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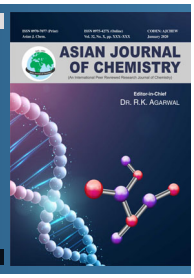

Synthesis, Anticonvulsant Evaluation and Molecular Docking Studies of Novel Benzo[1,3]dioxol-5-yloxy-N′-(4-substituted benzylidene)acetohydrazide Derivatives

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Novel (benzo[1,3]dioxol-5-yloxy)-N′-(4-substituted benzylidene)acetohydrazide derivatives were synthesized and their anticonvulsant activity evaluated by MES and scMET seizure models. Compound 2-(benzo[*d*][1,3]dioxol-5-yloxy)-N′-benzylideneacetohydrazide (**4a**) was found to be most potent in MES seizure test and showed no neurotoxicity at the highest administered dose. All the compounds showed high docking score with γ-aminobutyric acid receptor, GABAAR-β3 homopentamer (PDB ID: 4COF). Thus, the probable mechanism of action of benzo[1,3]dioxol-5-yloxy-N′-(4-substituted benzylidene)acetohydrazide derivatives (**4a-h**) may be augmentation of GABAergic activity.

Keywords: Acetohydrazide derivatives, Anticonvulsant activity, Neurotoxicity, Molecular docking.

INTRODUCTION

Epilepsy is a common chronic neurological disorder. Repeated seizures, which are a consequence of excessive nerve cell discharges in the brain, are a peculiar characteristic of epilepsy. The seizures disrupt consciousness, disturb sensation, interfere with movements or impair mental function. Various combinations of these characteristics are observed in patients with epilepsy. Globally, almost 50 millions cases are reported to be affected by epilepsy [1]. The ubiquity of epilepsy in India is approximately 6-10 patients per 1000 people. Sudden unexpected death in epilepsy (SUDEP) is the most common and directly epilepsy-related major cause of death. SUDEP is considered the primary mortality cause in patients with chronic uncontrolled epilepsy [2]. At least 20 compounds with potential antiepileptic activity are currently being developed and in different phases of clinical development. Many of these drugs have been subjected to clinical trials and have exhibited promising results [3]. The widespread use of antiepileptic drugs (AEDs) is causing safety concerns because they have a narrow therapeutic index. Moreover, they can adversely affect various organs and functions of a patient's body. Overall, 10-30 % of patients with epilepsy cannot tolerate prescribed AEDs. Consequently, they discontinue the treatment [4]. Among the patients receiving long term antiepileptic treatment, the prevalence of adverse effects lies in the range 10-40 %, if tolerability is evaluated using spontaneous reports or nonstructured interviews. However, the prevalence increases and lies between 60 and 95 % when adverse effects are evaluated using a checklist [5]. A large proportion of patients who experience seizures are resistant to drug treatment [6]. Several studies have shown that adverse effects are the primary determinants of low quality of life because adverse effects of the drugs hamper the quality of life to a greater extent than they increase the frequency of seizures [7]. Current design of new AEDs and development of strategies for the prevention of epilepsy are focused mainly on reducing uncontrolled seizures, severe side effects and drug toxicity in long-term therapy.

Hydrazones having an azomethine -NHN=CH- proton are an important class of compounds for new drug development. Hydrazone derivatives possess various biological activities such as anticonvulsant, vasodilator, antimycobacterial, antiviral, antitumoral, antimalarial, analgesic, anti-inflammatory, antiplatelet, antidepressant, antimicrobial and antischistosomiasis activities. Azomethine (-CO-NHN=CH-) group play an important role in the drug development of newer class of compounds with anticonvulsant activity [8]. There are about 40 hydrazone

