; F, 4.90; N, 10.84; O, 12.39. Found: C, 68.25; H, 3.62; F, 4.88; N, 10.84; O, 12.35. ESI-MS: [M^{+1}] 488.36.

Synthesis of 3-(3-ethoxy-4-hydroxybenzylidene)-7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (4v)

5.66 g (0.02 mol) of compound **3** and 0.02 mol (3.32 g) of 3-ethoxy-4-hydroxy benzaldehyde and 3-4 drops of piperidine were dissolved in 30 ml of absolute alcohol. The mixture after refluxing for 9h, were poured to crushed ice. The solid precipitate out was collected, dried and recrystallized from rectified spirit. R_f : 0.57, 46% yield, mp: 260-262^{0c}. IR (KBr) cm^{-1}: O-H stretching- 3648, N-H (amide) stretching- 3239, =C-H stretching-3040, C=O stretching- 1670, C=N stretching- 1605, N=O stretching- 1560, 1344, Ar C=C stretching- 1442, C-O stretching- 1258, CH_2 bending- 1410.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.38 (s, 1H, NH), 5.41 (s, 1H, -OH), 8.36-7.20 (m, 11H-ArH, 1H-CH=), 3.34 (s, 2H, $-CH_2-$), 2.82 (s, 3H, CH_3). Anal. calc. for ($C_{24}H_{19}N_3O_5$): C, 67.12; H, 4.45; N, 9.78; O, 18.62. Found: C, 67.08; H, 4.45; N, 9.75; O, 18.63. ESI-MS: [M^+] 430.42.

Antianxiety activity

Elevated plus maze test module was selected for evaluation of antianxiety activity. Male Swiss albino mice weighing around 20-25g were selected. The study was carried out in camera attenuated dark room while observations such as number of entries of animals in open as well as closed arm of apparatus along with the time spent in arms were made through camera from outside the room, for a period of 5 minutes. Animal were fed food and water as normal and maintained in air conditioner rooms. The entire maze was painted black and kept at a height of 50cm from surface. Various synthesized selected test drugs (20mg/kg) and standard drug diazepam (2mg/kg) were introduces in a single dose of 2ml/kg, by intraperitoneal route by facing the mouse towards centre of maze. The control group were supplemented by saline with 1% tween 80. Animal were grouped in 6 numbers[15].

