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Synthesis, molecular docking and CNS activity of 5,5-diphenylimidazolidine-2,4-dione derivatives

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The new Phenytoin derivatives have been synthesized, characterized, and compared for CNS activity. The synthesis was carried out in three steps. Firstly, the chloroacetylation of the 5,5-diphenyl hydantoin is carried out and then various substituted phenols are added into it and have been evaluated for anti-anxiety activity, muscle relaxant activity and anticonvulsant activity by using different models. The number of parameters have been optimized which reveal that the compound containing chloro group such as C3 and C6 show imperative potential when compared with the standard drug Diazepam. The newly synthesised compounds have the probability to be optimized further to engender new scaffolds to treat various CNS disorders.

Keywords: Phenytoin, Parameters, CNS activity, Blood brain barrier, Log P, CNS activity

The hydantoin and its derivatives are used for the treatment of convulsions and has many other importance too. It is speculated that this drug has high ability to cross the blood brain barrier and so it is significant and imperative for treatment of epilepsy and other related disorders. The literature review data revealed that this moiety is also found it used in treatment of several other disorders such as heart disease, arrhythmias, microbial infections, and CNS disorder[1,2]. Many anticonvulsants and anti-anxiety drugs were found to have activity towards GABA-A receptors[3-7]. The like $GABA_A$ receptor is an ionotropic receptor and a ligand-gated ion channel. Its endogenous molecule is c-aminobutyric acid (GABA), the chief inhibitory neurotransmitter in the central nervous system. On stimulation, the GABA-A receptor selectively conducts Cl through its pore which cause hyperpolarization of the neuron. This mechanism prevents neuronal firing by opening chloride channels. These receptors are mostly found in inferior areas of the central nervous system and are involved in the control of motor rhythm generation[8-11]. The protein also contains several different allosteric binding sites which modulate the activity of the receptor indirectly. These allosteric sites are the targets of various other drugs, including benzodiazepines, phenytoin, barbiturates and ethanol Consequently to this a rational drug design process for a new and competent anticonvulsant could be accomplished by the previously reported screening method and hybrid pharmacophore approach[12]. This approach involves the addition of two or more potent molecule which can be combined to give the compound with synergistic effect.

Previous studies showed that phenol pharmacophore containing compounds have potential anticonvulsant activity along with number of other pharmacological activities including pesticides, antioxidants, and various natural products as well as polymeric materials, such as natural organic matter (NOM), lignin, and some resins and plastics[13,14].

Aiming to the hybrid pharmacophore approach the phenytoin moiety was hybridized with phenol in our current work and the synthesized molecules were investigated their pharmacodynamic and pharmacokinetic benefits. The synthesized molecules were also evaluated for their anticonvulsant, anti-anxiety and skeletal muscle relaxant activity using different models.

Experimental Section

The chemicals were acquired from Sigma-Aldrich and were used as it is. The melting points were determined by open capillary tubes on Electrothermal capillary apparatus, the results obtained remain uncorrected. IR spectra of the derivatives were gained

on potassium bromide plates on Schimadzu FTIR Spectrophotometer 8300. ^{1}H NMR spectra of the derivatives were verified on a Bruker DRX-300 spectrophotometer and ^{13}C NMR spectra were confirmed on a Bruker NMR spectrometer (100 MHz). The purity and development of the reaction was observed and determined by TLC.

General procedure for the synthesis of 3-(2-chloroacetyl)-5,5-diphenylimidazolidine-2, 4-dione (B)

Phenytoin (0.001) was dissolved in 35 mL of DMF and the mixture was cooled in an ice bath to acquire the temperature 0-5°C. Chloroacetylchloride (0.01mol) was added drop by drop to the prepared solution and then the mixture was stirred for about 6-8 h. The progress of the reaction was determined by TLC. When the reaction gets completed, the mixture was allowed to stand at RT for about 1 h. Coldwater (100-150 mL) was added after 1 h and kept overnight to get the precipitate. The solid product was obtained in white color which was collected by vacuum filtration. Recrystallization was done by ethanol.

General method for synthesis of 3-(2-(substituted phenoxy) acetyl) -5,5-diphenylimidazolidine -2,4-dione (C1-C10)

A mixture of 0.01 mole of (3-(2-chloroacetyl)- 5,5-di phenyl imidazolidine–2,4-dione (2) in 30 mL of DMF along with 0.002 mole of anhydrous potassium carbonate (K_2CO_3), and 0.001 mole of potassium iodide (KI) was prepared. To this mixture, 0.01 moles of substituted phenol were added drop by drop and the mixture was stirred constantly at 50-55°C for 24-26 h. When the reaction gets completed, the mixture was dispensed into ice-cold water to obtain the final compounds. Final derivatives were filtered through vacuum filtration and washed with water. For the purification, recrystallization was done with ethanol.

The synthesized structure and their physical characterization data was represented in Table 1.