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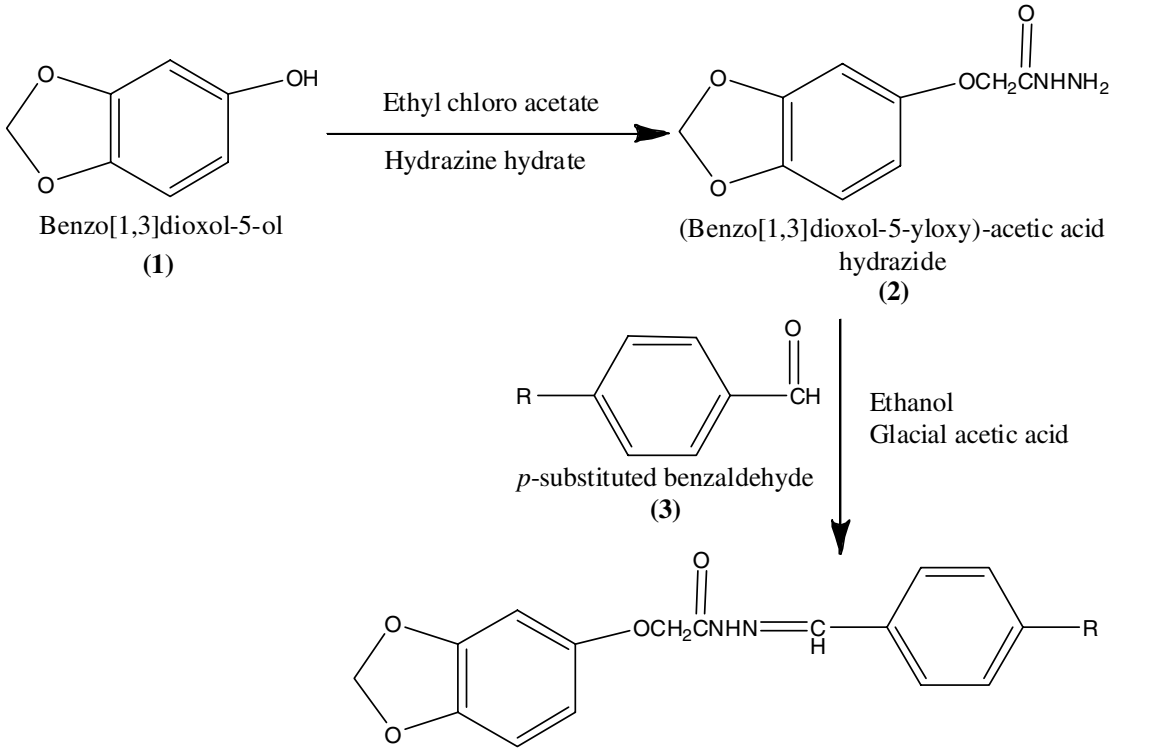
derivatives shown to exhibit anticonvulsant activity [9]. Sesamol is the major constituent of sesame seed oil obtained from *Sesamum indicum* Linn. Pedaliaceae. It is traditionally used as health supplement because of its antioxidant, antiaging, antidepressant, anti-inflammatory, chemopreventive, immunomodulatory and neuroptotective effects. Epilepsy is a chronic neurological disorder is associated with the generation of reactive oxygen species and cognitive impairment [10]. It was thought that sesamol might exert therapeutic potential in epilepsy because of its antioxidant effect. For this, the potential of sesamol as an anticonvulsant was evaluated against the pentylenetetrazol-induced seizures, pentylenetetrazole (PTZ)-induced kindling, cognitive impairment and oxidative stress. It showed protection against PTZ-induced seizures, notably delayed the development of kindling and exerted ameliorative effects against seizures, cognitive impairment and oxidative stress in the experimental model of epilepsy [11]. Keeping in mind the anticonvulsant potential of both hydrazones and sesamol, novel benzo[1,3]-dioxol-5-yloxy-N′-(4-substituted benzylidene)acetohydrazide derivatives were synthesized and evaluated for their anticonvulsant activity. Molecular docking study of designed molecules was also carried out in order to establish the binding ability of the newly synthesized compounds to GABAAR-β3 binding site.

EXPERIMENTAL

Chemicals used in the study were procured from Sigma and Himedia Chemicals. Melting points were recorded on a Tempo capillary melting point apparatus, Mumbai and are uncorrected. The FT-IR spectrum (cm^{-1}) was determined on Bruker Tensor 27 FT-IR spectrometer. ^1H NMR spectrum were recorded on Bruker Avance II 400 MHz, USA FT-NMR spectrometer at 300 MHz, after dissolving the sample in a suitable solvent (DMSO or D_2O) using tetramethylsilane as internal standard and chemical shifts (δ) are reported in parts per million (ppm). UV-visible spectra were recorded after dissolving the drug/conjugates in a suitable solvent using UV-visible spectrophotometer (Shimadzu 1700, Japan). Thin layer chromatography (TLC) and elemental analysis were used to establish the purity of the compounds. Plates for TLC were prepared with silica gel G and activated at 110 ºC for 30 min. Iodine vapours were used to develop the TLC plates. Elemental analyses were performed on Vario EL-III analyzer.

Synthesis of (benzo[1,3]dioxol-5-yloxy)acetic acid hydrazide (2): A mixture of benzo[1,3]dioxol-5-ol (**1**), anhydrous K_2CO_3, chloroethyl acetate and DMF was stirred at room temperature for 8 h. The reaction mixture was diluted with ice cold water. A solid separated out and completion of reaction was confirmed by TLC study. A mixture of obtained compound (0.019 M) and hydrazine hydrate (15 mL) was refluxed for 4 h. The excess of hydrazine hydrate was removed *in vacuo* and the residue was triturated with water, filtered off, dried and recrystallized from 70 % ethanol to give colourless crystals of (benzo[1,3]dioxol-5-yloxy)acetic acid hydrazide (**2**) [12].