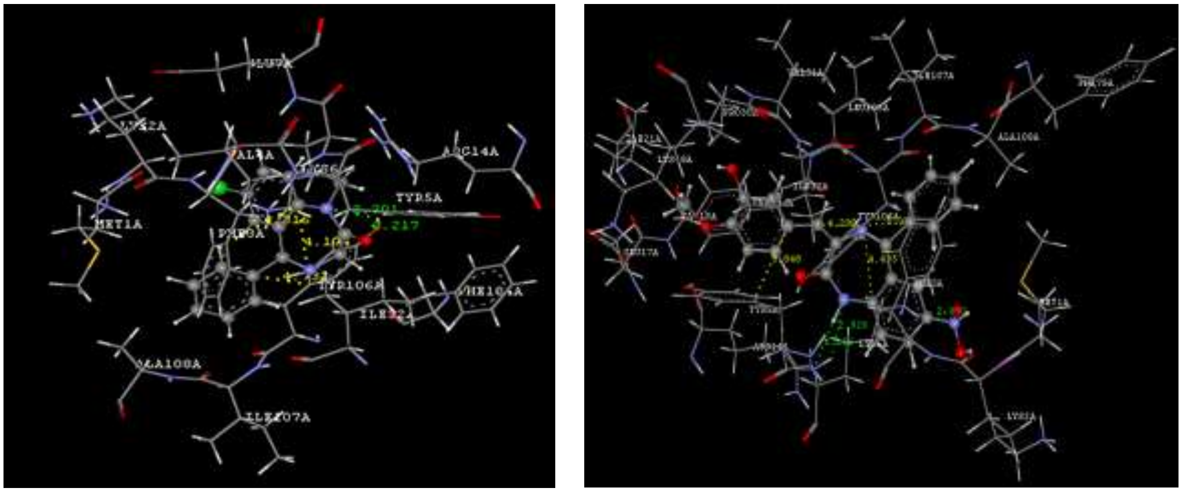
Anticonvulsant activity

Maximal Electroshock method was adopted to explore anticonvulsant effect. Swiss albino mice weighing around 25g were selected on approved from Institutional Animal Ethical Committee. Animals were exposed to an alternating current of 50 mA for 0.2 s through a pair of electrode to each ear. Animal were grouped in six numbers for standard, test and control group. Each group were treated with each selected test drugs (20mg/kg), diazepam (2mg/kg) and normal saline with 1% tween 80 respectively, in a single volume of 2ml/kg. Duration of seizure in each animal was noted and percentage inhibition of seizure with each selected tested drugs and standard were compared with control[13].

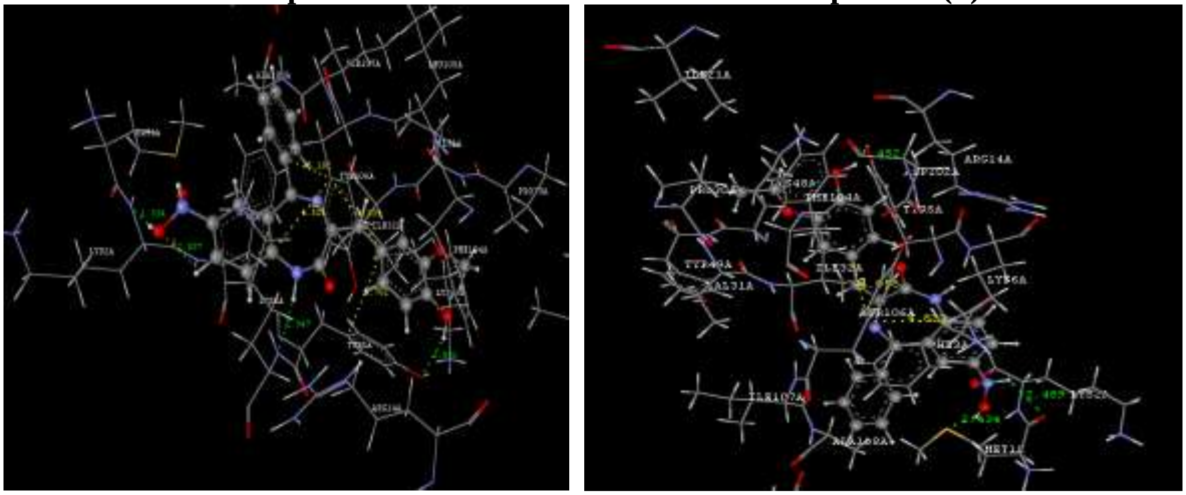
RESULTS AND DISCUSSION

In-silico Molecular docking study

Docking of exposed ligand with target receptor has been performed. Minimum dock score data were consider to be the best parameter to express protein-ligand affinity. Parallel docking study has been performed to with marketed available drug i.e. diazepam a commonly used benzodiazepine employed in both disorders. Diazepam found to develop two hydrogen bonds with TYR 106A amino acid residue whereas π-π stacking interaction with TYR 106A and PHE 3A protein moiety. It is capable to form hydrophobic interactions with PHE 104A, PHE 3A, VAL 4A, ILE-32A, TYR 6A, TYR 5A, LYS 6A, and ARG 14A. Our proposed molecules also found to target similar amino acids while binding to the receptor. Dock score obtained also found to be comparable to standard diazepam (Table 1). Ligand-receptor interactions of some ligands are illustrated in Figure 2 and Figure 3.

