European Journal of Medicinal Chemistry 73 (2014) 97–104

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, anticonvulsant activity and molecular properties prediction of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydropyridine-3,5-dicarboxylates

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ARTICLE INFO

Article history:
Received 25 January 2013
Received in revised form
22 November 2013
Accepted 3 December 2013
Available online 12 December 2013

Keywords:
Diethylmalonyl
Pyridine-3,5-dicarboxylates
Lipinski's "rule of five"
Maximal electroshock method
Subcutaneous pentylenetetrazole method

ABSTRACT

The synthesis and anticonvulsant properties of new N-diethylmalonyl derivatives of nifedipine and other isosteric analogues (**7a**–**7n**) were described. Anticonvulsant screening was performed by subcutaneous pentylenetetrazole (scPTZ) and maximal electroshock (MES) induced seizures tests. Majority of the compounds were effective in scPTZ and MES screens. Compound **7k** showed good activity displaying maximum protection, which may be due to the presence of styryl moiety at position 4 of 1,4-dihydropyridine nucleus and the methyl groups of diester functionality. Compounds **7a**–**7d**, **7g**, **7i** and **7k** obeyed the Lipinski's "rule of five" and have drug-likeness. Based on computational prediction of molecular and pharmacokinetic properties, it was found that the compounds have good oral absorption.

1. Introduction

Epilepsy is a group of serious disorders of the brain characterized by recurrent spontaneous seizures. The efficacy of many antiepileptic drugs (AEDs) is limited by the dose related toxicities and diverse array of adverse side effects, due to the limited access through the blood–brain barrier, which is a major impediment for the treatment of central nervous system (CNS) diseases [1,2]. The limitations for AEDs are also due to the drugs acting as substrates for P-glycoprotein (Pgp), which leads to multi drug resistance [3]. Various attempts have been made to overcome the limited access of drugs to the brain and to reduce the systemic side effects that are common with general treatments. The biologically active compounds linked to a lipoidal dihydropyridine carrier, which can easily penetrate the blood–brain barrier were reported as novel chemical delivery systems for the site specific and sustained delivery of drugs to the brain [4].

Nifedipine is a 1,4-dihydropyridine derivative, possessing calcium channel blocking and antihypertensive activities. It has also been reported to prolong the latent period and reduce the duration of tonic extensor phase of maximal electroshock induced seizure method [5,6]. 1-Malonyl-1,4-dihydropyridine derivatives (**1** and **2**) (Fig. 1) were reported as novel carrier systems for the site specific and sustained drug delivery to the brain which exhibited moderate antidepressant activity comparable to imipramine [7,8].

In the present work, diethylmalonyl ester derivatives of nifedipine bearing different substituted aryl moieties attached to position 4 of 1,4-dihydropyridine(1,4-DHP) were synthesized. It was planned to study the effect of phenyl ring and the nature of the spacer moiety consisting of 1–2 carbon atoms connected to the 1,4-DHP ring system. The compounds were evaluated for anticonvulsant activity by maximal electroshock and pentylenetetrazole induced methods and also predicted for drug-likeness. The topological polar surface area, *in vitro* oral bioavailability and blood–brain barrier permeability were calculated.

2. Results

2.1. Chemistry

The designed diethylmalonyl ester derivatives, **7a**–**7n** were successfully synthesized as illustrated in Scheme 1. The condensation of commercially available aldehydes (**5a**–**5e**), alkylacetoacetates (**3** or **4**) and ammonia solution in methanol provided compounds **6a**–**6n** in good yields. Compounds **6a**–**6n** were refluxed with diethyl-2-bromomalonate in absolute ethanol for

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http://dx.doi.org/10.1016/j.ejmech.2013.12.001

