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Design, Synthesis and evaluation of dialkyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-substituted)pyridine-3,5-dicarboxylates as potential anticonvulsants and their molecular properties prediction

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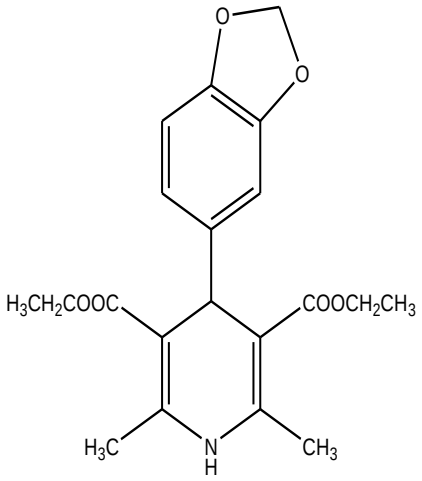
Graphical Abstract:



compound 8f

A novel series of 4-aryl-1,4-dihydropyridines (8a-8o) is designed and synthesized, which displayed good anticonvulsant and antioxidant activities. Compound **8f** exhibited good anticonvulsant activity comparable to diazepam and also passed the Lipinski's rule-of-five.

Research Highlights:

- A series of 4-aryl-1,4-dihydropyridines were synthesized by Hantzsch reaction.
- All the compounds were evaluated for anticonvulsant and antioxidant activities.
- Compounds **8a**, **8f** and **8k** showed good anticonvulsant activity.
- These compounds also obeyed the Lipinski's rule-of-five.
- Selected compounds were also evaluated for antinociceptive activity.

Design, Synthesis and evaluation of dialkyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-substituted)pyridine-3,5-dicarboxylates as potential anticonvulsants and their molecular properties prediction†.

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Abstract

The present study is on the development of dialkyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-substituted)pyridine-3,5-dicarboxylate derivatives as isosteric analogues of isradipine and nifedipine, by the replacement of benzofurazanyl and 2-nitrophenyl groups respectively with benzo[d][1,3]dioxo-6-yl group, as potential anticonvulsants. Fifteen new derivatives **(8a-8o)** were synthesized and tested for anticonvulsant activity using maximal electroshock and subcutaneous pentylenetetrazole induced seizure methods. Compound **8f** possessing free NH group in 1, 4-dihydropyridine ring, diethyl ester functionality at the positions 3 and 5 showed significant anticonvulsant and antioxidant activities. This was also supported by molecular properties prediction data. Selected compounds were evaluated for antinociceptive activity in capsaicin induced nociception assay at 10mg/kg body weight, but displayed no significant activity at the tested dose.

Key words: benzofurazanyl; benzo[d][1,3]dioxo-6-yl; 1,4-dihydropyridine; anticonvulsant activity; antinociceptive activity.

1. Introduction

4-Aryl-1,4-dihydropyridines (4-aryl-1,4-DHPs) form a major class of drugs used as potent calcium-channel blockers and were reported to be effective against majority of convulsive procedures including electro and pentylenetetrazole convulsions. The recent investigations on 1,4-dihydropyridines proved that they can modulate TRPV1 (transient receptor potential vanilloid1) channels in a positive fashion and thus represent a lead molecule for future pain therapeutics [1,2]. Seizures are the symptoms of epilepsy, which is a common neurological disorder affecting about 1% of the world population. Despite all the new antiepileptic drugs (AEDs) introduced in the past few decades, about 30% of the patients with epilepsy are still not seizure free [3]. Therefore, there is a significant need to develop new AEDs that are more potent and with fewer side effects.

Nifedipine and amlodipine are the prototype drugs of the class 4-aryl-1,4-DHPs and have been subjected to several modifications (**Fig 1**). Their analogue, isradipine has received particular attention as antihypertensive agent, calcium channel blocker and was also reported to possess anticonvulsant activities [4-6]. Isradipine (4-(4-benzofurazanyl-1,4-dihydro-2,6-dimethyl-3,5-pyridindicarboxylate, methyl 1-methylethyl) was evaluated for anticonvulsant activity by maximal electroshock method (MES) in mice and reported to be active at 5mg/kg dose [7,8]. Pharmacological and clinical studies have documented the pathophysiological similarities in epilepsy and neuropathic pain models. Thus, antiepileptic agents have good potential to manage neuropathic pain [9].

Based on these reports, we have recently begun a study on benzo[d][1,3] dioxole derivatives as isosteric analogues of isradipine molecule, where benzofurazanyl moiety of isradipine was replaced by benzo[d][1,3]dioxol-6-yl moiety. In this paper, we report the molecular properties prediction, synthesis, characterization and evaluation for anticonvulsant, antinociceptive and antioxidant activities of dialkyl4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-substituted)pyridine-3,5-dicarboxylate derivatives, **8a-8o** in which the benzo[d][1,3]dioxolyl moiety was attached to the fourth position of 1,4-dihydropyridine ring.

2. Results

2.1. Chemistry

Synthesis of benzo[d][1,3]dioxole derivatives of 1,4-dihydropyridine was carried out via a one-pot multicomponent reaction **(scheme 1)**. The condensation of commercially

available piperonal, alkylacetoacetates and different substituted anilines in methanol provided the compounds **8a-8j** in good yields [10]. In the synthesis of asymmetrical 1,4-dihydropyridine derivatives (**8k-8o),** the above method was modified to avoid the formation of symmetrical 1,4-dihydropyridines. The reaction was carried out by condensation of piperonal with ethylacetoacetate, followed by addition of methylacetoacetate and substituted anilines and ammonia to obtain asymmetrical 1,4-DHPs as explained in **scheme 2** [11,12]. The structures of compounds **8a-8o** were confirmed by spectral data and elemental analysis.

2.2. Pharmacological evaluation.

The preclinical discovery and development of the new drug candidates for the treatment of epilepsy are based mainly on the use of predictive animal models. In the present study there are two *in vivo* screens used those include subcutaneous pentylenetetrazole (scPTZ) seizures and maximal electroshock seizures.

The profile of anticonvulsant activity of compounds **8a-8o** was evaluated by maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) tests after the administration of test compounds (p.o) and reference standard drug (phenytoin, p.o; diazepam, i.p) to male Wistar albino rats at the dose of 50 mg/kg body weight [13,14]. Seizure inducing pentylenetetrazole (80mg/kg, s.c) injection or maximal electroshock (150mAmp for 0.2 sec) was applied 1h after the administration of the drug candidates. In scPTZ induced method, the seizure response was observed for a maximum period of 300sec. Compound 8f blocked the seizure activity at the maximum latency time used in the study. Compounds **8a, 8b, 8f-8m** and **8o** showed significant anti-scPTZ activity ($p<0.0001$ vs. control). Of these compounds, **8f, 8g** and **8h** exhibited good activity comparable to diazepam (5mg/kg, i.p) as reference standard (**Table 1**). Other compounds, **8a, 8b, 8i-8k, 8m** and **8o** showed moderate activity. Compounds **8d**, **8e** and **8n** were devoid of anti-scPTZ activity. The protection against PTZ induced mortality was also studied. Compound **8f** exhibited 100% protection and compounds, **8g, 8h** and **8j** showed moderate protection against mortality (66%). Other compounds did not exhibit significant protection.

In the MES model, all the test compounds showed significant decrease in the duration of limb extension and protection against electro convulsions except **8d** and **8e** ($p>0.05$ vs. control) (**Table 1**). Among these derivatives, **8f** and **8h** exhibited good anti-MES protection and other derivatives showed moderate protection. However, the activities of compounds **8a-8o** are less than that of phenytoin (30mg/kg, po).