Synthesis of (benzo[1,3]dioxol-5-yloxy)-N′-(4-substituted benzylidene)acetohydrazide derivatives (4a-h): Equimolar concentration of (benzo[1,3]dioxol-5-yloxy)acetic acid hydrazide (**2**) and *p*-substituted benzaldehydes (**3**) were dissolved in absolute ethanol and few drops of glacial acetic acid were added and the reaction mixture refluxed for 6 h. The reaction mixture was poured in ice-cold water and the synthesized compound was filtered. Finally, it was washed with water, dried



Scheme-I

at room temperature and recrystallized with ethanol (**Scheme-I**). The reaction process was monitored by TLC using ethyl acetate:ethanol (2:3) as mobile phase and iodine as detecting agent [13].

2-(Benzo[*d*][1,3]dioxol-5-yloxy)-N′-benzylideneacetohydrazide (4a): White solid, yield: 91.2 %, m.p. 199- 201 ºC, m.w. 298.29; R_f 0.72. FT-IR (KBr, ν_{max} cm^{-1}): 3256 (N-H), 3042 (arom. C-H), 2957 & 2855 (aliph. CH), 1649 (C=O), 1629 (phenyl ring *str.*), 1595 (C=N), 1507 & 1486 (phenyl C-H out of plane bending), 1466 (CH_2 bending), 1282 (C-O), 1186 (in-plane arom. bend.), 1129 (C-O-C, asym.), 1042 (C-O-C, sym), 929 (ethylene dioxide). ^{1}H NMR (DMSO, 400 MHz): δ in ppm: 8.64 (1H, s, -CH=N), 8.01 (1H, s, -NH), 7. 85 (2H, d, -CH-Ar, *J* = 8.32), 7.52 (3H, d, -CH-Ar, *J* = 7.12 Hz), 6.77 (1H, d, -CH-Ar, *J* = 5.6 Hz,), 6.48-6.42 (2H, dd, -CH-Ar, *J* = 10.36, 9.04), 6.06 (2H, s, -CH_2-O), 4.63 (2H, s, -CH_2-O). Elemental analysis of $C_{16}H_{14}N_2O_4$ calcd. (found) (%): C, 64.42 (65.02); H, 4.73 (4.03); N, 9.39 (8.89); O, 21.45 (22.05).

N′-(4-Chlorobenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4b): White solid, yield: 89.5 %, m.p. 213-215 ºC, m.w. 332.06, R_f 0.43. FT-IR (KBr, ν_{max}, cm^{-1}): 3261 (N-H), 3050 (arom. C-H), 2951 & 2850 (aliph. CH), 1646 (C=O), 1626 (phenyl ring *str.*), 1592 (C=N), 1504 & 1489 (phenyl C-H out of plane bend.), 1469 (CH_2 bend.), 1278 (C-O), 1183 (in-plane arom. bend.), 1126 (C-O-C, asym.), 1085 (C-Cl), 1039 (C-O-C, sym.), 927 (ethylene dioxide).^{1}H NMR (DMSO, 400 MHz): δ in ppm: 4.62 (s, 2H, CO-CH_2-O), 6.05 (s, 3H, O-CH_2-O), 6.46 (d, 1H, CH-Ar), 6.47 (s, 1H, CH-Ar), 6.77(d, 1H, CH_2-Ar), 7.52 (d, 2H, CH-Ar), 7.77(d, 2H, CH-Ar), 8.06 (s, 1H, NH), 8.21 (s, 1H, CH=N). MS (*m/z*, ESI): 332.06 (M^+, 79.3). Elemental analysis of $C_{16}H_{13}N_2O_4Cl$ calcd. (found) (%): C, 57.75 (57.78); H, 3.94 (3.94); Cl, 10.0 (10. 04); N 8.42 (8.39); O, 19.33 (19.29).