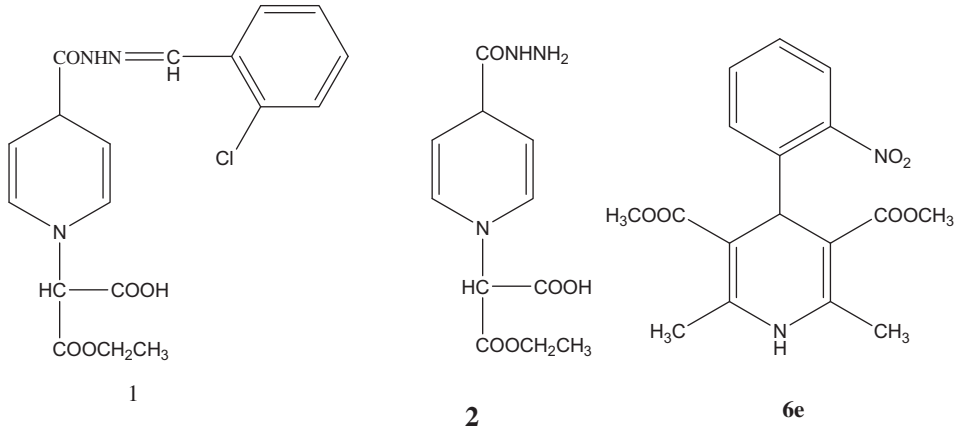
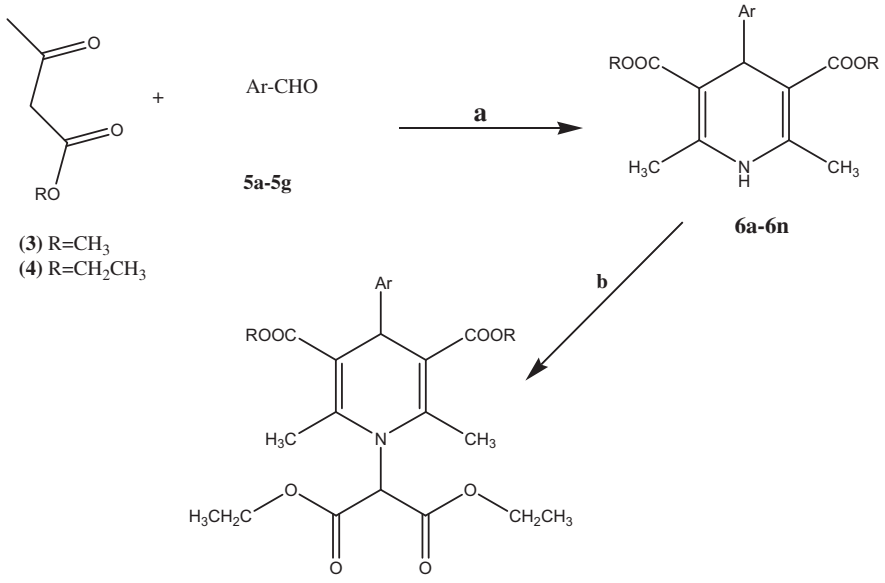


Fig. 1. Lead structures for target compounds **7a**–**7n**.

15 h in 1:2 stoichiometric ratios affording compounds, **7a**–**7n** in good yields. The purity of the compounds was assessed by thin layer chromatography. The detailed synthesis, physical data (Table 1), spectral and analytical data are listed in the Experimental protocol section.

2.2. Anticonvulsant activity

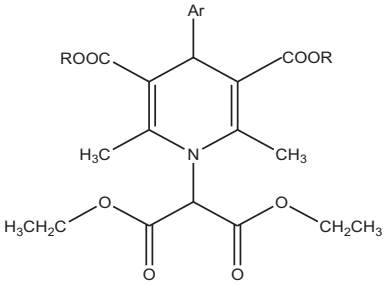
Acute toxicity studies were performed and all the compounds were found to be safe up to 100 mg/kg body weight as no deaths were observed. Hence, the safe dose was fixed as 10 mg/kg body weight.



	Ar	R		Ar	R
7a	H	CH_3	7h	$C_6H_5CH_2$	C_2H_5
7b	H	C_2H_5	7i	$C_6H_5CH_2CH_2$-	CH_3
7c	C_6H_5	CH_3	7j	$C_6H_5CH_2CH_2$-	C_2H_5
7d	C_6H_5	C_2H_5	7k	C_6H_5CH=CH-	CH_3
7e	C_6H_4-2-NO_2	CH_3	7l	C_6H_5CH=CH-	C_2H_5
7f	C_6H_4-2-NO_2	C_2H_5	7m	4-$NO_2C_6H_4CH$=CH-	CH_3
7g	$C_6H_5CH_2$-	CH_3	7n	4-$NO_2C_6H_4CH$=CH-	C_2H_5

Scheme 1. Synthetic protocol of the target compounds **7a**–**7n**. Reagents and conditions: (a) ammonia solution, methanol, reflux 8–16 h; (b) diethyl-2-bromomalonate, absolute ethanol, reflux 15 h.

Table 1
Physical data of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydropyridine-3,5-dicarboxylates (**7a**–**7n**)



Compound	R	Ar	Mol. formula	M.P (°C)
7a	CH_3	H	$C_{18}H_{25}NO_8$	220
7b	C_2H_5	H	$C_{20}H_{29}NO_8$	226
7c	CH_3	C_6H_5	$C_{24}H_{29}NO_8$	224
7d	C_2H_5	C_6H_5	$C_{26}H_{33}NO_8$	228
7e	CH_3	2-$NO_2C_6H_4$	$C_{24}H_{28}N_2O_{10}$	225
7f	C_2H_5	2-$NO_2C_6H_4$	$C_{26}H_{32}N_2O_{10}$	229
7g	CH_3	$CH_2C_6H_5$	$C_{25}H_{31}NO_8$	226
7h	C_2H_5	$CH_2C_6H_5$	$C_{27}H_{35}NO_8$	230
7i	CH_3	$CH_2CH_2C_6H_5$	$C_{26}H_{33}NO_8$	228
7j	C_2H_5	$CH_2CH_2C_6H_5$	$C_{28}H_{37}NO_8$	234
7k	CH_3	CH=CHC_6H_5	$C_{26}H_{31}NO_8$	237
7l	C_2H_5	CH=CHC_6H_5	$C_{28}H_{35}NO_8$	238
7m	CH_3	CH=CHC_6H_4-4-NO_2	$C_{26}H_{30}N_2O_{10}$	238
7n	C_2H_5	CH=CHC_6H_4-4-NO_2	$C_{28}H_{34}N_2O_{10}$	239