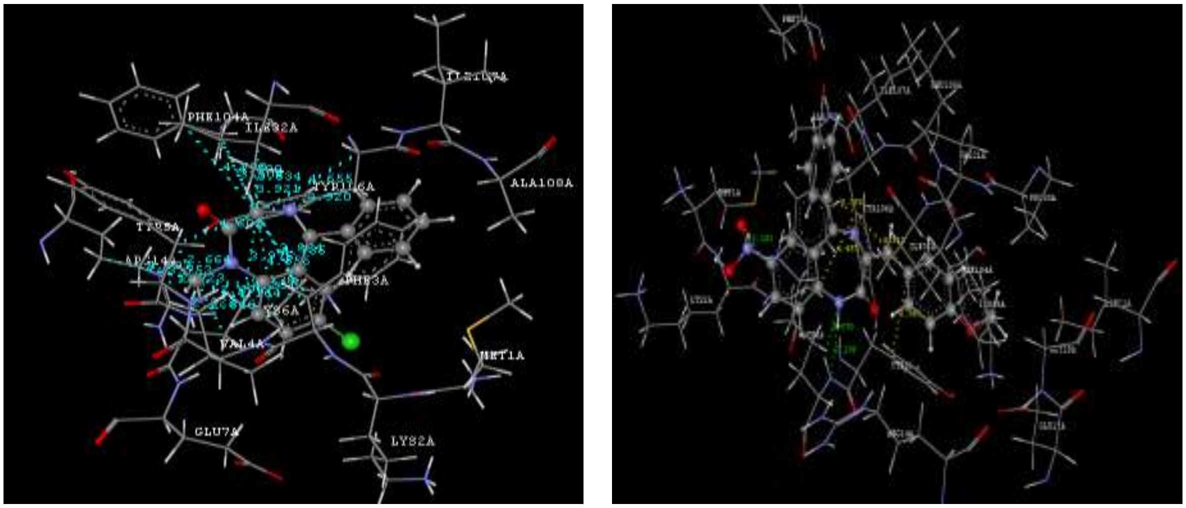


Diazepam **Compound 4(b)**

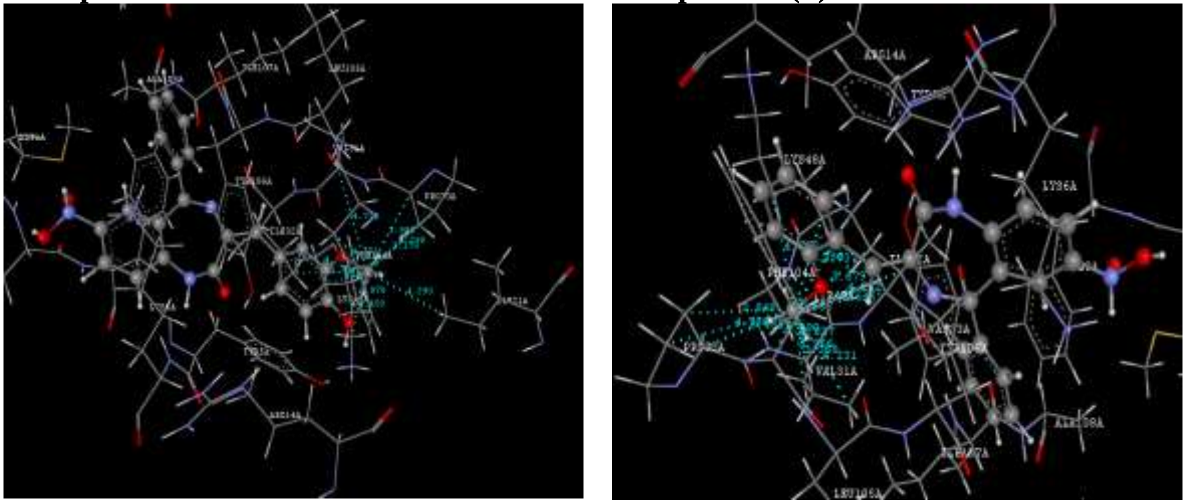


Compound 4(e) **Compound 4(v)**

Figure 2: Molecular docking results of Diazepam and compounds 4(b), 4(e) and 4(v) with receptor GABA$_A$ (1KJT). Green and yellow dotted lines refer to H-bonds and π-stacking respectively.