N′-(4-Hydroxybenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4c): Off-white solid, yield: 92.4 %, m.p. 216-218 ºC, m.w. 314.09, R_f 0.92. FT-IR (KBr, ν_{max}, cm^{-1}): 3472 (O-H), 3265 (N-H), 3048 (arom. C-H), 2947 & 2852 (Aliph. C-H), 1648 (C=O), 1628 (phenyl ring *str.*), 1599 (C=N), 1504 & 1486 (phenyl C-H out of plane bending), 1467 (CH_2 bend.), 1276 (C-O), 1185 (in-plane arom. bend.), 1127 (C-O-C, asym.), 1040 (C-O-C, sym), 926 (ethylene dioxide). ^{1}H NMR (DMSO, 400 MHz): δ in ppm 4.63 (s, 2H, CO-CH_2-O), 5.37 (s, 1H, OH), 6.05 (s, 2H, O-CH_2-O), 6.47(d, 1H, CH-Ar), 6.85 (d, 2H, CH-Ar), 6.76 (d, 1H, CH-Ar), 6.85 (d, 2H, CH-Ar), 7.78 (d, 2H, CH-Ar), 8.05 (s, 1H, NH), 8.22 (s, 1H, CH=N). MS (*m/z*, ESI): 341.09 (M^+, 82.1). Elemental analysis of $C_{16}H_{14}N_2O_5$ calcd. (found) (%): C, 61.14 (61.18); H, 4.49 (4.46); N, 8.91 (8.88); O, 25.45 (25.47).

N′-(4-Bromobenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4d): Yellowish white solid, yield: 76.6 %, m.p. 256-258 ºC, m.w. 376.01, R_f, 0.59. FT-IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3058 (arom. C-H), 2948 & 2854 (aliph. CH), 1642 (C=O), 1631 (phenyl ring *str.*), 1587 (C=N), 1505 & 1485 (phenyl C-H out of plane bend.), 1466 (CH_2 bend.), 1277 (C-O), 1179 (in-plane arom. bend.), 1129 (C-O-C, asym.), 1052 (C-Br) 1036 (C-O-C, sym.), 925 (ethylene dioxide). ^{1}H NMR (DMSO, 400 MHz): δ in ppm 8.53 (1H, s, -CH=N), 8.00 (1H, s, -NH), 7.71 (2H, d, -CH-Ar, *J* = 7. 92 Hz), 7.60 (2H, d, -CH-Ar, *J* = 7. 92 Hz), 6.77 (1H, d, -CH-Ar, *J* = 9.0Hz,), 6.46-6.41 (2H, dd, -CH-Ar, *J* = 11.56, 9.68), 6.09 (2H, s, -CH_2-O), 4.62 (2H, s, -CH_2-O). Elemental analysis of $C_{16}H_{13}N_2O_4Br$ calcd. (found) (%): C, 50.95 (51.40); H, 3.47 (3.17); Br, 21.18 (20.78); N, 7.43 (7.23); O, 16.97 (17.42).

N′-(4-Fluorobenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4e): White solid, yield: 74.8 %, m.p. 226-228 ºC, m.w. 316.09, R_f 0.57. FT-IR (KBr, ν_{max}, cm^{-1}): 3267 (N-H), 3056 (arom. C-H), 2957 & 2856 (aliph. CH), 1643 (C=O), 1623 (phenyl ring *str.*), 1588 (C=N), 1502 & 1487 (phenyl C-H out of plane bend.), 1465 (CH_2 bend.), 1283 (C-O), 1192 (C-F), 1178 (in-plane arom. bend.), 1129 (C-O-C, asym.), 1041 (C-O-C, sym.), 928 (ethylene dioxide). ^{1}H NMR (DMSO, 400 MHz): δ in ppm 8.35 (1H, s, -CH=N), 8.00 (1H, s, -NH), 7.85 (2H, d, -CH-Ar, *J* = 6.56Hz), 7.36 (2H, d, -CH-Ar, *J* = 5.6 Hz), 6.77 (1H, d, -CH-Ar, *J* = 9.36Hz,), 6.47-6.42 (2H, dd, -CH-Ar, *J* = 8.72, 9.52), 6.07 (2H, s, -CH_2-O), 4. 63 (2H, s, -CH_2-O). Elemental analysis of $C_{16}H_{13}N_2O_4F$ calcd. (found) (%): C, 60.76 (61.06); H, 4.14 (3.74); F, 6.01 (5.71); N, 8.86 (9.06); O, 20.23 (20.43).

N′-(4-Methoxybenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4f): Greyish white solid, yield: 82.8 %, m.p. 188-191 ºC, m.w. 328.11, R_f, 0.76. FT-IR (KBr, ν_{max}, cm^{-1}): 3268 (N-H), 3054 (arom. C-H), 2952 & 2857 (aliph. C-H), 1645 (C=O), 1632 (phenyl ring *str.*), 1595 (C=N), 1503 & 1481 (phenyl C-H out of plane bend.), 1462 (CH_2 bend.), 1452 (asym. CH_3 bend.), 1376 (sym. CH_3 bend.), 1275 (C-O), 1186 (in-plane arom. bend.), 1131 (C-O-C, asym.), 1044 (C-O-C, sym.), 923 (ethylene dioxide).^{1}H NMR (DMSO, 400 MHz): δ in ppm 8.36 (1H, s, -CH=N), 8.01 (1H, s, -NH), 7.85 (2H, d, -CH-Ar, *J* = 6.56Hz), 7. 08 (2H, d, -CH-Ar, *J* = 7. 92 Hz), 6.76 (1H, d, -CH-Ar, *J* = 8. 96 Hz,), 6.46-6.40 (2H, dd, -CH-Ar, *J* = 11.56, 7.76), 6.08 (2H, s, -CH_2-O), 4. 64 (2H, s, -CH_2-O), 3.83 (3H, s, -O-CH_3). Elemental analysis of $C_{17}H_{16}N_2O_5$ calcd. (found) (%): C, 62.19 (62.48); H, 4.91 (4.65); N, 8.53 (8.23); O, 24.37 (24.54).