The anticonvulsant activity of compounds **7a**–**7n** was evaluated by the subcutaneous pentylenetetrazole (scPTZ) and maximal electroshock (MES) screening methods, following oral administration of the compounds into male Wistar albino rats at the dose of 10 mg/kg and observations were made after 1 h [9,10]. The results were shown in Table 2 and Fig. 2. All the compounds showed significant protection against pentylenetetrazole induced convulsions,

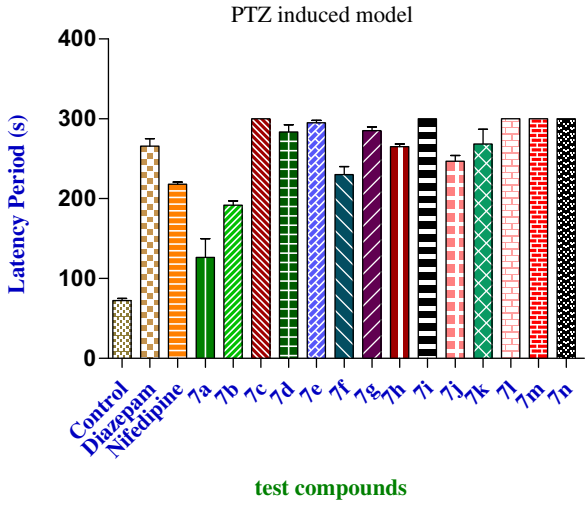


Fig. 2. Comparison of latency period with test compounds in PTZ method. Compounds **7a**–**7n** were used at the dose of 10 mg/kg, whereas, diazepam was used as a standard.

except **7a**. Compounds **7h** and **7k** showed increased latency period and protection against mortality comparable to diazepam (5 mg/kg, i.p). Compounds **7c**, **7d**, **7e**, **7g**, **7l**, **7m** and **7n** showed increased latency period better than diazepam, but exhibited moderate protection against mortality.

In the maximal electroshock method, compounds **7b**, **7c**, **7e**, **7k**, **7m** and **7n** showed significant protection against electroconvulsive seizures (Table 2 and Fig. 3). However, other compounds **7a**, **7d**, **7f**–**j** and **7l** did not show significant protection.

2.3. Calculation of drug-likeness properties

Drug-likeness appears as a promising paradigm to encode the balance among the molecular properties of a compound that

Table 2
Anticonvulsant activity of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydropyridine-3,5-dicarboxylates by scPTZ and MES methodsat 10 mg/kg dose (p.o).

Test compounds	scPTZ[a]		MES[b]	
	Latency period (s)	Protection against mortality (%)	Duration of limb extension (s)	Percentage protection against electroconvulsive seizures (%)
	Mean ± SEM		Mean ± SEM	
Control	72 ± 2	–	16 ± 1	–
Diazepam	266 ± 9***	66.6	–	–
Phenytoin	–	–	6 ± 1***	62.5
Nifedipine	215 ± 3***	33.3	7 ± 1***	56.8
7a	126 ± 23**	0	13 ± 1^{ns}	14.5
7b	192 ± 5***	0	9 ± 1***	41.6
7c	>300***	33.3	12 ± 1*	25
7d	283 ± 9***	0	13 ± 1^{ns}	16.6
7e	295 ± 3***	0	10 ± 1***	39.6
7f	230 ± 10***	0	12±1^{ns}	22.9
7g	285 ± 5***	33.3	14 ± 1^{ns}	14.5
7h	265 ± 3***	66.6	14 ± 1^{ns}	10.4
7i	>300***	66.6	13 ± 1^{ns}	20.8
7j	246 ± 7***	33.3	15 ± 1^{ns}	8.3
7k	268 ± 18***	100	9 ± 2***	41.6
7l	>300***	0	12 ± 1^{ns}	22.9
7m	>300***	0	7 ± 1***	52.1
7n	>300***	0	8 ± 1***	50

Values are expressed as mean ± SEM, $n = 6$. One way analysis of variance (ANOVA) followed by Dunnett's method.
[a] The test compounds were administered orally 1 h before the injection of PTZ (80 mg/kg, i.p) control: 0.5% sodium carboxymethylcellulose. Standard: nifedipine (30 mg/kg, p.o) and diazepam (5 mg/kg, i.p). ***$p < 0.0001$ vs. control, **$p < 0.05$ vs. control.
[b] The test compounds were administered 1 h before the application of maximal electroshock (150 mA, 0.2 s). Standard: nifedipine (30 mg/kg, p.o) and phenytoin (30 mg/kg, oral). ***$p < 0.0001$ vs control, *$p < 0.05$ vs control, ns $p > 0.05$ vs control (not significant).

