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

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Original article

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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-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. Fivfteen 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 10 mg/kg body weight, but displayed no significant activity at the tested dose.

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 ester) was evaluated for anticonvulsant activity by maximal electroshock method (MES) in mice and reported to be active at 5 mg/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 dialkyl 4-(benzo[*d*][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-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.

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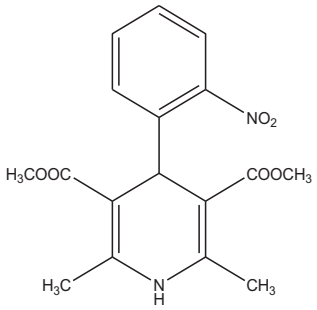
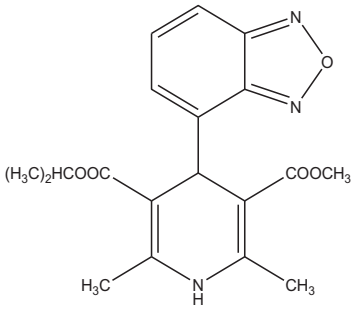
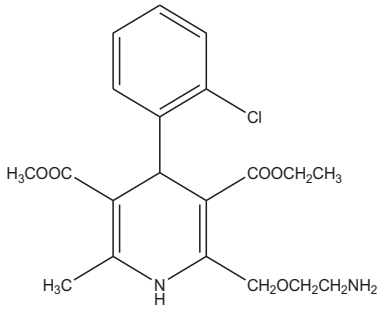


Nifedipine



Isradipine



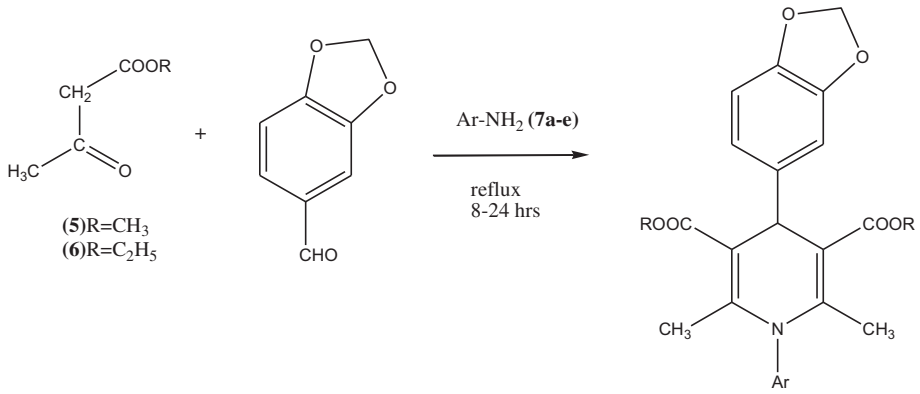
Amlodipine

Fig. 1. Lead structures for target compounds **8a**–**8n**.

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



(8a-j)

	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

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**).

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.