Diazepam **Compound 4(b)**



Compound 4(e) **Compound 4(v)**

Figure 3: Molecular docking results of Diazepam and compounds 4(b), 4(e) and 4(v) with receptor GABA$_A$ (1KJT). Hydrophobic interactions are represented by blue dotted lines.

Antianxiety activity

Only selected analogues have been tested for antianxiety potential. Most rising antianxiety effect was seen in compound **4l** in respect of number of entries and time spent in open arm and percent preference to open arm. Ligand **4b** and **4v** acted nearly same i.e. 67 percentage to preference to open arm. Compound **4d, 4e, 4f, 4h, 4i** and **4j** were found to act moderately **(Table 2).**

Table 2: Antianxiety and anticonvulsants activity of selected drugs on the basis of docking studies

Comp.	Antianxiety activity			Antiepileptic activity	
	No. of Entries in Open arm	Average time spent in open arm (s)	% preference to open arm	Duration of Seizure (s)	% Inhibition of Seizure

Control	2.5±0.28	8.75±1.91***	27.77	223.75±6.84***	00
4b	5.50±0.28***	41.50±3.46***	66.66	69.25±4.13***	69.05
4c	4.00±0.40***	35.75±3.78**	38.09	80.00±2.08***	64.24
4d	4.75±0.25***	38.5±3.46**	55.88	65.50±2.72***	70.72
4e	4.75±0.47***	37.75±6.40**	54.28	63.00±1.82***	71.84
4f	4.25±0.25***	29.25±4.12	54.83	79.50±2.53***	64.46
4g	2.75±0.25	19.00±4.32	32.35	86.75±3.17***	61.22
4h	4.75±0.25***	38.50±6.83**	59.37	82.75±3.27***	63.01
4i	4.25±0.47***	33.75±4.43*	58.62	107.25±3.32***	52.06
4j	4.25±0.47***	34.75±5.74*	56.66	79.50±2.39***	64.46
4k	2.50±0.28	17.75±6.60	45.45	109.25±2.46***	51.17
4l	6.00±0.40***	43.25±4.12***	69.36	74.25±2.32***	66.81
4s	1.75±0.25	16.00±7.83	19.44	207.75±5.85	07.15
4v	5.75±0.25***	40.25±3.46***	67.64	54.50±1.70***	75.66
Diazepam (Standard)	6.5±0.28	48.75±4.43	72.06	25.5±2.10	88.60

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 ± standard error of mean (S.E.M.).

Anticonvulsant activity

Same analogues were explored for this effect as antiepileptic activity. Compound **4v** was the most effective to normalize the impulse generation. Ligands **4b, 4d, 4e** acted to inhibit the seizure in the range of 69-72%, while **4c, 4f, 4h, 4j** and **4l** shown percent inhibition of seizure in the range of 63-67%. Compound **4s** acted desperately (Table 2).

CONCLUSION

All the proposed ligands were found to possess good dock score as a function of affinity but most of the compounds incapable to form intermolecular complex with the receptor site. Methoxy substituted ligands have shown interesting role in docking studies and also responded the pharmacological profile. This activity of methoxy groups may be attributed to *pi*-interactions with the receptor. Replacement of methoxy with ethoxy group shifted activity towards higher level. Halogens and nitro groups had not shown any role in docking interaction study. Hydroxyl groups also not showed any role. Two dimethyl amino substitutions on benzylidene moiety were performed well and able to reduce anxiety as well as epileptic episodes. Isopropyl group have also found to be potential CNS affects. The title compound thus may have an immense potential towards human benefit.

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