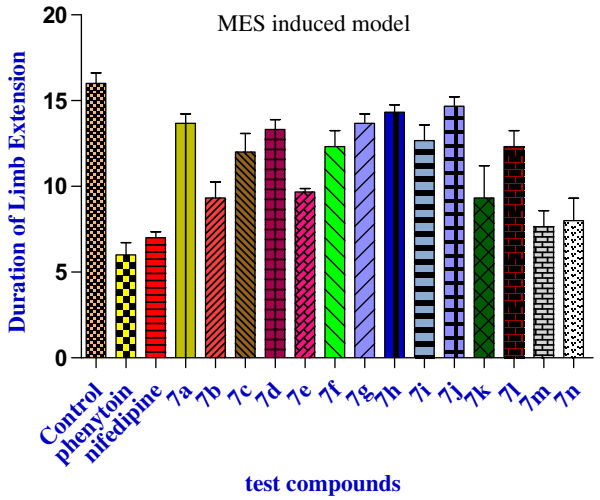


Fig. 3. Comparison of duration of limb extension with test compounds in MES method. Compounds **7a**–**7n** were used at the dose of 10 mg/kg, whereas, phenytoin and nifedipine (30 mg/kg) were used as standards.

influences its pharmacodynamics and pharmacokinetics and ultimately optimizes their absorption, distribution, metabolism and excretion (ADME) in human body like a drug [11]. These parameters allow to ascertaining oral absorption or membrane permeability that occurs when the evaluated molecule follows Lipinski's rule of five [molecular weight (MW) ≤500 Da, log $P \leq 5$, H-bond donors (HBD) ≤5 and H-bond acceptors (HBA) ≤10]. Molecules violating more than one of these parameters may have problems with bioavailability and high probability of failure to display drug-likeness [12,13]. Other parameters that included are number of rotatable bonds which indicate the flexibility of the molecule, volume and polar surface area. The *in silico* estimation of human intestinal absorption of a drug candidate involves numerous *in vitro* methods like percent human intestinal absorption (HIA), Caco2 (P_{Caco2}) and MDCK (P_{MDCK}) cell models and BBB (C_{brain}/C_{blood}) for the prediction of oral drug absorption and blood–brain barrier penetrations respectively.

The above mentioned parameters were calculated for **7a**–**7n** and the results were presented in Tables 3and 4. From the data obtained, it was observed that derivatives **7a**–**7d**, **7g**, **7i** and **7k** were found to obey the Lipinski rule, whereas **7e**, **7f**, **7h**, **7j**, **7l**, **7m** and **7n** were found to violate in HBA and/or MW. All the compounds displayed HIA more than 81% and blood–brain barrier penetration less than 2.0.

Table 4
Prediction of pharmacokinetic properties of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydropyridine-3,5-dicarboxylates (**7a**–**7n**).

Compound	HIA (%)	P_{Caco2} (nm/s)	P_{MDCK} (nm/s)	iPPB (%)	BB (C_{brain}/C_{blood})
7a	87.7	6.86	8.66	37.8	0.347
7b	90.8	5.95	18.69	41.6	0.629
7c	97.5	16.87	57.45	62.0	0.476
7d	98.4	19.62	9.29	72.2	0.900
7e	81.6	2.88	1.13	63.9	0.149
7f	85.5	2.00	0.15	73.3	0.250
7g	98.0	20.22	68.0	75.1	0.566
7h	98.7	24.36	1.58	81.7	0.988
7i	98.4	20.97	13.11	80.6	0.258
7j	99.0	24.53	0.19	85.2	0.441
7k	98.8	20.90	8.57	77.3	0.224
7l	99.3	24.37	0.14	83.2	0.411
7m	88.9	6.61	0.04	88.7	0.019
7n	91.4	3.58	0.04	89.6	0.032

HIA (%), percentage human intestinal absorption; P_{Caco2} (nm/s), Caco2 cell permeability in nm/s; PMDCK (nm/s), Madin–Darby canine kidney cell permeability in nm/s; iPPB(%), *in vitro* plasma protein binding (percentage); BB (C_{brain}/C_{blood}), in vivo blood–brain barrier penetration.

3. Discussion

We have synthesized fourteen derivatives of 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydro-pyridine-3,5-dicarboxylates, **7a**–**7n** with various substituents like phenyl, benzyl, phenethyl, styryl and 4-nitrostyryl at position 4 and increased the structural diversity at positions 3 and 5 by introducing methyl and ethyl groups. The reaction involved substitution of diethylmalonyl group on the nitrogen of the 1,4-DHP ring. The intermediate 1,4-dihydropyridine derivatives, **6a**–**6n** were prepared by Hantzsch condensation of methylacetoacetate (**3**) or ethylacetoacetate (**4**) with various commercially available aromatic aldehydes and ammonia solution [14,15].