Of these derivatives, compounds **8f-8k** and **8m** showed good seizure inhibition activity in scPTZ induced seizure method and are comparable to nifedipine at 30mg/kg, whereas in MES induced method compounds **8f** and **8h** are comparable to nifedipine and isradipine hind limb extension periods. Compound **8f** was further subjected to dose-dependent study at three doses (25, 50 and 100 mg/kg body weight) by PTZ induced and MES induced seizure models (**Table 2)**. The compound showed 41.6, 65.6 and 72.0 percentage protection respectively against electroconvulsive seizures. In PTZ model, compound 8f showed equal latency periods at 50mg and 100mg/kg doses, but no protection against mortality at 100mg/kg dose.

All the compounds were evaluated for *in vitro* antioxidant activity by DPPH free radical scavenging assay. IC_{50} values were calculated and are given in **table 3.** Among these, compounds **8f, 8i** and **8n** showed moderate radical scavenging activity (IC_{50} values ranging from 363-386µg/ml).

The pathophysiological similarity between epilepsy and neuropathic pain has prompted us to extend the study to capsaicin-induced nociception assay, to evaluate the ability of the compounds in reducing neuropathic pain. Perusal of the results showed that compounds **8a**, **8b**, **8f** and **8o** did not present any significant activity at the dose of 10mg/kg (**Table 4**).

2.3. Calculation of drug-likeness properties

Drug-likeness can be considered as a delicate balance among the molecular properties of a compound that influences its pharmacodynamics, pharmacokinetics and ultimately ADME (absorption, distribution, metabolism and excretion) in human body like a drug [15]. These parameters allow ascertaining oral absorption, or membrane permeability that occurs when evaluated molecules obey Lipinski's rule-of-five [16]. Other parameters that included are number of rotatable bonds, molecular volume, topological polar surface area, percentage absorption and in vitro plasma protein binding.

The above mentioned parameters were calculated for **8a-8o** and the results were presented in **table 5**. It was observed that compounds **8a**, **8f** and **8k** have optimum logP (<5), lower *in vitro* plasma protein binding (<60%) and no violations from Lipinski's rule-of-five.

3. Discussion

The title compounds, **8a-8o** were synthesized based on the lead molecule, isradipine. The present work is related to the isosteric replacement of benzofurazanyl group in isradipine with benzo[d][1,3]dioxo-6-yl moiety. Isradipine was reported to cause significant anticonvulsant activity in mice using maximal electroshock model in a dose-dependent manner at 1, 2.5 and 5.0 mg/kg [7,8].

Symmetric 1,4-DHP derivatives (**8a-8j**) were synthesized by following the Hantzsch synthesis and asymmetric derivatives (**8k-8o**) by the modified Hantzsch synthesis. The mechanism involved condensation of the aldehyde, β-ketoester (**5, 6**) and amines (**7a-e**) followed by cyclisation and simultaneous elimination of three moles of water [17]. The synthesis of symmetric 1,4-DHP derivatives (**8a-8j**) involves knoevenagel condensation of piperonal and one molecule of alkylacetoacetate, followed by Michael addition of aminoketone arising from the reaction of second molecule of alkylacetoacetate and amine. Asymmetric 1,4-DHP derivatives were synthesized by slight modification of the reaction, where piperonal reacts with ethylacetoacetate to produce knoevenagel product, which further reacts with the methylacetoacetate and substituted amines to produce the compounds **8k-8o** [18].

The IR spectra of the title compounds showed a broad band at 3400-3300 cm^{-1} assignable to secondary amine group, a strong band at 1710-1680 cm^{-1} due to unsaturated ester group on 1, 4-DHP basic nucleus at the positions 3 and 5. A band at 1450-1350cm^{-1} indicated symmetric and asymmetric stretching of nitro functional group and a band at 1050-1020cm^{-1} showed C-N stretching. The ^{1}HNMR spectrum of the compounds supported the structures of **8a-8o**. These compounds showed multiplets in the region of 6.4 to 8.1 ppm due to aryl protons and a singlet at 5.80-5.95 ppm representing methylene protons of the benzo[d][1,3]dioxole ring. The mass spectra of the compounds **(8a-8o)** showed the respective molecular ion peaks and the data of elemental analysis of the compounds are within the limits of ±0.4% of theoretical value.

In an attempt to study of the structure-activity relationships, the compounds are designed by introducing a phenyl or substituted phenyl at position 1, benzo[d][1,3]dioxole ring at position 4 and by varying the size of alkyl groups at positions 3 and 5 of 1,4-dihydropyridine. It was previously reported that anticonvulsant activities of 1,4-DHPs are strongly influenced by the nature of substitution at positions 1 and 4. The presence of phenyl ring at fourth position (compounds **3**& **4**) increased the anticonvulsant activity when

compared to 1,4-dihydropyridine derivatives with no substitution at position 4 (compounds **1**&**2; Fig 2**) [19]. In our study, introduction of benzo[d][1,3]dioxolyl moiety in place of phenyl group retained the activity indicating the importance of presence of 4-aryl substitution. It was found that the anticonvulsant activity profile of the 4-benzo[d][1,3]dioxole-1,4-dihydropyridines is significantly influenced by the nature of substitution on the nitrogen atom at position 1. Compounds possessing no substitution at position 1 and with free NH group available are found to be active (**8a**, **8f** and **8k**). Compound **8f** showed marked anticonvulsant activity in both the models and maximum protection against mortality. Similar results were also reported with N-methylation of 4-substituted-1,4-DHPs which caused a large decrease in anticonvulsant activity [20]. It is probable that the presence of free NH might augment binding to the target via hydrogen bonding.

Introduction of a phenyl group at position one resulted in decrease in the activity. The presence of a nitro group on the phenyl ring further influenced the activity. Compounds with 2-nitrophenyl substitution are more potent than those containing nitro groups at third and fourth positions of the phenyl ring. With an increase in size of the alkyl group of the ester functionality, the anticonvulsant activity is also increased. The presence of ethyl group of 3,5-diester functionality as in **8f-8j** resulted in active analogs. It was supported by the literature studies that bulkiness of ester functionality seems to be important for potency [20]. It is probable that the presence of larger alkyl groups in the ester could have better interaction with calcium channels and gain more potency. It was reported that existence of hindrance by substituted groups prevent hydrolysis of esteric groups on 1,4-dihydropyridine ring [21]. The presence of benzodioxolyl moiety at position 4 and 2,6-dimethyl groups on dihydropyridine ring can induce steric hindrance on ester groups at third and fifth positions of dihydropyridine. Hence, it is likely that the physiologically active compound in the seizure studies is indeed the test compound, instead of a metabolite. Compound **8f** was further evaluated at 25mg and 100mg as it exhibited promising seizure inhibition and protection against mortality at 50mg/kg dose. The activity was found to be dose dependent in both scPTZ and MES models. The isosteric replacement of 2-nitrophenyl and benzofurazanyl substitutions of nifedipine and isradipine respectively with benzodioxolyl group at position 4 of dihydropyridine ring retained the activity.

Perusal of the radical-scavenging ability data showed that the compounds exhibited only moderate scavenging of DPPH free radical **(Table 3)**. There was a good agreement

between anticonvulsant activity and radical scavenging activity for compound **8f**, thus suggesting that anticonvulsant activity of compound **8f** might involve antioxidant mechanisms.

The study was extended to evaluate selected compounds for antinociceptive activity by using capsaicin induced flinching assay in Swiss albino mice. Literature studies showed that capsaicin, resiniferatoxin, N-oleoyldopamine etc act on vanilloid receptors (TRPV1) producing nocicepton [22]. Compounds **8a, 8b, 8f** and **8o** which exhibited good anticonvulsant activity were evaluated for capsaicin induced flinching model at 10mg/kg body weight. None of the compounds displayed significant antinociceptive activity at the tested dose. As these compounds were not evaluated at other higher doses, further studies are required to test the antinociceptive profile of the compounds.