3-(2-Phenoxyacetyl)-5,5-diphenylimidazolidine-2, 4-dione, C1: IR (KBr): 3319 (str, NH amide), 3069 (str, CH arom), 2968 (str C-H ali), 1736 (str C=O amide), 1793 (str C=O alip), 1511 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.6 (15H, Ar-H), 11.25(s, 1H. NH), 3.321(s, 1H, CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 113.5-138.5 (15-H, Ar-H), 158.0- 168.2 (s, ^{13}C, NH), 157.2 (^{13}C, Ar-$OHCH_2$), 168.7-62.5 (s, ^{13}C, CH_2CO). Calcd for $C_{23}H_{19}N_3O_3$: C, 67.28; H, 4.47; N, 7.94. Found: C, 67.24; H, 4.44; N, 7.91%.

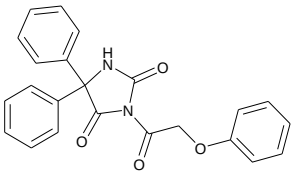
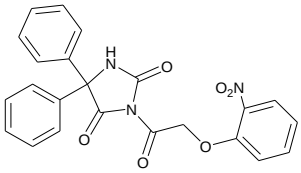
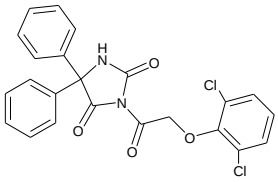
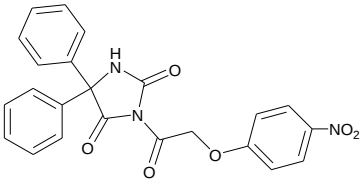
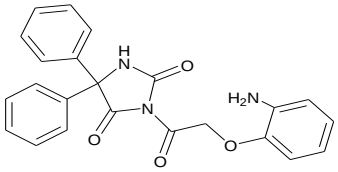
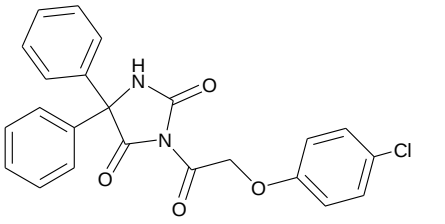
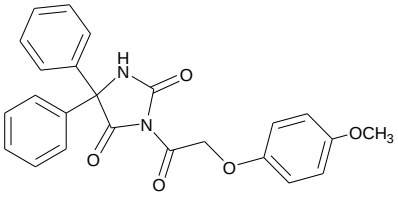
3-(2-(2-Nitrophenoxy) acetyl) -5,5-diphenylimidazolidine-2, 4-dione, C2: IR (KBr): 3318 (str, NH amide), 3066 (str, C-H arom), 2969 (str, C-H alip), 1733 (str, C=O amide), 1794 (str, C=O alip), 1496 (str, C=C arom), 1310 cm^{-1} (str, N-O arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.9 (14 H, Ar-H),11.317 (s ,1H, NH), 3.357(s, 1, H, CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 115.5-139.5 (14-H, Ar-H), 156.0- 167.4 (s, ^{13}C, NH), 155.2 (^{13}C, Ar-$OHCH_2$), 168.2-62.3 (s, ^{13}C, CH_2CO). Calcd for $C_{23}H_{19}N_3O_6$: C, 64.25; H, 4.02; N, 9.94. Found: C, 64.2;, H, 3.99;, N, 9.91%.

3-(2-(2,6-Dichlorophenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-one, C3: IR (KBr): 3323 (str, N-Hamide), 3065 (str, C-H arom), 2970 (str, C-H alip), 1732 (str C=C arom), 1795 (str, C=O alip), 1512 (str, C=C amide), 742 cm^{-1} (str, C-Cl arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ7.0-7.7(13 H, Ar-H),11.078(s, 1H, NH), 3.340 (s,1H, CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 126.5-139.5 (13-H, Ar-H), 158.2- 167.7 (s, ^{13}C, NH), 151.2 (^{13}C, Ar-$OHCH_2$), 168.2-65.2 (s, ^{13}C, CH_2CO), 128.5-130.0 (^{13}C, Ar-Cl). Calcd for $C_{23}H_{16}C_{12}N_2O_4$: C, 60.67; H, 3.60; N, 6.25. Found: C, 60.64; H, 3.58; N, 6.21.

3-(2-(4-Nitrophenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-dione, C4: IR (KBr): 3320 (str, NH amide), 3067 (str, C-H arom), 2968 (str, C-H alip), 1734 (str, C=O amide), 1795 (str, C=O alip), 1512 (str, C=C arom), 1310 cm^{-1} (str, N-O arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.9(14 H, Ar-H), 11.34(s, 1H, NH), 3.4(s, 1H, CH_2CO);^{13}C NMR (300 MHz, DMSO-d_6): δ 125.5-139.5 (14-H, Ar-H), 158.5- 168.7 (s, ^{13}C, NH), 165.5 (^{13}C, Ar-$OHCH_2$), 168.7-62.9 (s, ^{13}C, CH_2CO), 125.5 (^{13}C, Ar-NO_2). Calcd for $C_{23}H_{17}N_3O_3$: C, 64.15; H, 4.08; N, 9.96. Found: C, 64.11; H, 4.01; N, 9.92%.