The IR spectra of the synthesized compounds **7a**–**7n** showed a weak band at 3025–3015 cm^{-1}, due to the H–C=C–H stretch of trans styryl group and a strong absorption band at 1730–1715 cm^{-1}, due to the C=O stretching of the α,β-unsaturated ester moiety. The ^{1}H NMR spectra of compounds **7a**–**7n** showed a triplet corresponding to the CH_3 protons at δ 1.2–1.4 ppm and a quartet at δ 4.1–4.3 ppm indicating the 2 protons of CH_2. A characteristic

Table 3
Structural properties of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydropyridine-3,5-dicarboxylates (**7a**–**7n**).

Compd	%ABS	MW	*c* log *P*	HBD	HBA	nviolations	nrotb	Volume	TPSA
Rule	–	<500	≤5	<5	<10	≤1	–	–	–
7a	71.6	383	1.38	0	9	0	11	347.88	108.4
7b	71.6	411	1.88	0	9	0	13	381.48	108.4
7c	71.6	459	2.49	0	9	0	12	419.31	108.4
7d	71.6	487	3.27	0	9	0	14	452.92	108.4
7e	55.7	504	2.77	0	11	2	13	442.65	154.28
7f	55.7	532	3.55	0	11	2	15	476.25	154.28
7g	71.6	473	2.57	0	9	0	13	436.11	108.45
7h	71.6	501	3.349	0	9	1	15	469.72	108.45
7i	71.6	487	2.95	0	9	0	14	452.92	108.45
7j	71.6	515	3.73	0	9	1	16	486.52	108.45
7k	71.6	485	3.03	0	9	0	13	446.73	108.45
7l	71.6	513	3.82	0	9	1	15	480.33	108.45
7m	55.7	530	3.31	0	11	2	14	470.06	154.28
7n	55.7	558	4.09	0	11	2	16	503.67	154.28

%ABS, percentage of absorption; MW, molecular weight; HBD, number of H-bond donors; HBA, number of H-bond acceptors; nviolations, number of violations; nrotb, number of rotatable bonds; TPSA, topological polar surface area.

downfield shift of the CH protons attached to the nitrogen of the dihydropyridine moiety was observed at δ 5.1–5.6 ppm. The aryl protons were observed as multiplets at δ 6.5–7.9 ppm. A double doublet appeared at δ 6.1–6.5 ppm (J = 12–15 Hz) indicating the –CH=CH– of styryl moiety present in **7k**–**7n**. The mass spectra of compounds (**7a**–**7n**) showed the molecular ion peaks at their respective molecular weights and the elemental analysis for the compounds is within the limits of ±0.4% of theoretical values.

The compounds were evaluated for anticonvulsant activity at a dose of 10 mg/kg orally in scPTZ and MES models. The results obtained from scPTZ model revealed that compounds possessing 4-phenyl substitution and methyl or ethyl group of 3,5-diester functionality respectively as in **7c** and **7d** showed good activity than the unsubstituted derivatives, **7a** and **7b**. 4-Benzyl and 4-phenethyl substitutions were introduced as in **7g**, **7h** and **7i**, **7j** comprising of one and two carbon separations respectively, between the phenyl and 1,4-DHP ring system. This has led to retention of activity when compared to **7c** and **7d** possessing no carbon spacer between the phenyl and 1,4-DHP ring system, but improved the protection against mortality.

Introduction of a double bond between the two carbon ethyl spacer of **7i** and **7j** produced compounds **7k** and **7l**, which were also found to possess similar latency period, but in **7k** complete protection against mortality was observed. This may be attributed to the presence of styryl moiety and the methyl group of ester functionality. The electron withdrawing nitro group in the form of 2-nitrophenyl and 4-nitrostyryl groups was attached to the 1,4-DHP as in **7e**, **7f** and **7m**, **7n** respectively. There was no improvement in activity in case of **7e** and **7f**, whereas **7m** and **7n** showed increased latency period. The presence of styryl moiety attached to 1,4-DHP resulted in increase in the latency period and the activity is comparable to the standard drug diazepam. However, the presence of nitro group on styryl moiety improved the latency period, but decreased the percentage protection against mortality. Replacement of methyl group of ester functionality by ethyl did not influence the latency period as well as percentage protection.

Compounds exhibiting activity against pentylenetetrazole test can inhibit petit-mal seizures [16]. PTZ can induce seizures by depressing chloride channel function by binding to picrotoxin site in the GABA receptor complex. Drugs that can exhibit anticonvulsant activity against PTZ induced convulsions can raise seizure threshold in the brain, enhancing $GABA_A$ receptor mediated inhibitory neurotransmitter [17]. In the present study, the anticonvulsant activity of the test compounds against PTZ induced seizures may be due to inhibition of T-type Ca^{2+} currents and also GABA receptor mediated inhibitory neurotransmission.

In the maximal electroshock model, compounds **7m** and **7n** bearing 4-nitrostyryl group displayed good protection against seizures. Compounds containing styryl, 2-nitrophenyl and unsubstituted 1,4-dihydropyridine ring showed moderate activity. Other compounds did not exhibit significant activity. The activity of all the derivatives was less than phenytoin (30 mg/kg). Substitution by diethylmalonyl moiety at position 1 of nifedipine retained the anticonvulsant activity.