Calculation of drug-likeness properties

Drug-likeness of the compounds **8a-8o** were estimated from predicted ADME values and Molinspiration software and found to score well. In particular, optimum lipophilicity (<5) and presence of hydrogen bonding donor make the molecules **8a**, **8f** and **8k** likely to have good drug-likeness and absorption. Number of rotatable bonds is important to know the conformational changes, flexibility and for binding with receptors or channels [23]. Compounds **8a-8o** were found to possess rotatable bonds 5-9 (<10), indicating high conformational flexibility. It was observed from the data that the title compounds exhibited %ABS ranging from 67.5 to 83.3%, while standard isradipine and nifedipine displayed 73.2 and 70.9 %ABS respectively.

Lipinski's rule-of-five is widely used filter for drug-like properties and states that most molecules with good membrane permeability have logP≤5, MW≤500, HBD≤5 and HBA≤10. Of these compounds, **8a**, **8f** and **8k** obeyed the rule and other compounds were found to violate in one parameter i.e. partition co-efficient. Furthermore, compounds which violated the rule are also deficient in hydrogen bonding donors, whereas compounds **8a**, **8f** and **8k** were found to have one HBD, which may also be a necessary requirement to exhibit the activity.

The importance of presence of free NH group of 1,4-dihydropyridine is also supported by the estimation of *in vitro* plasma protein binding using preADME studies. *In vitro* plasma protein binding (%) for the compounds **8a-8o** was given in **table 5**. Compounds

containing free NH group i.e. **8a, 8f** and **8k** showed less than 90% *in vitro* plasma protein binding thus suggesting weak plasma protein binding of these compounds [24].

4. Conclusion

The present study revealed that compound **8f** showed promising anticonvulsant activity comparable to phenytoin. The presence of free NH group of 1,4-DHP, 4-aryl substitution and the size of the alkyl group of the ester functionality at positions 3 and 5 are probably the desirable features for good anticonvulsant and antioxidant activities. Further, the molecular properties prediction data supports that compounds **8a**, **8f** and **8k** might involve hydrogen bonding interaction with target site, displayed good *in silico* absorption and low plasma protein binding, thus making them as potential drug candidates for antiepileptic therapy.

5. Materials and methods

5.1 General

Aldehyde and esters were procured from Sigma-Aldrich and Merck chemicals. All other chemicals are of AR grade. Purity of the samples was monitored by TLC analysis using Precoated aluminium plates (Merck), coated with Silica Gel (Kieselgel 60) with F_{254} indicator. Melting points were determined in open capillaries using Analab melting point apparatus and were uncorrected. IR spectra were recorded as KBr diluted pellets on a Jasco FTIR (FTIR-4100) Spectrophotometer. ^{1}H NMR spectra were carried out on Jeol-400 MHz NMR Spectrophotometer (JNM-400) using TMS as internal reference. Chemical shifts (δ values are given in parts per million (ppm) using $CDCl_3$ as solvent coupling constants (J) in Hz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectral data was obtained on LCMS (schimadzu) APCI modelLC-2010 EV. Elemental analyses were performed on Perkin Elmer 2400 C, H and N elemental analyser.

5.1.1. General method for the synthesis of dialkyl 4-(benzo (d)[1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(substituted)pyridine-3,5-dicarboxylate ***(8a-8j). (Scheme-1)***

To a solution of piperonal (0.01mol, 1.5g), methylacetoacetate (**5**) (0.03mol, 3.2ml) or ethylacetoacetate (**6**) (0.03mol, 3.8ml) in methanol (20ml) was treated with ammonia solution or substituted anilines (**7a-e**) (0.02mol) and refluxed for 8-36 hours. The completion of the reaction was monitored by TLC using n-hexane: acetone (9:1). After the completion of the

reaction, the mixture was cooled and evaporated to separate the solid. The crude compound was purified by double recrystallization with methanol. The yield obtained was 52-60%.

5.1.1.1. Dimethyl 4-(benzo[d] [1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxyl ate ***(8a).*** Piperonal, methylacetoacetate and ammonia were refluxed in methanol for 8h and obtained as pale yellow crystals, yield 52%, mp 175-178°C. R_f: 0.315; **I**R (KBr, ν_{max}, cm^{-1}): 3391(N-H Str), 3070-3020(Ar C-H Str), 2294 (alk C-H Str), 1702(C=O Str), 1086 (aliphatic C-N Str), 771(N-H Wag). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.7 (s, 6H, 2(CH_3) at C-2 & C-6), 3.7 (s, 6H, 2 (CH_3) at C-3 & C-5), 4.45 (s, 1H, C-4), 5.05 (br s, 1H, NH), 5.9 (s, 2H, O-CH_2-O), 6.4 (s, 1H, Ar-H), 6.50-6.65 (d, 2H, J=7.48Hz, Ar-H). APCI-MS: m/z = 344.9 $(M)^+$, 345.9 $(M+H)^+$. Anal. Calc. for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 62.35; H, 5.41; N, 4.01.

5.1.1.2. Dimethyl 4-(benzo[d] [1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-phenyl pyridine -3,5-dicarboxylate ***(8b).*** Piperonal, methylacetoacetate and aniline were refluxed in methanol for 12h and obtained as pale yellow crystals, yield 52%. m.p 185-186°C. R_f: 0.31. IR (KBr, ν_{max}, cm^{-1}): 3070(Ar C-H Str), 2950(Alk C-H Str), 1702 & 1691(C=O Str, α, β unsaturated ester), 1036(Aliphatic C-N Str). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.7 (s, 6H, 2(CH_3) at C-2 & C-6) 3.7 (s, 6H, 2(CH_3) at C-3 & C-5), 4.45 (s, 1H, CH at C-4), 5.9 (s, 2H, O -CH2-O), 6.42 (d, 1H, J=8.0Hz, Ar -H), 6.5 (d, 1H, J=8.0Hz, Ar-H), 6.6 (t, 1H, J =7.6Hz, Ar-H), 6.7(d, 2H, J=7.44Hz, Ar-H), 7.01 (t, 2H, J=7.44Hz, Ar-H). APCI-MS: m/z = 421 $(M)^+$, 422 $(M+H)^+$. Anal. Calc. for $C_{24}H_{23}NO_6$: C, 68.40; H, 5.50; N, 3.32. Found: C, 68.35; H, 5.45; N, 3.26

5.1.1.3. Dimethyl4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(2-nitrophenyl) pyridine-3,5-dicarboxylate ***(8c).*** Piperonal, methylacetoacetate and 2-nitroaniline were refluxed in methanol for 18h and obtained as Orange colour crystals with 56% yield. m.p. 235-237°C. R_f:0.72; IR (KBr) ν_{max}, cm^{-1}: 3024 (Ar C-H Str), 2925(Alk C-H Str),1702 (C=O Str, α, β unsaturated ester), 1482 (N-O asym str),1326(N-O sym str).^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.75 (s, 6H, CH_3 at C-2 & C-6), 3.75 (s, 6H, CH_3 at C-3& C-5), 4.45 (s, 1H, CH at C-4), 5.95 (s, 2H, O-CH_2-O), 6.35 (s, 1H, Ar-H), 6.40-6.55 (d, 2H, J=8.0 Hz, Ar-H), 6.8 (d, 1H, J=7.3Hz, Ar-H), 7.2 (t, 1H, J=7.3Hz, Ar-H), 7.4 (t, 1H, J=7.3Hz, Ar-H), 7.5-7.6(d, 1H, J=7.3Hz, Ar-H). APCI-MS: m/z = 465.9 $(M)^+$, 466.9 $(M+H)^+$. Anal. Calc. for $C_{24}H_{22}N_2O_8$: C, 61.80; H, 4.75; N, 6.01. Found. C, 61.75; H, 4.71; N, 5.97