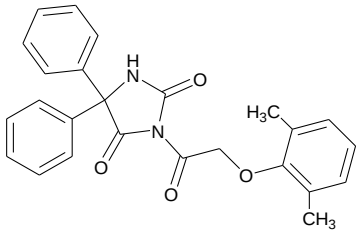
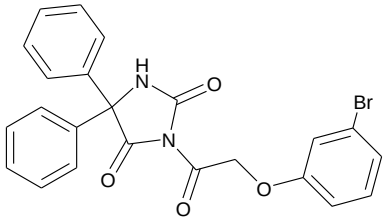
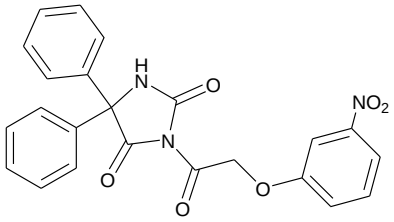
3-(2-(2-Aminophenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-dione, C5: IR (KBr): 3280 (str, N-Hamide), 3060 (str, C-H arom), 2955 (str, C-H alip), 1675 (str, C=O amide), 1755 (str, C=O alip), 1460 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.7(1 4H, Ar-H), 3.302 (s, 1H, CH_2CO).^{13}C NMR (300 MHz, DMSO-d_6): δ 114.5-139.5 (14-H, Ar-H), 158.2- 168.5 (s, ^{13}C, NH), 145.5 (^{13}C, Ar-$OHCH_2$), 168.5-63.4 (s, ^{13}C, CH_2CO), 135.5

Table 1 — Physical characterization data and chemical structure of C1-C10

Compd	Structure	m.p. (°C)	Yield (%)
C1	 3-(2-phenoxyacetyl)-5,5-diphenylimidazolidine-2,4-dione	230-233	67
C2	 3-(2-(2-nitrophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-dione	238-240	70
C3	 3-(2-(2,6-dichlorophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-dione	246-248	78
C4	 3-(2-(4-nitrophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-dione	228-230	65
C5	 3-(2-(2-aminophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-dione	262-264	69
C6	 3-(2-(4-chlorophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-	271-273	76
C7	 3-(2-(4-methoxyphenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-dione	282-284	73

(*Contd*.)

Table 1 — Physical characterization data and chemical structure of C1-C10 (*Contd*.)

Compd	Structure	m.p. (°C)	Yield (%)
C8	 3-(2-(2,6-dimethylphenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-	241-243	68
C9	 3-(2-(3-bromophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4	250-252	53
C10	 3-(2-(3-nitrophenoxy)acetyl)-5,5-diphenylimidazolidine-2,4-d	254-257	69

(^{13}C, Ar-NH_2). Calcd for $C_{23}H_{19}N_3O_4$: C, 69.02; H, 4.82; N, 10.56. Found: C, 68.99; H, 4.80; N, 10.53%.

3-(2-(4-Chlorophenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-dione, C6: IR (KBr): 3452 (str, CH amide), 3039 (str, C H arom), 1269 (str, C-N arom), 766 (str, C-Cl arom), 2900 (str, C-H alip), 1722 cm^{-1} (str C=O alip); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.9 (15H, Ar-H), 11.085(s,1H, NH),3.33(s,1H, CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 117.5-139.8 (15-H, Ar-H), 157.4- 168.5 (s, ^{13}C, NH), 155.2 (^{13}C, Ar-$OHCH_2$), 168.5-63.4 (s, ^{13}C, CH_2CO), 125.5 (^{13}C, Ar-Cl). Calcd for $C_{23}H_{17}NClN_2O_4$: C, 65.74; H, 4.15; N, 6.76. Found: C, 65.70; H, 4.11; N, 6.72%.

3-(2-(4-Methoxyphenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-dione, C7: IR (KBr): 3322 (str, NH amide), 3067 (str, C-H arom), 2968 (str, C-H alip), 1732 (str, C=O amide), 1795 (str, C=O alip), 1511 (str, C=C arom), 2821 cm^{-1} (str, C-H methoxy); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.7(14H, Ar-H) 11.116 (s, 1H, NH)3.6(s,1H, CH_2CO), ^{13}C NMR (300 MHz, DMSO-d_6): δ 126.5-139.8 (14-H, Ar-H), 158.4- 168.7 (s, ^{13}C, NH), 150.2 (^{13}C, Ar-$OHCH_2$), 168.9-63.5 (s, ^{13}C, CH_2CO). Calcd for $C_{24}H_{20}N_2O_5$: C, 69.10; H, 4.69; N, 9.01. Found: C, 69.07; H, 4.66; N, 8.98%.

3-(2-(2, 6-Dimethylphenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-dione, C8: IR (KBr): 3322 (str, NH amide), 3066 (str, C-H arom), 2967 (str, C-H alip), 1783 (str, C=C amide), 1794 (str, C=O alip), 1512 (str, C=C arom), 2820 cm^{-1} (str, C-H methyl); ^{1}H NMR (300 MHz, DMSO-d_6): δ 70-7.7(13 H, Ar-H), 11.108(s, 1H, NH), 3.345(s, 1H, CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 125.5-139.2 (13-H, Ar-H), 158.2- 168.5 (s, ^{13}C, NH), 155.3 (^{13}C, Ar-$OHCH_2$), 168.5-63.9 (s, ^{13}C, CH_2CO). Calcd for $C_{25}H_{22}N_2O_4$: C, 59.45; H, 3.73; N, 6.08. Found: C, 59.43;, H, 3.69;, N, 5.97%.