Drug-likeness was calculated by using Molinspiration and pre-ADMET softwares and the data indicated that compounds **7a**–**7d**, **7g**, **7i** and **7k** obeyed the Lipinski's rule-of-five (Table 3). Other compounds such as **7e**, **7f**, **7h**, **7j**, **7l**, **7m** and **7n** were found not to have drug-likeness and not complied with more than one descriptor. Topological polar surface area (TPSA) is recognized as a good indicator of drug absorption in the intestine (TPSA less than 140 Angstroms squared [Å^2]) and blood–brain barrier penetration (TPSA less than 60 Å^2) [18,19]. The compounds exhibited computational topological polar surface area values less than 140 Å^2 and have good intestinal absorption except **7e**, **7f**, **7m** and **7n**. However, the derivatives were found not to have adequate blood–brain barrier penetration, as the TPSA values are more than 60 Å^2.

Compounds **7a**–**7n** showed human intestinal absorption of more than 81% and are well absorbed (Table 4). All the compounds are weakly bound to plasma proteins with optimum concentration of unbound drug available for cell penetration. Compounds, **7a**–**7l** were found to have moderate penetration to CNS (0.98–0.14), whereas **7m** and **7n** have low penetration (less than 0.1). However, these compounds exhibited good anticonvulsant activity. There is lack of correlation between anticonvulsant activity and poor brain penetration of compounds **7m** and **7n**.

The computational data pertaining to the good oral absorption of the compounds supported the oral anticonvulsant activity of this series of compounds. A previous study on a series of 1-malonyl-1,4-dihydropyridine derivatives demonstrated their ability to cross the blood–brain barrier at detectable concentrations [8]. But in our study, computational TPSA calculations indicated that the compounds containing 1-diethylmalonyl-1,4-dihydropyridine moiety did not show adequate blood–brain barrier penetration. The calculated values did not correlate with the reported biological activities of 1-malonyl-1,4-dihydropyridine derivatives as novel carrier systems for the site specific and sustained drug delivery to the brain and requires further experimental studies.

4. Conclusion

The results obtained revealed that majority of the compounds synthesized exhibited potent anticonvulsant activity in the PTZ model. Only compound **7k** showed significant protection against mortality. Introduction of styryl and 4-nitrostyryl group on 1,4-dihydropyridine nucleus resulted in analogues possessing good anticonvulsant activity. Computational drug-likeness and TPSA calculations revealed that the compounds showed good intestinal absorption.

5. Experimental protocol

5.1. Chemistry

Aldehydes 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; dd, doublet of doublet; m, multiplet.

Accurate masses were obtained on LCMS (Schimadzu) APCI model LC-2010 EV. Elemental analyses were performed on Perkin Elmer 2400 C, H, N elemental analyser.

5.1.1. General method for the synthesis of dialkyl 2,6-dimethyl-4-substituted-1,4-dihydro-pyridine-3,5-dicarboxylates (**6a**–**6n**)

A solution of aldehyde (0.01 mol), methylacetoacetate (0.03 mol, 3.2 ml) or ethylacetoacetate (0.03 mol, 3.8 ml) in methanol (20 ml) was treated with ammonia solution (0.02 mol) and refluxed for 8–16 h. After the completion of the reaction, the mixture was cooled and evaporated to separate compounds **6a**–**6n**. The crude compound was purified by double recrystallization with methanol. The yield obtained was 52–60%.

5.1.2. General method for the synthesis of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydro-pyridine-3,5-dicarboxylates, ***7a–7n*** *(Scheme 1)*

To a stirred solution of **6a–6n** (10 mmol) in 30 ml absolute ethanol, a solution of diethyl-2-bromomalonate (20 mmol, 3.2 g) in 20 ml of absolute ethanol was added. The mixture was refluxed for 15 h; the completion of the reaction was monitored by TLC using *n*-hexane:acetone (8:2). The solution was concentrated and the solid was separated and recrystallized with aqueous ethanol to obtain compounds **7a–7n** [8].

*5.1.2.1. Dimethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethylpyridine-3,5- dicarboxylate (**7a**).* 0.03 mol (3.2 ml) of methylacetoacetate and 0.01 mol (0.55 ml) of formaldehyde were used and obtained the compound as brown crystals with 48% yield; IR (KBr) ν_{max}, cm^{-1}: 3099 and 3083 (Ar–C–H str), 2996 and 2952 (Alk, C–H str), 1731 (ester, –C=O str), 1721 (C=O str, α,β-unsaturated ester), 1120 (Aliph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 6H, $J = 7.0$ Hz, 2(CH_3) of $COOCH_2CH_3$), 1.71 (s, 6H, 2(CH_3) of CH_3 at C-2 and C-6), 3.15 (s, 2H, CH_2 on C-4), 3.76 (s, 6H, 2(CH_3) of $COOCH_3$), 4.2 (q, 4H, $J = 7$ Hz, 2(CH_2) of $COOCH_2CH_3$), 4.31 (s, 1H, CH at N-1). Mass *m/z*: 383.9 $(M + H)^+$. Anal. Calc. for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65. Found: C, 56.18; H, 5.41; N, 4.01.