N′-(4-Nitrobenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4g): Off-white solid, yield: 86.2 %, m.p. 159-161 ºC, m.w. 343.08, R_f, 0.81. FT-IR (KBr, ν_{max}, cm^{-1}): 3262 (N-H *str.*), 3044 (arom. C-H), 2954 & 2858 (aliph. C-H), 1643 (C=O), 1624 (phenyl ring *str.*), 1595 (C=N), 1554 (asym. N=O *str.*), 1503 & 1489 (phenyl C-H out of plane bend.), 1464 (CH_2 bend.), 1348 (asym. N=O *str.*), 1281 (C-O), 1188 (in-plane arom. bend.), 1132 (C-O-C, asym.), 1036 (C-O-C, sym.), 929 (ethylene dioxide). ^{1}H NMR (DMSO, 400 MHz,): δ in ppm 8.36(1H, s, -CH=N), 8.35 (2H, d, -CH-Ar, *J* = 9.16Hz), 8.09 (2H, d, -CH-Ar, *J* = 8.24 Hz), 8.01 (1H, s, -NH), 6.76 (1H, d, -CH-Ar, *J* = 5.12 Hz,), 6.46-6.40 (2H, dd, -CH-Ar, *J* = 7.68, 6.96), 6.07 (2H, s, -CH_2-O), 4. 62 (2H, s, -CH_2-O). Elemental analysis of $C_{16}H_{13}N_3O_6$ calcd. (found) (%): C, 55.98 (56.31); H, 3.82 (4.01); N, 12.24 (11.63); O, 27.96 (28.05).

N′-(4-Ethylbenzylidene)-2-(benzo[*d*][1,3]dioxol-5-yloxy)acetohydrazide (4h): White solid, yield: 88.3 %, m.p. 205-207 ºC, m.w. 326.13, R_f, 0.68. FT-IR (KBr, ν_{max}, cm^{-1}): 3259 (N-H), 3058 (arom. C-H), 2952 & 2858 (aliph. C-H), 1644 (C=O), 1624 (phenyl ring *str.*), 1595 (C=N), 1507 & 1489 (phenyl C-H out of plane bend.), 1467 (CH_2 bend.), 1447 (asym. CH_3 bend.), 1377 (sym. CH_3 bending), 1279 (C-O), 1189 (in-plane arom. bend.), 1131 (C-O-C, asym.), 1045 (C-O-C, sym),

928 (ethylene dioxide). ^{1}H NMR (DMSO, 400 MHz): δ in ppm 8.56(1H, s, -CH=N), 8.00 (1H, s, -NH), 7.78 (2H, d, -CH-Ar, *J* = 7.24 Hz), 7.34 (2H, d, -CH-Ar, *J* = 9.36 Hz), 6.78 (1H, d, -CH-Ar, *J* = 8.88 Hz), 6.46-6.40 (2H, dd, -CH-Ar, *J* = 6.4, 6.04), 6.07 (2H, s, -CH_2-O), 4.64 (2H, s, -CH_2-O), 2.60 (2H, q, -CH_2-), 1.25 (3H, t, *J* = 1.4, 6.64, -CH_3). Elemental analysis of $C_{18}H_{18}N_2O_4$ calcd. (found) (%): C, 66.25 (65.96); H, 5.56 (5.26); N, 8.58 (8.69); O, 19.61 (20.09).