3-(2-(3-Bromophenoxy)-5,5-diphenylimidazolidine-2,4-dione, C9: IR (KBr): 3502 (str, NH amide), 3066 (str, C-H arom), 2966 (str, C-H alip), 1794 (str, C=O amide), 1733 (str, C=O

alip), 1231 (str, C=C arom), 1443 (str, C-N arom), 698 cm^{-1} (str, C-Br arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.9(14 H, Ar-H), 11.116(s, 1H, NH), 3.35(s,1H, CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 115.5-139.2 (14-H, Ar-H), 157.2- 168.7 (s, ^{13}C, NH), 155.3 (^{13}C, Ar-$OHCH_2$), 168.5-63.9 (s, ^{13}C, CH_2CO), 120.5 (^{13}C, Ar-Br). Calcd for $C_{23}H_{17}BrN_2O_4$: C, 61.48; H, 4.13; N, 5.18. Found: C, 61.43; H, 4.10; N, 5.12%.

3-(2-(3-Aminophenoxy) acetyl)-5,5-diphenylimidazolidine-2, 4-dione, C10: IR (KBr): 3040 (str, NH amide), 3150 (str, C-H arom), 2976 (str, C-H alip), 1660 (str, C=O amide), 1720 (str, C=O alip), 1460 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.0-7.7 (14 H,Ar-H), 11.108 (s,1H,NH), 3.345(s, 1 H,CH_2CO); ^{13}C NMR (300 MHz, DMSO-d_6): δ 125.5-139.2 (14-H, Ar-H), 158.2- 168.7 (s, ^{13}C, NH), 165.3 (^{13}C, Ar-$OHCH_2$), 168.5-63.9 (s, ^{13}C, CH_2CO), 120.5 (^{13}C, Ar-Br). Calcd for $C_{23}H_{19}N_3O_4$: C, 59.45; H, 3.73; N, 6.08. Found: C, 59.41; H, 3.70; N, 6.03%.

Pharmacological Evaluation

Animals and Treatment

The permission was taken by Animal Ethics Committee earlier before carrying out the experiments and all procedures were accepted and done by protocol. Wistar rats, weighing 220–240 g each, were selected from the stock colony maintained in the central animal facility with free access to food and water and were maintained in an air-conditioned room. Concentration of each compound (5 mg/kg) was used in the form of freshly prepared suspensions in 1% Tween 80 and given i. p. in a volume of 0.5 mL/20-24g body weight of rats. The experimental animals were treated with diazepam (4 mg/ kg, n =6), or the test compounds (5 mg/kg) 60 min before evaluation in the maze. The control group was given saline with 1% Tween 80. Diazepam was used as a standard (positive control) drug. Different experimental models were used for different activities.

Anxiolytic Activity (Elevated Plus Maze Method)

According to the method reported in the literature[15-19] the anti-anxiety activity of the compounds was evaluated by elevated plus maze method. The numbers of entries into open and closed arms and the time spent in the open arms were recorded during a 5 min period. The percentage of number of entries into open arms [(open/open +closed)×100] was calculated for each rat.

Skeletal Muscle Relaxant Activity (Rota Rod Method)

Male Wistar rats (220-240 g) acquired from Animal Facility Centre were utilized for this activity. The activity was done by Rota rod method.

Anticonvulsant Screening Method

This method involves the use Pentylenetetrazol (PTZ) that induced convulsion in animals last for 5-10 seconds. At the predicted time of testing, the subcutaneous technique is used to induce convulsions. The derivatives were administered by oral route and standard drugs were administered by intraperitoneal (i. p.) route. Immediately after PTZ administration mice were observed for (1) onset of convulsions (elapsed time from PTZ injection until convulsion occurred), (2) duration of convulsion (number of mice showing convulsions) and (3) mortality for the duration of 30 minutes[22-26].

Molecular Docking Studies and ADME Properties Prediction

In this paper, the $GABA_A$ receptor was selected as the binding region through molecular docking using Molecular Operating Environment. The target protein structure was taken from the reported work[27]. The structure of protein was prepared by ‘‘Protein Preparation Wizard” of Schrodinger suite with default options. The synthesized derivatives were structurally drawn and the energy was minimized using MMFF94 force field followed by conformational search with Low Mode MD. The ‘‘triangle matcher” was used for placing the molecule into the binding pocket, which were ranked by London dG scoring function followed by structure refinement with GBVI/WSA dG.

Computational Calculation

The ADME parameters were predicted by using Chem 3D Ultra version 11.0, and Schrodinger software[28,29]. The parameters include Blood brain barrier (BBB) and Absorption level permeability Clog P and topological polar surface area (TPSA) are the various important parameters for the CNS activity. Other parameters are molecular weight (M.W), molar refractivity (M.R), number of rotatable bonds (RotB), number of hydrogen bond donors (nHBD), number of hydrogen acceptors (nHBA). The study of these parameters showed that the synthesized drugs can cross the CNS membrane and can produce efficient therapeutic effect.

Similarity Calculation

The next parameter studied was similarity calculation done by using the formula given below.

$$di^2 = \sum_{j=1}^{n} \frac{\left(\frac{1 - X_{i,j}}{X_{i,std}}\right)^2}{n}$$

Where, Xi, j is the estimation of atomic boundary 'I' for compound 'j',

Xi, std. is the estimation of a similar subatomic boundary for the standard drug, Diazepam.