*5.1.2.2. Diethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethylpyridine3,5- dicarboxylate (**7b**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (0.55 ml) of formaldehyde were used and obtained the compound as pale brown colour crystals with 50% yield; IR (KBr) ν_{max}, cm^{-1}: 3101 and 3082 (Ar–C–H str), 2996 and 2952, 2852 (Alk, C–H str), 1735 (ester, –C=O str), 1723 and 1715 (C=O str, α,β-unsaturated ester), 1150 (Alph, C–N str). ^1H NMR (400 MHz, $CHCl_3$) δ (ppm): 1.3 (t, 12H, $J = 7.3$ Hz, 2(CH_3) of $COOCH_2CH_2$), 1.07 (s, 6H, 2(CH_3) of CH_3 at C-2 and C-6), 3.2 (s, 2H, CH_2(C-4)), 4.13 (m, 8H, 4(CH_2) of $COOCH_2CH_3$), 4.29 (s, 1H, CH of N–$CH(COOEt)_2$). Mass *m/z*: 410.9 $(M)^+$. Anal. Calc. for $C_{20}H_{29}NO_8$: C, 58.38; H, 7.08; N, 3.40. Found: C, 58.18; H, 7.08; N, 3.39.

*5.1.2.3. Dimethyl-1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-phenyl pyridine-3,5-dicarboxylate (**7c**).* 0.03 mol (3.2 ml) of methylacetoacetate and 0.01 mol (1.1 ml) of benzaldehyde were used and obtained the compound as yellow fine crystals with 51% yield; IR (KBr) ν_{max}, cm^{-1}: 3101 and 3082 (Ar–C–H str), 2996 and 2952, 2852 (Alk, C–H str), 1735 (ester, –C=O str), 1723 and 1715 (–CH=CH–C=O, C=O str), 1150 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.3 (t, 6H, $J = 7.1$ Hz, 2(CH_3) of $COOCH_2CH_3$), 1.69 (s, 6H, 2(CH_3) (C-2 and C-6)), 3.75 (s, 6H, 2(CH_3) of $COOCH_3$ (C-3 and C-5)), 4.22 (q, 4H, $J = 7.1$ Hz, 2(CH_2) of $COOCH_2CH_3$), 4.31 (s, 1H, CH of $CH(COOEt)_2$), 4.46 (s, 1H, CH on C-4), 7.2–6.98 (m, 5H, Ar–H of C_6H_5). Mass *m/z*: 458.9 $(M)^+$. Anal. Calc. for $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.05. Found: C, 62.48; H, 6.35; N, 3.04.

*5.1.2.4. Diethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**7d**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.1 ml) of benzaldehyde were used and obtained the compound as yellow bulky crystals with 49% yield; IR (KBr) ν_{max}, cm^{-1}: 3099 and 3081 (Ar–C–H str), 2996 and 2852 (Alk, C–H str), 1732 (ester, –C=O str), 1715 (–CH=CH–C=O, C=O str), 1150 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.23 (12H, t, $J = 7.3$ Hz, 4(CH_3) of $COOCH_2CH_3$), 1.71 (s, 6H, 2(CH_3) on C-2 and C-6), 4.15 (m, 8H, 4(CH_2) of $COOCH_2CH_3$), 4.25 (s, 1H, N–CH), 4.45 (s, 1H, CH on C-4), 7.25–6.9 (m, 5H, Ar–H of C_6H_5). Mass *m/z*: 487.9 $(M + H)^+$. Anal. Calc. for $C_{26}H_{33}NO_8$: C, 64.05; H, 6.82; N, 2.87. Found: C, 63.80; H, 6.80; N, 2.88.

*5.1.2.5. Dimethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate (**7e**).* 0.03 mol (3.2 ml) of methylacetoacetate and 0.01 mol (1.51 g) of 2-nitrobenzaldehyde were used and obtained the compound as bright yellow crystals with 55% yield; IR (KBr) ν_{max}, cm^{-1}: 3083 (Ar–C–H str), 2986 and 2955 (Alk, C–H str), 1731 (ester, –C=O str), 1725 and 1717 (C=O str, α,β unsaturated ester), 1433 (N–O asym. str, NO_2), 1349 (N–O sym. str, NO_2), 1164 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 6H, $J = 7.0$ Hz, 2(CH_3) of CH_2CH_3), 1.73 (s, 6H, 2(CH_3) at C-2 and C-6), 3.74 (s, 6H, 2(CH_3) of $COOCH_3$ at C-3 and C-5), 4.2 (q, 4H, $J = 7.0$ Hz, 2(CH_2) of $COOCH_2CH_3$), 4.29 (s, 1H, CH), 4.49 (s, 1H, CH(C-4)), 8.05 (m, 4H, $C_6H_4NO_2$). Mass *m/z*: 502.9 (M^+), 503.9 $(M + H)^+$. Anal. Calcd for $C_{24}H_{28}N_2O_{10}$: C, 57.14; H, 5.59; N, 5.55. Found: C, 57.08; H, 5.56; N, 5.51.