5.1.1.4. Dimethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(3-nitrophenyl) pyridine-3,5-dicarboxylate ***(8d).*** Piperonal, methylacetoacetate and 3-nitroaniline were refluxed in methanol for 24h and obtained as yellowish orange crystals with 57% yield. m.p. 251-253°C. R_f : 0.33; IR (KBr, ν_{max}, cm^{-1}) : 3070 (Ar C-H Str), 2994 (alk C-H Str), 1702 (C=C Str, α, β unsaturated ester), 1482 (N-O asym.str), 1341(N-O sym str).^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.75 (s, 6H, CH_3 at C-2 & C-6), 3.75 (s, 6H, CH_3 at C-3& C-5), 4.45 (s, 1H, CH at C-4), 5.95 (s, 2H, O-CH_2-O), 6.35 (s, 1H, Ar-H), 6.49-6.55 (d, 2H, J=8.0 Hz, Ar-H), 6.8 (d, 1H, J=7.5Hz, Ar-H), 7.2 (t, 1H, J=7.5Hz, Ar-H), 7.4 (s, 1H, J=7.5Hz, Ar-H), 7.55-(d, 1H, J=7.5Hz, Ar-H). APCI-MS: m/z = 466.9 $(M+H)^+$. Anal. Calc. for $C_{24}H_{22}N_2O_8$: C, 61.80; H, 4.75; N, 6.01. Found C, 61.76; H, 4.69; N, 5.95;

5.1.1.5. Dimethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-nitrophenyl) pyridine -3,5-dicarboxylate ***(8e).*** Piperonal, methylacetoacetate and 4-nitroaniline were refluxed in methanol for 18h and obtained as dark yellow crystals with 55% yield. m.p. 265-268°C. R_f : 0.305. IR (KBr, ν_{max}, cm^{-1}): 3101 (Ar C-H Str), 2893 (alk C-H Str), 1705 (C=O Str, α, β unsaturated), 1489 (N-O asym. str), 1349 (N-O sym. str).^{1}H NMR(400MHz, $CDCl_3$) δ(ppm): 1.73 (s, 6H, CH_3 at C-2 & C-6) , 3.75 (s, 6H, CH_3 at C-3 & C-5), 4.5 (s, 1H, CH at C-4), 5.9(s, 2H, -O-(CH_2)-O), 6.37 (s, 1H, Ar-H), 6.45-6.65 (d, 2H, J=6.7Hz, Ar-H), 6.8-7.9 (m, 4H, Ar-H). APCI-MS: m/z = 465.9 $(M)^+$. Anal. Calc. for $C_{24}H_{22}N_2O_8$: C, 61.80; H, 4.75; N, 6.01. Found C, 61.74; H, 4.71; N, 5.97;

5.1.1.6. Diethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl pyridine-3,5-dicarboxylate ***(8f).*** Piperonal, ethylacetoacetate and ammonia were refluxed in methanol for 12h and obtained as fine yellow crystals with 56% yield. m.p. 198-201°C. R_f :0.3 IR (KBr, ν_{max}, cm^{-1}): 3401 (N-H Str), 3070 (Ar C-H Str), 2925(Alk C-H Str), 1691 (C=O Str, α, β unsaturated), 800(N-H Wag).^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.3 (t, 6H,, J=6Hz, CH_3 at C-3 & C-5) ,1.71(s, 6H, CH_3 gp at C-2 & C-6), 4.2 (q, 4H, J=6Hz, CH_2 at C-3 & C-5), 4.44 (s, 1H, CH at C-4), 5.08 (br s, 1H, NH), 5.9 (s, 2H, O-CH_2-O), 6.4 (s, 1H, Ar-H), 6.48-6.65 (d, 2H, J=7.8Hz, Ar-H). APCI-MS: m/z = 372.9 $(M)^+$, 373.9 $(M+H)^+$. Anal. Calc. for $C_{20}H_{23}NO_6$: C, 64.33; H, 6.21; N, 3.75. Found: C, 64.08; H, 6.09; N, 3.85.

5.1.1.7. Diethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-phenylpyridine-3,5-dicarboxylate ***(8g).*** Piperonal, ethylacetoacetate and aniline were refluxed in methanol for 16h and obtained as pale yellow crystals with 53% yield. m.p. 215-216°C. R_f :0.317. IR (KBr, ν_{max}, cm^{-1}): 3070(Ar C-H Str), 2950(Alk C-H Str), 1702 & 1691(C=O Str, α, β unsaturated

ester), 1036(Aliphatic C-N Str). ^{1}H NMR(400MHz, $CDCl_3$) δ (ppm):1.33 (t, 6H, CH_3 at C-3 & C-5), 1.72 (s, 6H, CH_3 at C-2 & C-6), 4.2 (q, 4H, J=5.8Hz, CH_2 gp at C-3 & C-5), 4.65 (s, 1H, CH at C-4), 5.75 (s, 2H, -O-CH_2-O-), 6.37(s, 1H, Ar-H), 6.40-6.55 (d, 2H, J=7.1Hz, Ar-H),7.0-6.6 (m, 5H, Ar-H). APCI-MS: m/z = 449 $(M)^+$. Anal. Calc. for $C_{26}H_{27}NO_6$: C, 69.47; H, 6.05; N, 3.12. Found: C, 69.48; H, 6.01; N, 3.25.

5.1.1.8. Diethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(2-nitrophenyl) pyridine-3,5-dicarboxylate ***(8h).*** Piperonal, ethylacetoacetate and 2-nitroaniline were refluxed in methanol for 28h and obtained as light orange crystals with 58% yield. m.p. 258-259°C. R_f :0.68. IR (KBr, ν_{max}, cm^{-1}): 3024 (Ar C-H Str), 2925(Alk C-H Str),1702 (C=O Str, α, β unsaturated ester), 1482 (N-O Asym str),1326(N-O sym str),1203(C-phenolic gp), 1149(C-O-C Ester).^{1}H NMR(400MHz, $CDCl_3$) δ (ppm): 1.28 (t, 6H, J=4Hz CH_3 at C-3 & C-5),1.7 (s, 6H, CH_3 at C-2 & C-6), 4.15 (q, 4H, J=4Hz, CH_2 gp at C-3 & C-5),4.45 (s, 1H, CH at C-4), 5.9 (s, 2H, O-CH_2-O-), 6.37 (s, 1H, Ar-H), 6.48-6.65 (d, 2H, J=6.9Hz, Ar-H),7.95-6.8 (m, 4H, Ar-H). APCI-MS: m/z = 494.9 $(M+H)^+$. Anal. Calc. for $C_{26}H_{26}N_2O_8$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.08; H, 5.36; N, 5.85.

5.1.1.9. Diethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(3-nitrophenyl) pyridine-3,5-dicarboxylate ***(8i).*** Piperonal, ethylacetoacetate and 3-nitroaniline were refluxed in methanol for 36h and obtained as pale orange crystals with 53% yield. m.p. 273-275°C. R_f :0.35. IR (KBr ν_{max}, cm^{-1}): 3070 (Ar C-H Str), 2994 (alk C-H Str), 1702 (C=C Str, α, β unsaturated ester), 1482 (N-O asym.str), 1341(N-O sym str). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.2 (t, 6H, J=4.4Hz, CH_3 at C-3 & C-5),1.7(s, 6H, CH_3 at C-2 & C-6), 4.15(q, 4H, J=4.4Hz CH_2 at C-3 & C-5),4.45 (s, 1H, CH at C-4), 5.95 (s, 2H, O-CH_2-O-), 6.38 (s, 1H, Ar-H), 6.7-6.45 (d, 2H, J=7.2Hz Ar -H),7.4 (s, 1H, Ar-H),7.6-6.68 (m, 3H, Ar-H). APCI-MS: m/z = 494.9 $(M+H)^+$. Anal. Calc. for $C_{26}H_{26}N_2O_8$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.21; H, 5.16; N, 5.55.