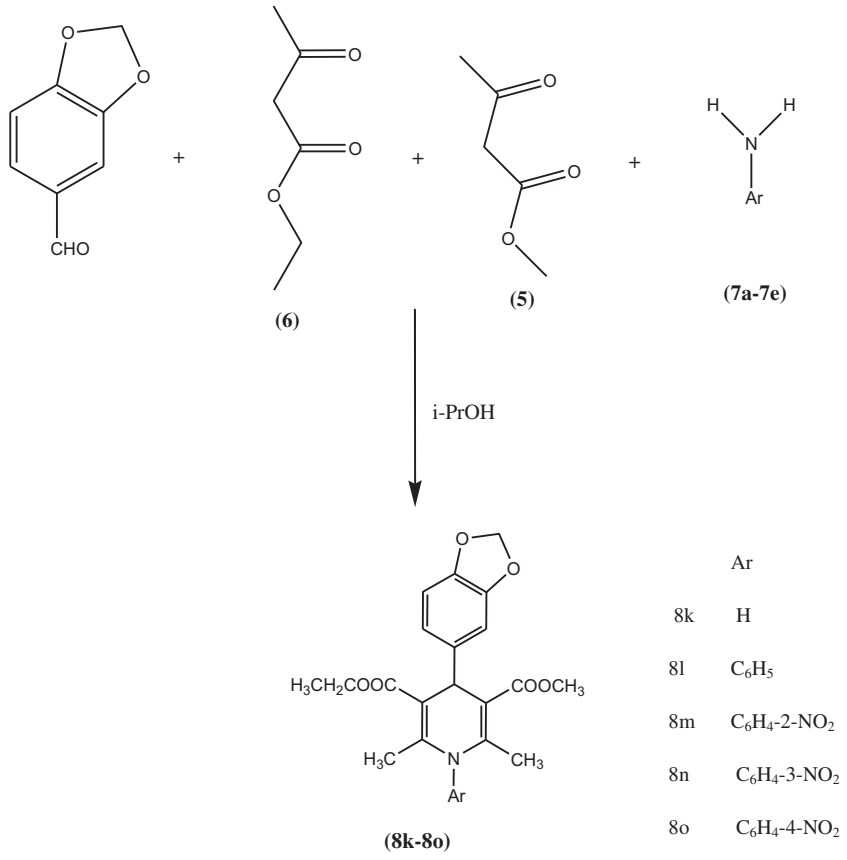
The profile of anticonvulsant activity of compounds **8a**–**8o** was evaluated by maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) tests after the administration of test compounds (p.o) and reference standard drug (phenytoin, p.o; diazepam, i.p) to male Wistar albino rats at the dose of 50 mg/kg body weight [13,14]. Seizure inducing pentylenetetrazole (80 mg/kg, s.c) injection or maximal electroshock (150 mA for 0.2 s) was applied 1 h after the administration of the drug candidates. In scPTZ induced method, the seizure response was observed for a maximum period of 300 s. 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 (5 mg/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 (30 mg/kg, p.o).

Of these derivatives, compounds **8f**–**8k** and **8m** showed good seizure inhibition activity in scPTZ induced seizure method and are comparable to nifedipine at 30 mg/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 50 mg and 100 mg/kg doses, but no protection against mortality at 100 mg/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 to 386 μg/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



Scheme 2. 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**).

Table 1
Anticonvulsant activity of dialkyl 4-[(benzo[*d*][1,3]-dioxol)-6-yl]-1,4-dihydro-2,6-dimethyl-1-substituted pyridine-3,5-dicarboxylates (**8a**–**8o**) at 50 mg/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 ± 13ns	0	18±1ns	11.1
8e	93±7ns	0	19±2ns	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 ± 14ns	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	–	–	–	–

#Isradipine was reported to show limb extension period 9 ± 1 (mean ± SEM) [8].
***$p < 0.0001$ vs control, **$p < 0.05$ vs control, ns$p > 0.05$ vs control.
[a] The test compounds were administered 1 h before the injection of PTZ (80 mg/kg, sc), MES (150 mA, 0.2 s), control: 0.5% sodium carboxymethylcellulose. Reference standard: diazepam (5 mg/kg, ip) and phenytoin (30 mg/kg, oral). Latency time was observed for 300 s in scPTZ. Values are expressed as mean ± SEM, $n = 6$. One way analysis of variance (ANOVA) followed by Dunnett's method.

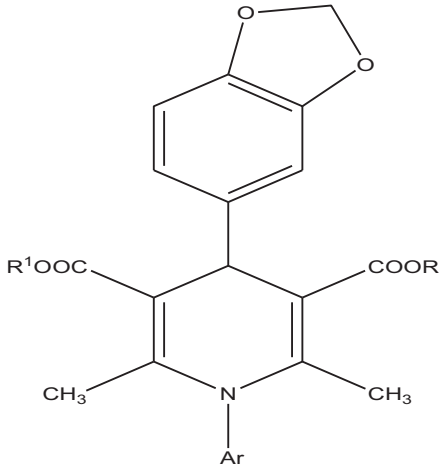
compounds **8a**, **8b**, **8f** and **8o** did not present any significant activity at the dose of 10 mg/kg (Table 4).