Table 2 represents the similarity index with respect to standard drug Diazepam.

Table 2 — Similarity of target compounds (C1-C10) with respect to the standard drugs.

Compd	Similarity[a,b] (in %) to Diazepam
C1	82
C2	67
C3	87
C4	67
C5	99
C6	91
C7	73
C8	76
C9	61
C10	93

[a] (1 – R)×100, where R = quadratic mean (root mean square mean), [b] Calculated from physicochemical properties: Molecular weight; Molar refractivity; Connolly dissolvable available surface region; Connolly subatomic surface territory; Connolly dissolvable barred volume; Topological polar surface region; Molecular topological file.

Evaluation of Physicochemical Parameters

Various other factors like molecular weight, n ON value, n OHNH value, *n*-violations were determined through online software[30].

Results and Discussions

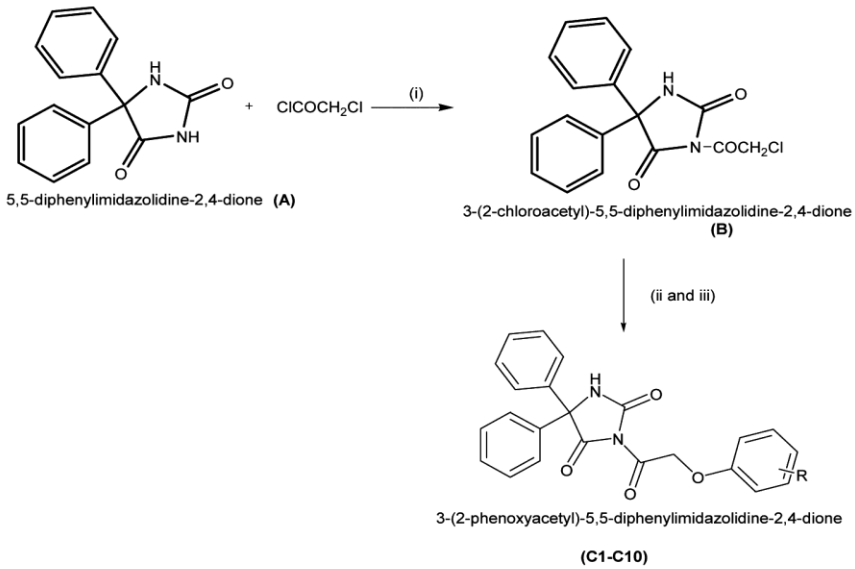
Chemistry

The synthesis of the final derivatives is outlined in Scheme 1, in which phenytoin was chloroacetylated in the solvent DMF to yield intermediate 3-(2-chloroacetyl)-5,5-diphenylimidazolidine-2,4-dione **B** further this intermediate was allowed to react with different substituted phenol to give target compounds **C1-C10**. The structures of the compounds were characterized by spectral methods *i.e.*, IR, ^{1}H and ^{13}C NMR, and elemental analysis.

Pharmacology

Anxiolytic Activity (Elevated Plus Maze Method)

Wistar rats, weighing 220–240 g each, were selected from the stock colony maintained in the central animal facility with free access to food and water and were maintained in an air-conditioned room. Concentration of each compound (5 mg/kg) was used in the form of freshly prepared suspensions in 1% Tween 80 and given i. p. in a volume of 0.5 mL/20-24g body weight of rats. The experimental animals were treated with diazepam (4 mg/ kg, n =6), or the test compounds (5 mg/kg) 60 min before evaluation in the maze. The control group was given saline with 1% Tween 80. The numbers of entries into



Reagents and conditions: (i) DMF, stirring 6-8 h; (ii) DMF, K_2CO_3 and KI, stirring 24-26 h.

Scheme 1 — Synthesis of the target compounds **C1-C10**

Table 3 — Anti-anxiety activity of the target compound 3-(2-(substituted phenoxy) acetyl)-5,5-diphenylimidazolidine-2,4-dione (C1-C10) by Elevated plus maze method.

	Open arm		
Compd	Spent time	Number of entries	Number of entries(%)
C1	64.85±1.45	6.62±0.56	54.25
C2	67.63±2.64	7.73±0.39	45.21
C3	85.35±0.62	11.01±0.75	57.86
C4	50.07±3.46	4.08±0.78	43.35
C5	52.89±2.45	3.66±0.36	36.23
C6	83.54±0.65	10.06±0.72	58.98
C7	63.57±1.76	6.02±0.65	46.59
C8	48.65±3.55	3.29±0.34	29.25
C9	69.74±1.65	7.96±0.65	47.40
C10	71.15±1.35	8.53±0.72	42.26
Diazepam	91.83±2.33	10.09±76	63.46
Vehicle	41.14±3.45	2.84±0.92	21.25

Data represent the mean ± SEM; n= 6; P< 0.05 compared with vehicle

open and closed arms and the time spent in the open arms were recorded during a 5 min period. The percentage of number of entries into open arms [(open/open +closed) x 100] was calculated for each rat and given in Table 3.