5.1.1.10. Diethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-nitrophenyl) pyridine -3,5-dicarboxylate ***(8j).*** Piperonal, ethylacetoacetate and 4-nitroaniline were refluxed in methanol for 30h and obtained as yellow fine crystals with 52% yield. m.p. 287-289°C. R_f : 0.325. IR (KBr, ν_{max}, cm^{-1}): 3101 (Ar C-H Str), 2893 (Alk C-H Str), 1705 (C=C, C=O Str, α, β unsaturated), 1489 (N-O Asym. str), 1349 (N-O sym. str). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.29 (t, 6H, J=6Hz CH_3 at C-3 & C-5), 1.71 (s, 6H, CH_3 at C-2 & C-6), 4.17 (q, 4H, J=6Hz, CH_2 at C-3 & C-5),4.43 (s, 1H, CH at C-H), 5.93 (s, 2H, -O-CH_2-

O-), 6.37 (s, 1H, Ar-H), 6.48-6.62 (d, 2H, J=7.8Hz, Ar-H), 6.8-7.9 (m, 4H, Ar-H). APCI-MS: m/z = 493.9 $(M)^+$. Anal. Calc. for $C_{26}H_{26}N_2O_8$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.25; H, 5.18; N, 5.65.

5.1.2. General method for the synthesis of 3-ethyl, 5-methyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(substituted)pyridine-3,5-dicarboxylate ***(8k-8o)***. **(Scheme-2)**

The synthesis of asymmetrical 1,4-DHPs (**8k- 8o)** were synthesized by the modified Hantzsch method to avoid the formation of symmetrical1,4-DHPs.

To an ice-cooled solution of 10mmol (1.5g) of piperonal in 30ml of isopropanol, 10mmol (1.1ml) of the ethylacetoacetate were added and refluxed for five hours. Then six mmol (0.8ml) of methylacetoacetate was added and the mixture was refluxed for 8 hours. Without separation 2mmol of amine was added and the mixture was refluxed for 4-6 hours. After cooling, the solvent was evaporated and the separated solid was purified by recrystallization. (Yield: 30-35 %)

5.1.2.1. 3-ethyl, 5-methyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate ***(8k).*** Fine yellow crystals with 57% yield. m.p. 186-189°C. R_f: 0.3. IR (KBr, ν_{max}, cm^{-1}): 3401 (N-H Str), 3070 (Ar C-H Str), 2925(Alk C-H Str), 1691 (C=O Str, α,β-unsaturated), 800(N-H Wag). ^{1}H NMR (400MHz, $CDCl_3$) δ(ppm): 1.23 (t, 3H, J=6Hz, CH_3 at C-3), 1.7 (s, 6H, CH_3 at C-2 & C-6), 3.75 (s, 3H, CH_3 at C-5), 4.15 (q, 2H, J=6Hz, CH_2 at C-3), 4.45 (s, 1H, CH at C-4), 5.1(br s, 1H, NH), 5.75 (s, 2H, O-CH_2-O), 6.4 (s, 1H, Ar-H), 6.62-6.5(d, 2H, J=7.8Hz, Ar-H). APCI-MS: m/z = 358.9 $(M)^+$, 359.9 $(M+H)^+$. Anal. Calc. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.9. Found: C, 63.15; H, 5.91; N, 3.95.

5.1.2.2. 3-ethyl,5-methyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-phenyl-pyridine-3,5-dicarboxylate ***(8l).*** Pale yellow colour fine crystals with 52% yield. m.p. 196-197°C. R_f : 0.319. IR (KBr, ν_{max}, cm^{-1}): 3070 (Ar C-H Str), 2950 (alk C-H Str), 1702 & 1691(C=O Str, α, β unsaturated ester), 1036(Aliphatic C-N Str). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 1.2-1.3 (t, 3H, CH_3 gp at C-3), 1.7 (s, 6H, CH_3 at C-2 & C-6), 3.75 (s, 3H, CH_3 at C-5), 4.14 (q, 2H, J=8Hz CH_2 gp at C-3), 4.45 (s, 1H, CH at C-4), 5.7 (s, 2H, O-CH_2-O-), 6.36 (s, 1H, Ar-H), 6.4-6.5 (d, 2H, J=8Hz, Ar-H), 6.55-7.0 (m, 5H, Ar -H). APCI-MS: m/z = 435.1 $(M)^+$, 436.1 $(M+H)^+$. Anal. Calc. for $C_{25}H_{25}NO_6$: C, 68.95; H, 5.79; N, 3.22. Found: C, 69.48; H, 5.71; N, 3.25.

5.1.2.3. 3-ethyl, 5-methyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(2-nitro phenyl)pyridine-3,5-dicarboxylate ***(8m).*** Orange fine crystals with 55% yield. m.p. 246-247°C. R_f: 0.63; IR (KBr, ν_{max}, cm^{-1}): 3024 (Ar C-H Str), 2925(Alk C-H Str),1702 (C=O Str, α, β unsaturated ester), 1482 (N-O Asym str),1326(N-O sym str). ^1H NMR(400MHz, $CDCl_3$) δ (ppm): 1.3 (t, 3H, J=4Hz CH_3 at C-3), 1.75 (s, 6H, CH_3 at C-2 & C-6), 3.72 (s, 3H, CH_3 at C-5), 4.17 (q, 2H, J=4Hz, CH_2 at C-3), 4.45 (s, 1H, CH at C-4), 5.95 (s, 2H, -O-CH_2-O-), 6.37 (s, 1H, Ar-H), 6.45-6.75 (d, 2H, J=8.5Hz, Ar-H), 8.0-6.75 (m, 4H, J=7.8Hz, Ar-H). APCI-MS: m/z = 479.9 $(M)^+$, 480.9 $(M+H)^+$. Anal. Calc. for $C_{25}H_{24}N_2O_8$: C, 62.49; H, 5.03; N, 5.83. Found: C, 62.09; H, 5.01; N, 5.75.

5.1.2.4. 3-ethyl, 5-methyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(3-nitro phenyl) pyridine-3,5-dicarboxylate ***(8n).*** Yellow crystals with 59% yield. m.p. 252-254°C. R_f: 0.4; IR (KBr, ν_{max}, cm^{-1}) : 3070(Ar C-H Str), 2950(Alk C-H Str), 1702 & 1691(C=O Str, α, β unsaturated ester), 1036(Aliphatic C-N Str). ^1H NMR (400MHz, $CHCl_3$) δ(ppm): 1.23 (t, 3H, J=4.2Hz, CH_3 gp at C-3), 1.7 (s, 6H, CH_3 gp at C-2 & C-6), 3.7 (s, 3H, CH_3 at C-5), 4.2 (q, 2H, J= 4.2Hz, CH_2 gp at C-3), 4.45 (s, 1H, CH at C-4), 5.9 (s, 2H, -O-CH_2-O-), 6.35 (s, 1H, Ar- H), 6.45-6.65 (d, 2H, J=7.2Hz, Ar-H), 6.7-7.6 (m, 4H, Ar-H). APCI-MS: m/z = 480.1 $(M+H)^+$. Anal. Calc. for $C_{25}H_{24}N_2O_8$: C, 62.49; H, 5.03; N, 5.83. Found: C, 62.15; H, 5.04; N, 5.05.

5.1.2.5. 3-ethyl, 5-methyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-(4-nitro phenyl)pyridine-3,5-dicarboxylate ***(8o).*** Pale yellow crystals with 57% yield. m.p. 267-269°C. R_f :0.31; IR (KBr, ν_{max}, cm^{-1}): 3101 (Ar C-H Str), 2893 (Alk C-H Str), 1705 (C=C, C=O Str, α, β unsaturated), 1489 (N-O Asym. str), 1349 (N-O sym. str). ^1H NMR (400MHz, $CDCl_3$) δ (ppm): 1.2 (t, 3H, J=4.4Hz, CH_3 at C-3), 1.68 (s, 6H, CH_3 at C-2&C-6), 3.75 (s, 3H, CH_3 at C-5), 4.2 (q, 2H, J=4.4Hz ,CH_2 at C-3), 4.47 (s, 1H, CH at C-4), 5.9 (s, 2H, -O-CH_2-O-), 6.37 (s, 1H, Ar-H), 6.45-6.7 (d, 2H, J=8.1Hz, Ar-H), 6.75-7.98 (m, 4H, J=7.6Hz, Ar-H). APCI-MS: m/z = 481.9 $(M+H)^+$. Anal. Calc. for $C_{25}H_{24}N_2O_8$: C, 62.49; H, 5.03; N, 5.83. Found: C, 62.59; H, 5.10; N, 5.85.