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 log *P* (<5), lower *in vitro* plasma protein binding (<60%) and no violations from Lipinski's rule-of-five.

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



Compound	R	R^1	Ar	IC_{50} (μg/ml)[a]
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

[a] 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.

3. Discussion

The title compounds, **8a**–**8o** were synthesized based on the lead molecule, isradipine. The present work is related to the isosteric

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

Compound	Dose mg/kg	scPTZ induced model[a]		MES method[a]	
		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

***$p < 0.0001$ vs control.
[a] The test compounds were administered 1 h before application of MES (150 mA, 0.2 s)/the injection of PTZ (80 mg/kg, sc), control: 0.5% sodium carboxymethylcellulose. Reference standard: diazepam (5 mg/kg, ip)/phenytoin (30 mg/kg, oral). Values are expressed as mean ± SEM, $n = 6$. One way analysis of variance (ANOVA) followed by Dunnett's method.

Table 4
Antinociceptive activity of dialkyl 4-[(benzo[*d*][1,3]-dioxol)-6-yl]-1,4-dihydro-2,6-dimethyl-1-substituted pyridine-3,5-dicarboxylates (**8a**, **8b**, **8f** and **8o**) in capsaicin induced flinching assay at 10 mg/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 1 h before the injection of capsaicin (20 μl, 1 nmol/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.

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 cyclization and simultaneous elimination of 3 mol 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 1450–1350 cm^{-1} indicated symmetric and asymmetric stretching of nitro functional group and a band at 1050–1020 cm^{-1} showed C–N stretching. The ^{1}H NMR spectrum of the compounds supported the structures of **8a**–**8o**. These compounds showed multiplets in the region of 6.4–8.1 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 analogues. 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

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

Compound	%ABS	M.W	cLog *P*	nrotb	HBA	HBD	Volume	MR	TPSA	Violations	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

cLog *P* – 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's rule-of-five, iPPB – *in vitro* plasma protein binding (%), %ABS – percentage absorption.

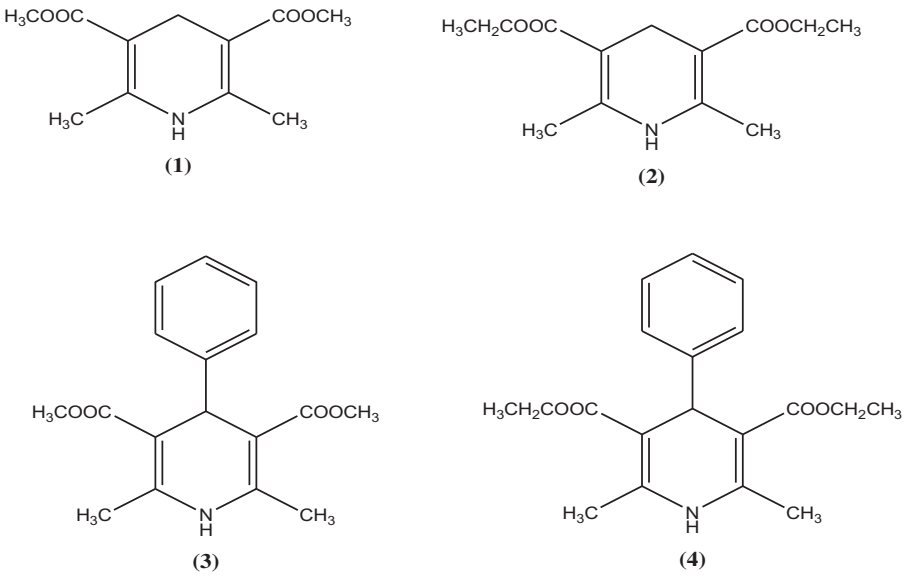


Fig. 2. Structures of 1,4-dihydropyridine derived pharmacologically active drug candidates.