Skeletal Muscle Relaxant Activity (Rota Rod Method)

Male Wistar rats (220-240 g) acquired from Animal Facility Centre were utilized for this activity. The activity was done by Rota rod method. The rats were placed on a horizontal wooden rod which rotates at a speed of 25 rpm. The rats which are capable of lasting on the top for 1 min or more were selected for the study. The selected animals were divided into groups (n= 6). The stock solutions were also prepared for the test samples by suspending in 0.5% CMC. CMC (0.5%, 10 mL/kg) and diazepam (4 mg/kg, i. p.) were given to the control and standard groups. 100 and 200 mg/ kg (p. o.) of the synthesized derivatives was given orally to the test groups. Then the procedure was repeated for each group of animals at an interval of 2 h. The animals which remain for more than 1 minute on Rota rod are considered as passes. The result of the screening data was given in Table 4.

Anticonvulsant Screening Method

Pentylenetetrazole (PTZ) induced convulsion Animals were divided into different groups (n=6 Wistar rats of either sex in one group). Control Group received the respective vehicles; standard group received diazepam 5 mg/kg and the other groups received synthetic derivatives (C1-C10) at a dose level 5mg/kg. All treatment groups were statistically compared with standard group except the control

Table 4 — Skeletal muscle relaxant activity of target compounds (C1-C9) by Rotarod method

Compd	Dose	Rotarod test
C1	25mg/kg	47.26±1.89
C2	25mg/kg	57.65±1.45
C3	25mg/kg	44.16±1.82
C4	25mg/kg	60.15±1.54
C5	25mg/kg	68.70±1.36
C6	25mg/kg	43.16±2.82
C7	25mg/kg	53.82±1.098
C8	25mg/kg	73.06±1.65
C9	25mg/kg	51.45±1.65
C10	25mg/kg	65.98±1.65
Diazepam	2mg/kg	38.20±1.34
Vehicle	10 mL/kg	83.12±1.97

Data represent the mean ± SEM; n= 6; P< 0.05 compared with vehicle

group. The derivatives were administered by oral route and standard drugs were administered by intraperitoneal (i. p.) route. Wistar rats were administered synthetic derivatives for seven days and on the experimental day, PTZ 65 mg/kg was injected intraperitoneal to mice 45 min after derivatives and 30 min after the standard drug. Immediately after PTZ administration mice were observed for (1) onset of convulsions (elapsed time from PTZ injection until convulsion occurred), (2) duration of convulsion (number of mice showing convulsions) and (3) mortality for the duration of 30 minutes. The data of the synthesized compounds were given in Table 5.

Statistical analysis

Observed data from the biochemical analyses and other parameters were expressed as mean ± SEM. One-way ANOVA was used to test the means. Values

Table 5 — Effect of synthetic derivatives (C1-C10) on the Pentylenetetrazol-induced convulsion in rats

Compd	Dose	Onset of clonic convulsion (min)	Duration of convulsion (min)	Mortality/ used (%)
Control	Vehicle	0.00±0.10	0.00±0.00	0/6 (0%)
Diazepam (Standard)	4mg/kg	1.00±0.10***	0.50±0.30***	0/6 (0%)
C1	5mg/kg	8.57±2.10	5.30±1.22	2/6 (33.33%)
C2	5mg/kg	8.30±2.10	5.54±2.10	2/6 (33.33%)
C3	5mg/kg	2.66±0.40**	1.10±0.44**	0/6 (0%)
C4	5mg/kg	8.20±2.10	4.10±1.10	2/6 (33.33%)
C5	5mg/kg	8.20±1.10	4.30±2.20	3/6 (50.00%)
C6	5mg/kg	2.34±0.40**	0.46±0.44**	0/6 (0%)
C7	5mg/kg	8.70±1.10	5.30±1.34	3/6 (50.00%)
C8	5mg/kg	8.90±2.12	4.30±2.57	2/6 (33.33%)
C9	5mg/kg	8.89±2.33	6.30±0.10	3/6 (50.00%)
C10	5mg/kg	9.22±0.20	6.20±1.10	2/6 (33.33%)

Data represent the mean ± SEM; n= 6; P< 0.05 compared with vehicle

were considered statistically significant at $P < 0.05$. All results were represented as mean ± SEM (n = 6). Values with different superscripts were significantly different ($P < 0.05$).

Structure Activity Relationship

In our research work the derivatives of Phenytoin were synthesized. Different CNS activity including anticonvulsant, anti-anxiety and skeletal muscle relaxant activity were evaluated. By analyzing the different activities, the following structure activity relationship (SARs) were generated.

It can be observed that different groups have different effects on the activity. Groups such as electron withdrawing and electron donating have different potency towards the CNS activity. It can be observed that compounds containing chloro group *i.e*. electron withdrawing groups shows good CNS activity. The change in the position of the substituents to the *ortho*, *meta* or *para* also have mild to moderate activity when compared to drug Diazepam. Whereas, other groups show mild to moderate CNS activity.

In brief, compounds containing chloro group can have higher efficacy when compared to other group containing compounds.