5.2. Pharmacology

5.2.1. Experimental animals

Male Swiss albino mice (18-22g) and male Wistar rats (150-200g) were used as experimental animals. They were obtained from King Institute of preventive medicine, Guindy, Chennai-32. The animals were acclimatized at least a week under standard

husbandary conditions, room temperature of 24±1°C, relative humidity 45-55% and a 12: 12 h light/dark cycle. The animals had free access to rodent pellet diet (Pranav Agro Industry, Bangalore) and water under strict hygienic conditions. All animal experiment protocols were approved by the Institutional Animal Ethical Committee (IAEC) of Annamacharya college of Pharmacy, Rajampet, India (1220/a/08/CPCSEA/ANCP/06).

5.2.2. Acute toxicity studies

The study was conducted as per OECD-425 guidelines for testing of chemicals acute oral toxicity [25]. The test was used to fix the safe dose for the compounds **8a-8o**. Swiss albino mice were divided into six groups each containing 10 animals and repeated for all the drugs. Drugs were administered by oral route in different concentrations (2000, 1000, 500, 250, 100 and 50mg/kg body weight). The animals were observed for their death over a period of 7days. The LD_{50} values were calculated by median lethal dose calculations and dose were fixed as 50mg/kg body weight.

5.2.3. Evaluation of Anticonvulsant Activity

5.2.3.1. The Maximal Electric Shock test (MES)

The anticonvulsant property of the test compounds in this model was assessed by its ability to protect against maximal electric shock induced convulsions. Male Wistar albino rats were divided into 17 groups of six rats each. Group 1 was the control group received vehicle (0.5% sodium carboxymethylcellulose); Group 2 received phenytoin (30 mg/kg, oral), Group 3 -17 received each of the test compounds **8a-8o** respectively (50 mg/kg, oral), which were prepared by suspending in 0.5% sodium carboxymethylcellulose. One hour after the administration of vehicle, phenytoin or test compounds, maximal electric shock of 150mA current for 0.2 sec was applied through corneal electrodes to induce convulsions using an electroconvulsiometer (INCO, Ambala, India) and duration of hind limb tonic extension was noted. Abolition or reduction in the duration of tonic extension was considered as the index for anticonvulsant activity [26].

5.2.3.2. The Subcutaneous Pentylenetetrazole Seizure test (Sc PTZ)

This method utilizes a dose of pentylenetetrazole (PTZ), 80mg/kg subcutaneously in rats that produces clonic seizures. The rats were divided into 17 groups of six rats each. Group 1 animals were kept as control and were received vehicle; Group 2 received diazepam (5 mg/kg, intraperitoneally), Group 3 -17 received the test compounds **8a-8o** respectively

(50mg/kg, oral), which were prepared by suspending in 0.5% sodium carboxymethylcellulose. One hour after administration of vehicle, diazepam or test compounds **8a-8o**, PTZ (80mg/kg) was injected subcutaneously. The time of onset of clonic convulsions and the protection against mortality were observed [27,28]. The maximum latency time used in the study was fixed as 300sec. Further, compound **8f** was subjected to the dose-dependent activity in both PTZ and MES models at 25, 50 and 100 mg/kg doses.

5.2.4. Evaluation of antioxidant activity

5.2.4.1. Assay for scavenging of DPPH free radicals

The ability to scavenge 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) stable free radical was determined by using DPPH method. In this method, 1 ml of test compound (10, 50, 100, 250, 500 µg/ml) in ethanol was added to 3.9 ml of 0.004% methanol solution of DPPH and incubated in a dark place for 30 min. The absorbance of the samples was read at 517 nm. Ascorbic acid was used as reference standard. Percentage inhibition of DPPH free radical by the test compounds was calculated [29].

5.3. Antinociceptive activity

Capsaicin-induced nociceptive assay

Male Swiss mice (18-22g) were used for the method. Following the adaptation to the experimental conditions, 20µl of capsaicin (1nmol/paw) was injected intraplantarly in to the right hind paw, and the total number of flinches of the injected paw was measured individually for 5 min and used as a measurement of nociception. The animals were treated with control and test compounds **8a**, **8b**, **8f** and **8o** using oral gavages (50mg/kg in a dose volume of 0.5% sodium carboxymethylcellulose) 1 h prior to capsaicin injection [30].

5.4. Statistical Analysis

The results of anticonvulsant and antinociceptive activities were expressed as Mean ± SEM. The statistical significance of the differences between the groups was analyzed by one-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test.

5.5. Calculation of drug-likeness and ADME properties

The molecular properties like TPSA, cLogP, number of rotatable bonds and violations of Lipinski's rule-of-five were calculated using Molinspiration online property calculator tool kit

[31]. Topological polar surface area was used to calculate the percentage of absorption (%ABS) according to the equation: %ABS = 109 – [0.345× TPSA] [23]. In vitro plasma protein binding values were obtained from ADME calculator [32].

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Table 1: Anticonvulsant activity of dialkyl 4-[benzo [d][1,3]-dioxol)-6-yl]-1, 4-dihydro-2, 6-dimethyl-1-substitutedpyridine-3,5-dicarboxylates (8a-8o) at 50mg/kg body weight.

compounds	scPTZ [a]		MES [a]	
	Latency period (s) mean±SEM	Protection against mortality (%)	Limb extension (s) mean±SEM	% protection
8a	200±33***	0	15±1**	27
8b	200±12***	0	12±1***	41.2
8c	174±20**	0	14±1**	33.3
8d	138±13^{ns}	0	18±1^{ns}	11.1
8e	93±7^{ns}	0	19±2^{ns}	07.9
8f	>300***	100	8±1***	59.0
8g	275±9***	66.6	11±1***	46.0
8h	293±4***	66.6	8±1***	60.3
8i	250±17***	33.3	10±1***	49.2
8j	237±28***	66.6	11±1***	46.0
8k	233±21***	33.3	15±1**	28.5
8l	221±25***	0	12±1***	41.2
8m	251±16***	33.3	11±1***	44.5
8n	136±14^{ns}	0	15±**	28.5
8o	225±10***	33.3	14±1**	31.7
diazepam	262±13***	33.3	--------	-------
phenytoin	--------	---------	6±1***	69.8
nifedipine	215±3***	33.3	7±1***	66.6
control	83±4	-----	21±1	-----
Ascorbic acid	-----	-----	------	-----

a The test compounds were administered 1h before the injection of PTZ (80 mg/kg, sc), MES (150 m.A, 0.2 sec), control: 0.5% sodium carboxymethylcellulose. Reference stanadard: diazepam (5mg/kg, ip) and phenytoin (30mg/kg, oral). Latency time was observed for 300sec in scPTZ. Values are expressed as mean±SEM, n=6. One way analysis of variance (ANOVA) followed by Dunnett's method.*** $p<0.0001$ vs control, ** $p<0.05$ vs control, ns $p>0.05$ vs control.

#Isradipine was reported to show limb extension period 9±1 (mean±SEM) [8].