Anticonvulsant evaluation: Newly synthesized benzo[1,3]-dioxol-5-yloxy-N′-(4-substituted benzylidene)acetohydrazide derivatives (**4a-h**) were evaluated and screened for anticonvulsant activity by using the conventional anticonvulsant drug development scheme recommended by National Institute of Health, Bethesda, USA. Male and female albino mice (weight: 25-30 g) were used for the study. All the animal study protocols were approved by Institutional Animal Ethics Committee of Sapience Bioanalytical Research Laboratory, Bhopal (1413/PO/E/S/11/cpcsea) and the experiments were conducted in accordance with the CPCSEA guidelines. The mice were housed in spacious sanitary cages throughout the experimental period. Animals had access to standard diet and water *ad libitum*. The mice were maintained at 22 ± 1 ºC conditions with 12 h light-dark cycle. The mice were divided into three groups comprising six animals each. Group I was the control group or distilled water-treated group. Group II was the test group, which received solutions of test compounds prepared in PEG-400. The intraperitoneal (i.p.) doses administered to the mice were 30, 100, and 300 mg/kg. Group III was the standard group, which received a reference drug (phenytoin 30 mg/kg i.p.). The anticonvulsant activity of test compounds was evaluated using maximal electroshock and the subcutaneous metrazole (scMET) test model at three time intervals and at three doses, namely 30, 100 and 300 mg/kg. The neurotoxicity of the test compounds was evaluated using the rotarod method.

Maximum electroshock seizure (MES) test: Vehicle control group and treatment group consisted of six animals. Using a ear-clip electrodes, 60 Hz of alternating current (50 mA in mice) was applied for 0.2 s. Readings were observed at 0.5 and 4 h after test compound doses of 30, 100 and 300 mg/kg. Polyethylene glycol (PEG) was used to dissolve the test compounds and injected intraperitoneally (i.p.) 0.5 h before seizures induction. Reference drug used was phenytoin. Eradication of hind limb tonic extensor showed the ability of test compound to prevent MES induced seizure spread and defined as protection [14].

Subcutaneous pentylenetetrazole (scPTZ) seizure test: Three groups of animals each comprising of six animals were subjected to scPTZ test. One was used for observing the effect of PTZ, the second for control and the third group to determine the effect with reference to phenytoin. Dose-percent effect curve determined the s.c. dose of PTZ (85mg/kg) at which 95 % of the animals manifest convulsive reaction. The synthesized compounds were administered at 30, 100 and 300 mg/Kg, i.p. At the calculated time, PTZ was administered s.c. in posterior midline of mice. Absence of clonic spasm in the observed time periods among half or more of the animals shows the compounds ability to conclude the effect of PTZ on seizure threshold. Results of synthesized compound were compared with control and standard group [15].

Neurotoxicity screening: Rotarod test was used to check the activity of the drugs interfering with motor coordination. Initially mice were trained to keep balance on an accelerating rotarod (diameter was 3.2 cm) rotating at a rate of six revolutions per minute. Trained animals were given i.p. injection of test compounds in doses of 30, 100 and 300 mg/kg. The inability of animal to maintain equilibrium on the rotarod for atleast 1 min in each of three trials indicated neurotoxicity. The dose at which the animals were unable to hold on to the rotarod, was determined as neurotoxic dose [16].

Molecular docking: The interaction and binding ability of the newer synthesized compounds was assessed by molecular docking study. Docking was performed at the $GABA_AR$-β3 binding site. The study was carried out using Glide extra precision (XP) Maestro 10.1 Schrodinger software. It runs on Windows i5 (intel core processor) operating system [Schrodinger, Version 10.1, 2016]. The 2D & 3D structure of synthesized compounds was prepared using Ligplot. The X-ray crystal structure of the human γ-aminobutyric acid receptor, $GABA_AR$-β3 homopentamer (PDB ID: 4COF) was obtained from RCSB Protein Data Bank. The protein preparation wizard was used to prepare protein and grid was generated for co crystal ligand, benzamidine using receptor grid generation. Elimination of water residues beyond 5 Å was done. The protein was optimized by assigning H-bonds and minimization at OPLS 2005 force field. The docked pose of ligands and their interactions were analyzed.

RESULTS AND DISCUSSION

Benzo[1,3]dioxol-5-yloxy-N′-(4-substituted benzylidene)-acetohydrazide derivatives (**4a-h**) were synthesized by reacting (benzo[1,3]dioxol-5-yloxy)acetic acid hydrazide and *p*-substituted benzaldehydes. The synthesized compounds were purified and characterized by spectroscopic techniques. Synthesized compounds were evaluated for anti-convulsant activity by MES and scMET seizure test model. Rotarod test was used to determine the neurotoxicity of the compounds.

Anticonvulsant activity: Compounds **4a**, **4b** and **4c** were found to be active in MES model. They showed protection (1/1, 4 h) at a dose of 300 mg/kg. Protection in scMET test was not shown by any of the compounds. At the highest administered dose *i.e.* 300 mg/kg; minor neurotoxicity (1/2, 4 h) was observed with compounds **4b** and **4c**. Thus, it may be concluded that compounds **4a**, **4b** and **4c** showed protection from MES induced seizures indicating anticonvulsant activity (Table-1). This activity of compounds against MES induced seizures showed their potential to prevent seizure spread and so they can be active in generalized tonic clonic seizures.