Docking Studies

The mechanism of drug interaction with receptor can be easily studied and understand by docking studies. This is considered to be imperative as it can highlight the possible interaction of the drug with receptor. The molecular docking of all compounds was carried out with $GABA_A$ receptors using Discovery Studio. The target protein structure was taken from the previous reported work. The structure of protein was prepared by ‘‘Protein Preparation

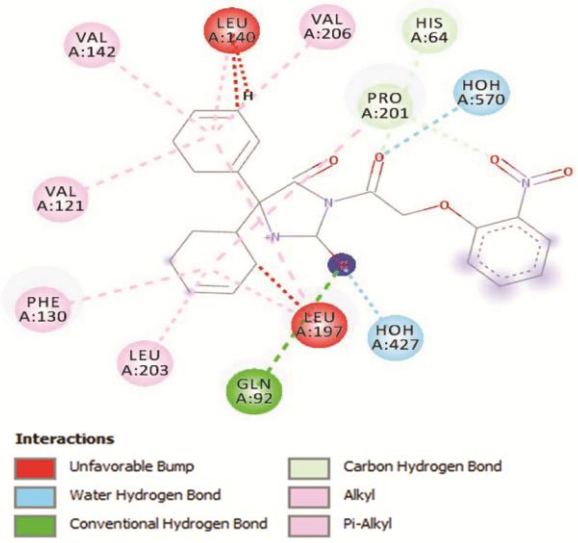


Fig. 1 — Docked poses and binding interaction of Compound C-2

Wizard” of Schrodinger suite with default options. The synthesized derivatives were drawn structurally and the energy of the structures were minimized using MMFF94 forcefield followed by conformational search with Low Mode MD. The compounds were docked into $GABA_A$ receptor using rigid receptor docking protocol. The methodology of ‘‘triangle matcher” was applied for binding the molecule into the pocket and ranked by London dG scoring function. The structure refinement was done with GBVI/WSA dG. The predicted interactions of the derivatives are shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4.

Computational analysis

The various molecular parameters of the synthesized drug along with the standard drug

Table 6 — Molecular, pharmacokinetic and toxicity parameters for synthesized compds. (C1-C10)

Compd	Log P	Mol.Wt.[a]	MR[b]	TPSA[c]	nHBD	nHBA	nRotB	BBB **(level)**	ABS **(level)**
C1	3.73	386.40	105.459	75.71	1	1	1	1	0
C2	3.49	431.40	0	127.52	2	2	1	1	0
C3	4.46	454.29	11.069	75.71	1	1	1	1	0
C4	3.78	431.40	0	127.52	2	2	2	1	0
C5	2.77	401.14	110.16	101.73	3	2	2	2	0
C6	4.52	420.84	110.264	75.71	1	1	1	1	0
C7	4.61	464.11	111.923	84.94	1	1	1	1	0
C8	4.70	414.46	115.102	75.71	1	1	1	1	0
C9	4.73	465.30	113.082	75.71	1	1	1	1	0
C10	3.77	431.11	110.16	101.73	2	2	1	1	0

[a]Molecular weight; [b]Molar refractivity; [c]Topological polar surface region; [d]Molecular topological record; [e]Wienner file; [f]Ovality; s