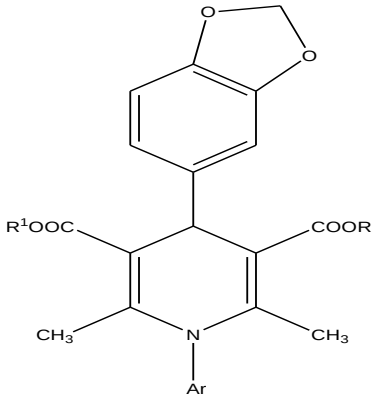
Table 2: Anticonvulsant activity of diethyl 4-[benzo [d][1,3]-dioxol)-6-yl]-1, 4-dihydro-2, 6-dimethyl-1-substitutedpyridine-3,5-dicarboxylate (8f) at 25 and 100mg/kg body weight.

compound	dose mg/kg	scPTZ induced model [b]		MES method [b]	
		Latency period Mean±SEM(s)	% Protection against mortality	Limb extension Mean±SEM(s)	% protection
Control		83±3.8	---	25±1	---
Diazepam	5	262±13***	66.6	------	----
Phenytoin	30	-----	-----	6±1***	74.6
8f	25	194±3***	100	14±1***	41.6
8f	50	>300***	100	8±1***	65.6
8f	100	>300***	0	7±1***	72.0

b The test compounds were administered 1h before application of MES (150 m.A, 0.2 sec)/ the injection of PTZ (80mg/kg, sc), control: 0.5% sodium carboxymethylcellulose. Reference standard: diazepam (5mg/kg, ip) / phenytoin (30mg/kg, oral). Values are expressed as mean ± SEM, n=6. One way analysis of variance (ANOVA) followed by Dunnett's method.

*** $p < 0.0001$ vs control.

Table 3: Antioxidant activity of dialkyl 4-[benzo[d][1,3]-dioxol)-6-yl]-1, 4-dihydro-2, 6-dimethyl-1-substitutedpyridine-3,5-dicarboxylates (8a-8o) in DPPH free radical scavenging activity



compound	R	R^1	Ar	IC_{50}(µg/ml)[c]
8a	CH_3	CH_3	H	604
8b	CH_3	CH_3	C_6H_5	542
8c	CH_3	CH_3	C_6H_4-2-NO_2	2354
8d	CH_3	CH_3	C_6H_4-3-NO_2	521
8e	CH_3	CH_3	C_6H_4-4-NO_2	1289
8f	C_2H_5	C_2H_5	H	375
8g	C_2H_5	C_2H_5	C_6H_5	443
8h	C_2H_5	C_2H_5	C_6H_4-2-NO_2	874
8i	C_2H_5	C_2H_5	C_6H_4-3-NO_2	363
8j	C_2H_5	C_2H_5	C_6H_4-4-NO_2	597
8k	CH_3	C_2H_5	H	467
8l	CH_3	C_2H_5	C_6H_5	740
8m	CH_3	C_2H_5	C_6H_4-2-NO_2	933
8n	CH_3	C_2H_5	C_6H_4-3-NO_2	386
8o	CH_3	C_2H_5	C_6H_4-4-NO_2	854
Ascorbic acid	---	----	----	32

c Reduction of DPPH free radical by the test compounds at various concentrations was expressed as IC_{50} value, which was estimated in ethanol solution, absorbance was measured at 517 nm.

Table: 4: Antinociceptive activity of dialkyl 4-[benzo[d][1,3]-dioxol)-6-yl]-1, 4-dihydro-2, 6-dimethyl-1-substitutedpyridine-3,5-dicarboxylates (8a, 8b, 8f and **8o) in capsaicin induced flinching assay at 10mg/kg**

Compound	Number of flinches (Mean ± SEM)	% of activity
control	12±2	------
8a	9.5 ± 1^{ns}	21
8b	12 ± 1^{ns}	0
8f	11 ± 1^{ns}	8
8o	11 ± 1^{ns}	4

The test compounds were administered 1h before the injection of capsaicin (20µl, 1nmol/paw), control: 0.5% sodium carboxymethylcellulose. Values are expressed as mean ± SEM, n=6. One way analysis of variance (ANOVA) followed by Dunnett's method.

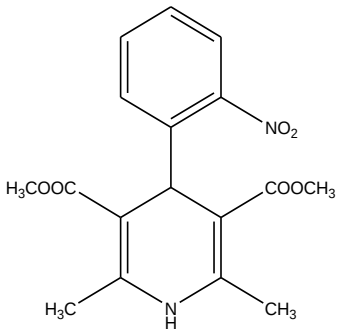
Table 5: Structural and pharmacokinetic properties of the 1,4-dihydropyridine derivatives 8a-8o.

compound	%ABS	M.W	cLogP	nrotb	HBA	HBD	volume	MR	TPSA	Viola-tions	iPPB
Rule	-----	<500	≤5	---	<10	<5	-----	40-130	----	≤1	----
8a	80.3	345.3	3.524	5	7	1	303.37	87.87	83.10	0	61.0
8b	83.3	421.4	5.466	6	7	0	375.16	113.65	74.31	1	91.1
8c	67.5	466.4	5.377	7	10	0	398.49	119.80	120.1	1	93.4
8d	67.5	466.4	5.401	7	10	0	398.49	119.80	120.1	1	93.4
8e	67.5	466.4	5.425	7	10	0	398.49	119.80	120.1	1	92.3
8f	80.3	373.4	4.276	7	7	1	336.97	97.10	83.1	0	69.5
8g	83.3	449.5	6.218	8	7	0	408.76	122.89	74.3	1	91.1
8h	67.5	494.5	6.129	9	10	0	432.10	129.03	120.1	1	92.4
8i	67.5	494.5	6.153	9	10	0	432.10	129.03	120.1	1	92.2
8j	67.5	494.5	6.177	9	10	0	432.10	129.03	120.1	1	91.4
8k	80.3	359.3	3.90	6	7	1	320.17	92.49	83.1	0	65.1
8l	83.3	435.4	5.842	7	7	0	391.96	118.27	74.3	1	91.1
8m	67.5	480.4	5.753	8	10	0	415.30	124.4	120.1	1	92.8
8n	67.5	480.4	5.777	8	10	0	415.30	124.4	120.1	1	92.4
8o	67.5	480.4	5.801	8	10	0	415.30	124.4	120.1	1	91.8
isradipine	73.2	343.3	3.571	5	8	1	296.69	87.11	103.5	0	72.8
nifedipine	70.9	346.3	3.072	6	8	1	302.78	87.89	110.4	0	41.4

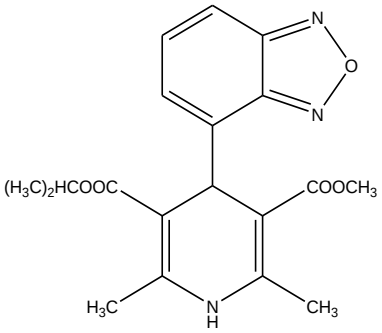
cLogP- calculated partition co-efficient, nrotb- number of rotatable bond, HBA-number of hydrogen bond acceptors, HBD-number of hydrogen bond acceptors, MR-molar refractivity, TPSA-molecular polar surface area, violations- number of violations from Lipinski rule-of-five, iPPB- in vitro plasma protein binding (%), %ABS- percentage absorption.