Molecular docking: Molecular docking study was carried out for the all synthesized compounds. They were found to strongly inhibit GABAAR-β3, by completely occupying the active sites (at the site of benzamine, co-crystal ligand of the protein as predicted by SiteMap) in the target protein. Many of the compounds showed high docking scores than the standard anticonvulsant drug valproic acid (Table-2).

Among all the synthesized compounds, compound **4b** was found to be most potent with highest docking score of (-6.33). The binding mode of compound **4b** assumes favourable orien-

TABLE -1
ANTICONVULSANT ACTIVITY AND NEUROTOXICITY OF BENZO[1,3]DIOXOL-5-YLOXY-N'-(4-SUBSTITUTED BENZYLIDENE)ACETOHYDRAZIDE DERIVATIVES (**4a-h**)

Compounds	Intraperitoneal injection in rat[a]							
	MES screen (h)			scMET screen (h)			Neurotoxicity screen (h)	
	0.5	2.0	4.0	0.5	2.0	4.0	0.5	4.0
4a	–	–	300[b]	–	–	–	–	–
4b	–	–	300[b]	–	–	–	–	300[c]
4c	–	–	300[b]	–	–	–	–	300[c]
4d	–	–	–	–	–	–	–	–
4e	–	–	–	–	–	–	–	–
4f	–	–	–	–	–	–	–	–
4g	–	–	–	–	–	–	–	–
4h	–	–	–	–	–	–	–	–
Phenytoin	30	30	30	–	–	–	100[c]	100[c]
Normal saline	–	–	–	–	–	–	–	–

[a]Doses of 30, 100 and 300 mg/kg were administered. The values indicate the minimum dose whereby bioactivity was demonstrated in half or more of the rat. The dash (-) indicates an absence of activity at maximum dose administered (300 mg/kg).
[b]Compounds showing protection (1/1, 4 h) at a dose of 300 mg/kg.
[c]Minor neurotoxicity (1/2, 4 h) observed.