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 25 mg and 100 mg as it exhibited promising seizure inhibition and protection against mortality at 50 mg/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 nociception [22]. Compounds **8a**, **8b**, **8f** and **8o** which exhibited good anticonvulsant activity were evaluated for capsaicin induced flinching model at 10 mg/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.

3.1. 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 log $P \leq 5$, 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 (Shimadzu) 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-dicarboxylates (**8a**–**8j**) (Scheme 1)*

To a solution of piperonal (0.01 mol, 1.5 g), methylacetoacetate (**5**) (0.03 mol, 3.2 ml) or ethylacetoacetate (**6**) (0.03 mol, 3.8 ml) in methanol (20 ml) was treated with ammonia solution or substituted anilines (**7a**–**e**) (0.02 mol) and refluxed for 8–36 h. 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-dimethyl pyridine-3,5-dicarboxylate (**8a**).* Piperonal, methylacetoacetate and ammonia (**7a**) were refluxed in methanol for 8 h and obtained as pale yellow crystals, yield 52%, mp 175–178 °C. R_f: 0.315; IR (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 (400 MHz, $CDCl_3$) 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 = 7.48 Hz, 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, 5.54; N, 4.05.

*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 (**7b**) were refluxed in methanol for 12 h 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 (400 MHz, $CDCl_3$) 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–CH_2–O), 6.42 (d, 1H, J = 8.0 Hz, Ar–H), 6.5 (d, 1H, J = 8.0 Hz, Ar–H), 6.6 (t, 1H, J = 7.6 Hz, Ar–H), 6.7 (d, 2H, J = 7.44 Hz, Ar–H), 7.01 (t, 2H, J = 7.44 Hz, 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, 5.49; N, 3.31.

*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 (**7c**) were refluxed in methanol for 18 h 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 (400 MHz, $CDCl_3$) 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 = 7.3 Hz, Ar–H), 7.2 (t, 1H, J = 7.3 Hz, Ar–H), 7.4 (t, 1H, J = 7.3 Hz, Ar–H), 7.5–7.6 (d, 1H, J = 7.3 Hz, 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, 4.73; N, 5.99.

*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 (**7d**) were refluxed in methanol for 24 h 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 (400 MHz, $CDCl_3$) 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 = 7.5 Hz, Ar–H), 7.2 (t, 1H, J = 7.5 Hz, Ar–H), 7.4 (s, 1H, J = 7.5 Hz, Ar–H), 7.55 (d, 1H, J = 7.5 Hz, 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, 4.76; N, 5.99.

*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 (**7e**) were refluxed in methanol for 18 h 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 (400 MHz, $CDCl_3$) 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 = 6.7 Hz, 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, 4.73; N, 5.99.

*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 (**7a**) were refluxed in methanol for 12 h 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 (400 MHz, $CDCl_3$) δ (ppm): 1.3 (t, 6H, J = 6 Hz, CH_3 at C-3 & C-5), 1.71 (s, 6H, CH_3 gp at C-2 & C-6), 4.2 (q, 4H, J = 6 Hz, 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 = 7.8 Hz, 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, 6.19; N, 3.74.

*5.1.1.7. Diethyl 4-(benzo[d][1,3]dioxol-6-yl)-1,4-dihydro-2,6-dimethyl-1-phenyl pyridine-3,5-dicarboxylate (**8g**).* Piperonal, ethylaceto-acetate and aniline (**7b**) were refluxed in methanol for 16 h 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 (400 MHz, $CDCl_3$) 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 = 5.8 Hz, 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 = 7.1 Hz, 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, 3.13.

*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 (**7c**) were refluxed in methanol for 28 h 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 (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 6H, J = 4 Hz CH_3 at

C-3 & C-5), 1.7 (s, 6H, CH_3 at C-2 & C-6), 4.15 (q, 4H, $J = 4$ Hz, 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, $J = 6.9$ Hz, 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, 5.31; N, 5.865.