Figure(s)

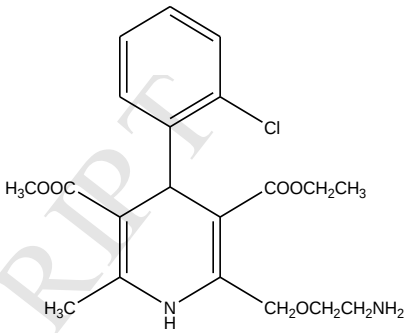
Figure 1. Lead structures for target compounds **8a-8n.**



Nifedipine

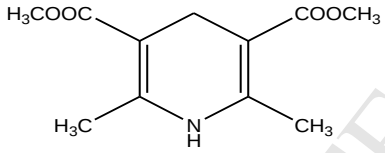


Isradipine

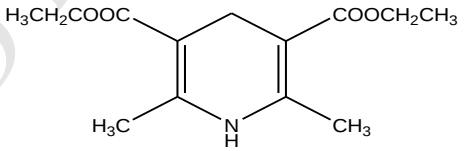


Amlodipine

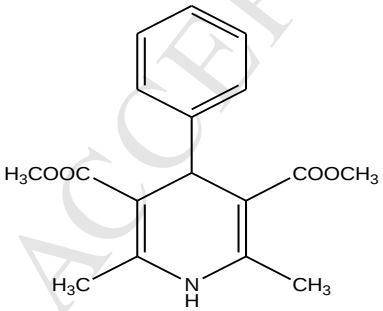
Figure 2. Structures of 1,4-dihydropyridine derived pharmacologically active drug candidates



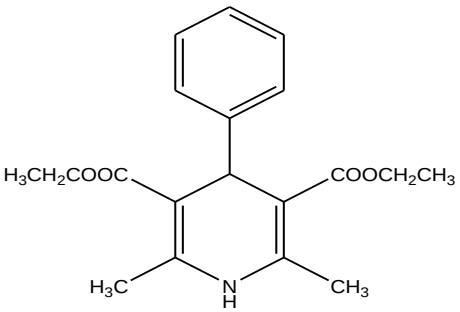
(1)



(2)

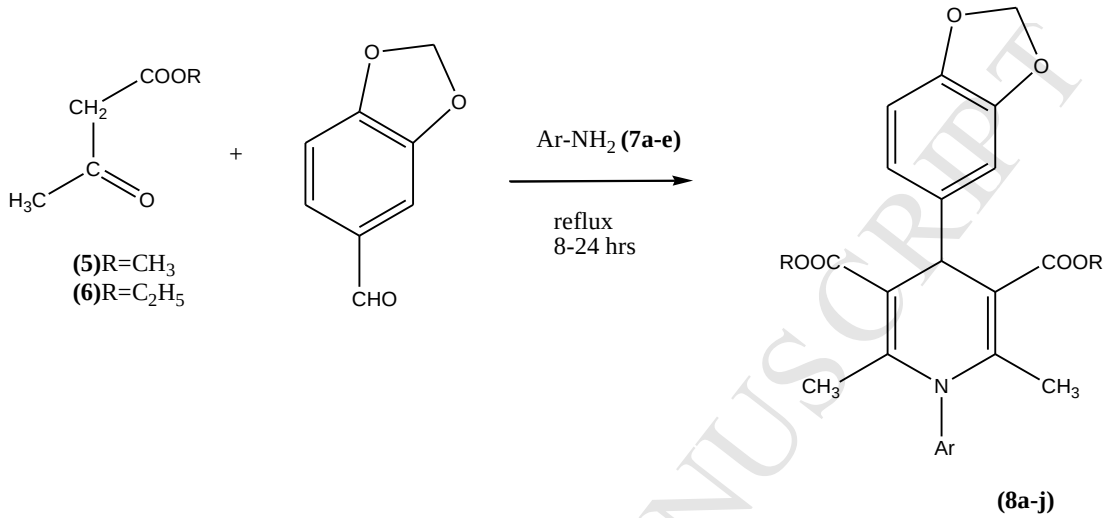


(3)



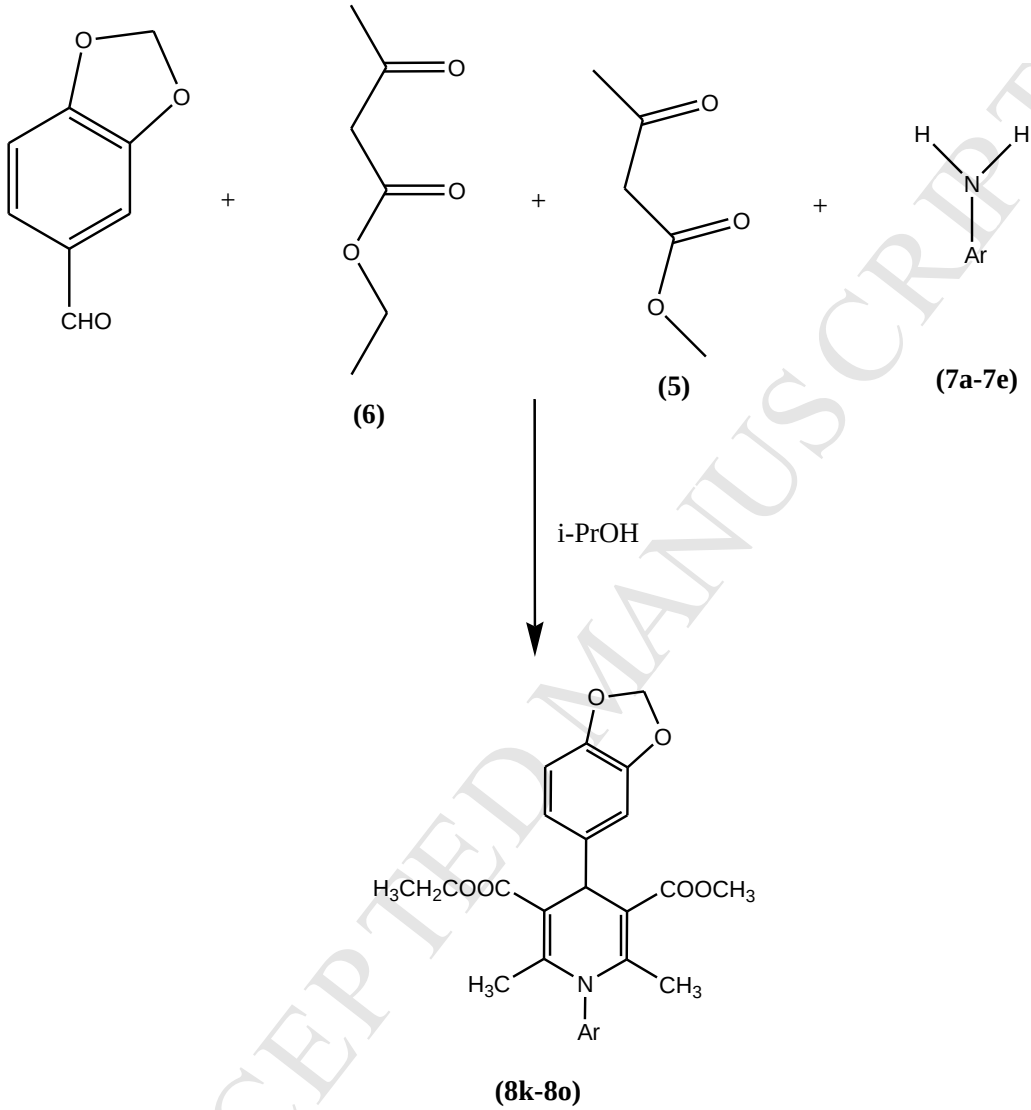
(4)

Scheme 1. Synthetic protocol of the symmetric dialkyl 4-{(benzo (d)[1,3]dioxo)-6-yl}-1,4-dihydro-2,6-dimethyl-1-(substituted)pyridine-3,5-dicarboxylates (8a-8j)



	R	Ar
8a	CH_3	H
8b	CH_3	C_6H_5
8c	CH_3	C_6H_4-2-NO_2
8d	CH_3	C_6H_4-3-NO_2
8e	CH_3	C_6H_4-4-NO_2
8f	C_2H_5	H
8g	C_2H_5	C_6H_5
8h	C_2H_5	C_6H_4-2-NO_2
8i	C_2H_5	C_6H_4-3-NO_2
8j	C_2H_5	C_6H_4-4-NO_2

Scheme2. Synthetic protocol of the asymmetric 3-ethyl, 5-methyl-4-[(benzo(d)[1,3] dioxo)-6-yl]-1,4-dihydro-2,6-dimethyl-1-(substituted)pyridine-3,5-dicarboxylates (8k-8o)



	Ar
8k	H
8l	C_6H_5
8m	C_6H_4-2-NO_2
8n	C_6H_4-3-NO_2
8o	C_6H_4-4-NO_2