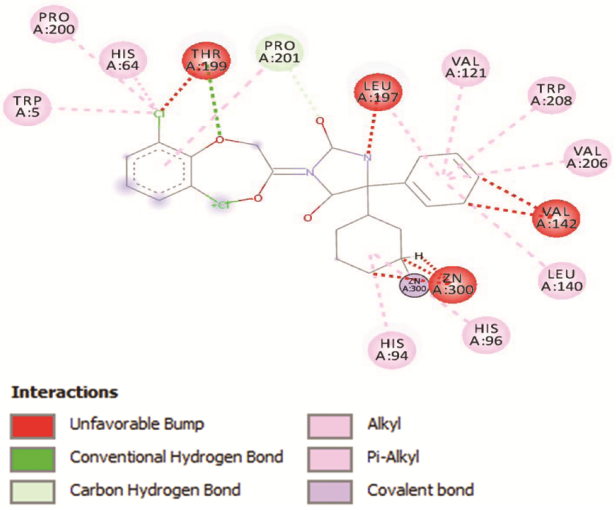


Fig. 2 — Docked poses and binding interaction of Compound C-

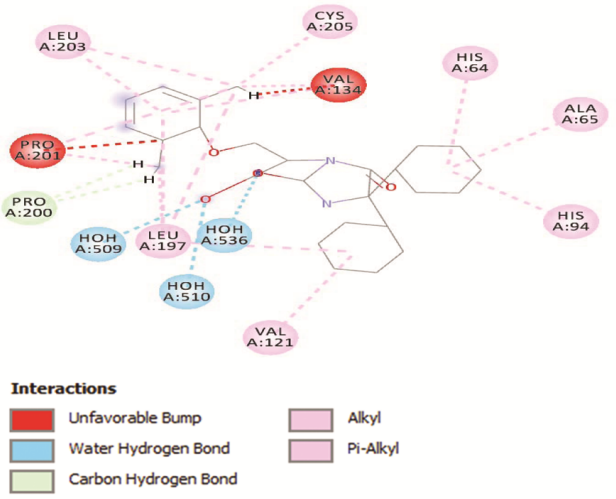


Fig. 3 — Docked poses and binding interaction of Compound C-8

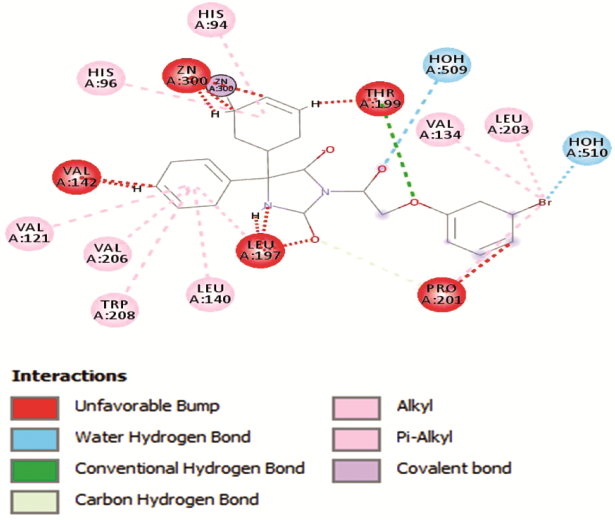


Fig. 4 — Docked poses and binding interaction of Compound C-9

diazepam were calculated to investigate the various important factors required for potent CNS activity. The study revealed that the synthesized drugs can cross the Blood Brain Barrier. All optimized parameters were summarized in Table 6.

Similarity Studies

The similarity studies were carried out with respect to the standard drug diazepam. The study was determined from 7 physicochemical properties and was given in Table 2.

Evaluation of Molinspiration

Various factors like molecular weight, n ON-value, n OHNH-value, *n-violations* and number of rotatable bonds are determined through online software's and all parameters were summed up in Table 7.

Table 7 — Physico-chemical Parameters values 3-(2-(substituted phenoxy) acetyl)-5,5-diphenylimidazolidine-2,4-dione (C1-C10)

Compd	n ON	n OHNH	n-violation
C1	6	1	0
C2	9	1	0
C3	6	1	0
C4	9	1	0
C5	6	3	0
C6	6	1	0
C7	7	1	0
C8	6	1	0
C9	6	1	0
C10	7	3	0

Conclusion

In conclusion synthesis of phenytoin derivatives was portrayed in the synthetic Scheme 1. The target compounds (**C1-C10)** were synthesized by the reaction of 5,5- diphenylimidazolidine-2, 4-dione (**A**) with chloroacetyl chloride in DMSO to obtain 3-(2-chloroacetyl)-5,5-diphenylimidazolidine-2, 4-dione (**B**) which was allowed to react with different substituted phenols to obtain the product (**C1-C10)**. The FT-IR and 1H NMR spectra of the compounds were shown peaks due to different functional groups and protons present in the synthesized compounds. The number of protons present in the target compounds was recorded by 1H NMR spectroscopy using Bruker DRX-300spectrophotometer. The spectra showed a multiplet at δ 7.0-7.6 ppm corresponding to aromatic hydrogen's (Ar-H); a singlet at δ 3.27 ppm corresponding to aromatic protons CH_2CO. ^{13}C NMR spectra were recorded on a Bruker NMR spectrometer (100 MHz) and element analysis of the synthetic compound were confirmed by the Shimadzu mass spectrometer and Perkin Elmer 2400 CHN elemental analyzer. Finally, these synthesized compounds were screened for different CNS activities such as anti-anxiety, skeletal muscle relaxant activity and anticonvulsant activity. Most of the synthesized compounds were shown promising activity. Some of the derivatives were found to have potent CNS activity when compared to the standard drug Diazepam. Synthesized compounds containing chloro groups were found to possess better CNS activity when comparing with other compounds. Therefore, compounds C3 and C6 were constituted with chloro groups on the phenyl ring had shown admirable activity. The result of the different CNS activity of synthesized analogues was denoted in tables. In molecular docking studies the target compounds were docked with $GABA_A$ receptor to find the likely mechanism of action. The compounds showed significant interaction at the BZD-binding site on the $GABA_A$ receptor. The similarity study was carried out which also displayed mild to moderate similarity with respect to standard drugs. The physicochemical parameters including pharmacokinetic and toxicity studies confirmed with the standard compounds and suggested its possibility of being drug-like candidates and can cross the blood-brain barrier.

Therefore, it was depicted that the synthesis, computational studies, and evaluation of anticonvulsant, anti-anxiety, and skeletal muscle relaxant activity of new phenytoin derivatives were carried out. The compounds were successfully synthesized and well described by various physicochemical properties. Through studies, it was revealed that some of the compounds exhibit good CNS activity. The compounds also display mild to moderate similarity with respect to the standard drugs. The pharmacokinetic and toxicity studies confirmed with the standard compounds and suggested its possibility of being drug-like candidates. From the pharmacological activity, it was found that some of the target compounds have significant CNS activity. However, future optimization might be beneficial in the prospect research and expansion of the target compounds for the enhancement of the CNS activity.

Ethics approval and consent to participate

The experiments were approved by the Research Ethics Committee, IFTM University, Moradabad 244 001, India.

Human and animal rights

No humans were used for the studies that are the base of this research. All animals used were in accordance with the US Public Health Service's "Policy on Humane Care and Use of Laboratory Animals", and "Guide for the Care and Use of Laboratory Animals".

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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