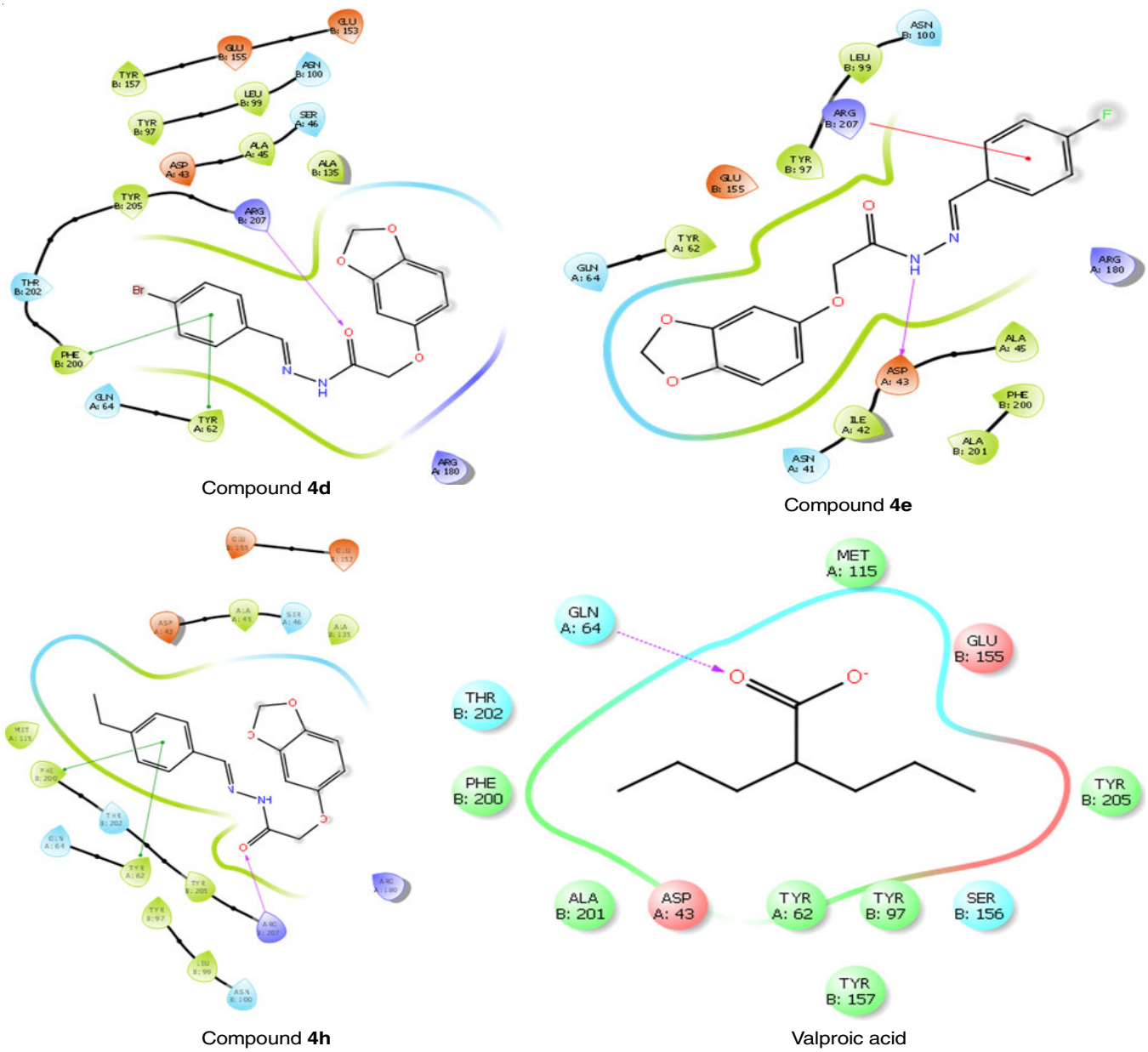


Fig. 1. 2D Ligplot diagram of comparative interactions of some synthesized compound and valproic acid at the binding site of $GABA_AR$-β3

TABLE - 2
DOCKING SCORES OF (BENZO[1,3]DIOXOL-5-YLOXY)-N'-(4-SUBSTITUTED BENZYLIDENE)ACETO HYDRAZIDE DERIVATIVES (**4a-h**) AND VALPROIC ACID (VPA)

Ligand	G Score	Dock Score	Lipophilic EvdW	PhobEn	Phob EnHB	Phob EnPair HB	H Bond	HB Penal	Expos Penal	Rot Penal
4a	-6.32	-6.32	-2.88	-1.73	-0.5	0	-0.85	0	0.46	0.35
4b	-6.33	-6.33	-2.43	-2.13	0	0	-0.82	0	0	0.29
4c	-6.32	-6.32	-2.88	-1.73	-0.5	0	-0.85	0	0.46	0.35
4d	-4.34	-4.34	-2.92	-1.10	0	0	-0.35	0	0.75	0.23
4e	-4.08	-4.08	-3.59	0	0	0	-0.35	0	0.68	0.32
4f	-3.26	-3.26	-3.26	-0.89	0	0	-0.66	0	0.81	0.26
4g	-3.22	-3.22	-3.22	-0.97	0	0	-0.69	0	0.88	0.29
4f	-3.50	-3.50	-2.76	-1.52	0	0	-0.30	0	0.74	0.38
VPA	-4.03	-4.03	-1.29	-1.40	-1	0	-0.35	0	0	0.60

Note: Results are obtained in XP visualizer (application which shows results obtained by extra precision docking) and the short forms used stands for Gscore: Glide score, PhobEnHB: Reward for hydrophobically packed H-bond, PhobEn: Hydrophobic enclosure reward Low MW: Reward for ligands with low molecular weight, Penalties: Polar atom burial and desolvation penalties, and penalty for intra-ligand contacts, Expos Penal: Penalty for solvent-exposed ligand groups; cancels van der Waals terms, Rot Penal: Rotatable bond penalty.

tation due to hydrogen bonds (-0.85) compared with reference valproic acid (VPA) (-4.03 as Dock Score), -1.29 (H bonds), respectively as represented in 2D ligand interaction diagram (Fig. 1). Compounds **4a** and **4c** also showed good docking score of (-6.32) each and H bond (-0.85) each which are more compared to that of standard drug valproic acid used.

Conclusion

Compounds **4a**, **4b** and **4c** were found to be most potent among the synthesized (benzo[1,3]dioxol-5-yloxy)-N′-(4-substituted benzylidene)acetohydrazide derivatives (**4a-h**) as all the three showed protection in MES seizure test. But on comparing all the three potent molecules, compound **4a** was found to be most promising as no neurotoxicity was observed at the highest administered dose. The docking results are well co-related with the pharmacological activity of the compounds. All the three compounds showing high docking score with γ-aminobutyric acid receptor, $GABA_AR$-β3 homopentamer (PDB ID: 4COF) are also showing protection in MES seizure test. This makes us to conclude that the probable mechanism of action of (benzo[1,3]dioxol-5-yloxy)-N′-(4-substituted benzylidene)aceto hydrazide derivatives (**4a-h**) is augmentation of GABAergic activity through binding with γ-aminobutyric acid receptor. Further, biochemical test are required to fully validate the proposed mechanism of action.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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