*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 (**7d**) were refluxed in methanol for 36 h 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 (400 MHz, $CDCl_3$) δ (ppm): 1.2 (t, 6H, $J = 4.4$ Hz, CH_3 at C-3 & C-5), 1.7 (s, 6H, CH_3 at C-2 & C-6), 4.15 (q, 4H, $J = 4.4$ Hz 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, $J = 7.2$ Hz 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, 5.31; N, 5.65.

*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 (**7e**) were refluxed in methanol for 30 h 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 (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 6H, $J = 6$ Hz CH_3 at C-3 & C-5), 1.71 (s, 6H, CH_3 at C-2 & C-6), 4.17 (q, 4H, $J = 6$ Hz, 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, $J = 7.8$ Hz, 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, 5.28; 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-dicarboxylates (**8k**–**8o**) (Scheme 2)*

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

To an ice-cooled solution of 10 mmol (1.5 g) of piperonal in 30 ml of isopropanol, 10 mmol (1.1 ml) of the ethylacetoacetate were added and refluxed for 5 h. Then 6 mmol (0.8 ml) of methylacetoacetate was added and the mixture was refluxed for 8 h. Without separation 2 mmol of amine (**7a**–**7e**) was added and the mixture was refluxed for 4–6 h. 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 (400 MHz, $CDCl_3$) δ (ppm): 1.23 (t, 3H, $J = 6$ Hz, 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, $J = 6$ Hz, 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, $J = 7.8$ Hz, 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, 3.90. Found: C, 63.15; H, 5.91; N, 3.91.

*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 (400 MHz, $CDCl_3$) 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, $J = 8$ Hz 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, $J = 8$ Hz, 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, 68.84; H, 5.77; N, 3.21.

*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). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.3 (t, 3H, $J = 4$ Hz 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, $J = 4$ Hz, 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, $J = 8.5$ Hz, Ar–H), 8.0–6.75 (m, 4H, $J = 7.8$ Hz, 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, 5.85.

*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). ^{1}H NMR (400 MHz, $CHCl_3$) δ (ppm): 1.23 (t, 3H, $J = 4.2$ Hz, 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, $J = 4.2$ Hz, 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, $J = 7.2$ Hz, 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, 5.81.

*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). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.2 (t, 3H, $J = 4.4$ Hz, 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, $J = 4.4$ Hz, 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, $J = 8.1$ Hz, Ar–H), 6.75–7.98 (m, 4H, $J = 7.6$ Hz, 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, 5.01; N, 5.85.

5.2. Pharmacology

5.2.1. Experimental animals

Male Swiss albino mice (18–22 g) and male Wistar rats (150–200 g) 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 husbandry 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 50 mg/kg body weight). The animals were observed for their death over a period of 7 days. The LD_{50} values were calculated by median lethal dose calculations and dose were fixed as 50 mg/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 150 mA current for 0.2 s 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 (scPTZ). This method utilizes a dose of pentylenetetrazole (PTZ), 80 mg/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 (50 mg/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 (80 mg/kg) was injected subcutaneously. The time of onset of clonic convulsions and the protection against mortality were observed [19,27]. The maximum latency time used in the study was fixed as 300 s. 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 [28].

5.3. Antinociceptive activity

5.3.1. Capsaicin-induced nociceptive assay

Male Swiss mice (18–22 g) were used for the method. Following the adaptation to the experimental conditions, 20 μl of capsaicin (1 nmol/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 (50 mg/kg in a dose volume of 0.5% sodium carboxymethylcellulose) 1 h prior to capsaicin injection [29].

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 analysed 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, cLog *P*, number of rotatable bonds and violations of Lipinski's rule-of-five were calculated using Molinspiration online property calculator tool kit [30]. Topological polar surface area was used to calculate the percentage of absorption (%ABS) according to the equation: $\%ABS = 109 - [0.345 \times TPSA]$ [23]. *In vitro* plasma protein binding values were obtained from ADME calculator [31].

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.06.006. These data include MOL files and InChiKeys of the most important compounds described in this article

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