*5.1.2.6. Diethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate (**7f**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.51 g) of 2-nitrobenzaldehyde were used and obtained the compound as pale yellow colour crystals with 53% yield; IR (KBr) ν_{max}, cm^{-1}: 3083 (Ar–C–H str), 2986 and 2955 (Alk, C–H str), 1731 (ester, –C=O str), 1725 and 1717 (C=O str, α,β unsaturated ester), 1433 (N–O asym. str, NO_2), 1349 (N–O sym. str, NO_2), 1164 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.27 (t, 12H, $J = 7.1$ Hz, 4(CH_3) of $COOCH_2CH_3$), 1.71 (s, 6H, 2CH_3 on C-2 and C-6), 4.21 (m, 8H, 4(CH_2) of $COOCH_2CH_3$), 4.35 (s, 1H, CH of N–$CH(COOEt)_2$), 4.47 (s, 1H, CH on C-4), 8.1–6.75 (m, 4H, Ar–H $C_6H_4NO_2$). Mass *m/z*: 531.9 (M^+), 532.9 $(M + H)^+$. Anal. Calc. for $C_{26}H_{32}N_2O_{10}$: C, 58.64; H, 604; N, 5.26. Found: C, 58.41; H, 6.04; N, 5.24.

*5.1.2.7. Dimethyl 1-(di(ethoxycarbonyl)methyl)-4-benzyl-1,4-dihydro-2,6-dimethyl pyridine-3,5-dicarboxylate (**7g**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.20 g) of phenylacetaldehyde were used and obtained the compound as pale yellow crystals with 51% yield; IR (KBr) ν_{max}, cm^{-1}: 3081 (Ar–C–H str), 2996 and 2852 (Alk, C–H str), 1732 (ester, –C=O str), 1715 (–CH=CH–C=O, C=O str), 1150 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.32 (t, 6H, $J = 6.9$ Hz, 2(CH_3) of CH_2CH_3), 1.7 (s, 6H, 2(CH_3) C-2 and C-6), 2.59 (d, 2H, $J = 6.4$ Hz, CH_2 of $CH_2C_6H_5$), 3.52 (t, 1H, $J = 6.4$ Hz, CH on C-4), 4.2 (q, 4H, $J = 6.9$ Hz, 2(CH_2) of $COOCH_2CH_3$), 4.31 (s, 1H, CH of $CH(COOEt)_2$), 7.21–7.0 (m, 5H, Ar–H in $CH_2C_6H_5$). Mass *m/z*: 473.9 $(M + H)^+$. Anal. Calc. for $C_{25}H_{31}NO_8$: C, 63.41; H, 6.66; N, 2.96. Found: C, 63.28; H, 6.58; N, 2.95.

*5.1.2.8. Diethyl 1-(di(ethoxycarbonyl)methyl)-4-benzyl-1,4-dihydro-2,6-dimethyl pyridine-3,5-dicarboxylate (**7h**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.20 g) of phenylacetaldehyde were used and obtained the compound as pale yellow fine crystals with 50% yield; IR (KBr) ν_{max}, cm^{-1}: 3099 (Ar–C–H str), 2852 (Alk, C–H str), 1735 (ester, –C=O str), 1718 (–CH=CH–C=O, C=O str), 1155 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.25 (t, 12H, $J = 7.1$ Hz, 4CH_3 of $COOCH_2CH_3$), 1.72 (s, 6H, 2CH_3 on C-2 and C-6), 2.61 (d, 2H, $J = 6.8$ Hz, CH_2 of $CH_2C_6H_5$), 3.53 (t, 1H, $J = 6.8$ Hz, CH on C-4), 4.12 (m, 8H, 4CH_2 of $CH_2COOCH_2CH_3$), 4.31 (s, 1H, CH of N–$CH(COOEt)_2$), 4.5 (s, 1H, CH on C-4), 7.25–7.05 (m, 5H, Ar–H of $CH_2C_6H_5$). Mass *m/z*: 501.9 $(M + H)^+$. Anal. Calc. for $C_{27}H_{35}NO_8$: C, 64.65; H, 7.03; N, 2.79. Found: C, 64.42; H, 7.01; N, 2.77.

*5.1.2.9. Dimethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-phenethyl pyridine-3,5-dicarboxylate (**7i**).* 0.03 mol (3.2 ml) of methylacetoacetate and 0.01 mol (1.34 g) of 3-phenylpropionaldehyde were used and obtained the compound as dull yellow crystals with 52% yield; IR (KBr) ν_{max}, cm^{-1}: 3089 (Ar–C–H str), 2862 (Alk, C–H str), 1736 (ester, –C=O str), 1725 (–CH=CH–C=O, C=O str), 1158 (Alph, C–N str). ^1H NMR (400 MHz,

$CDCl_3$) δ (ppm): 1.29 (t, 6H, J = 7.3 Hz, $2CH_3$ of $COOCH_2CH_3$), 1.6 (t, 2H, J = 6.9 Hz, CH_2 of $-CH_2-CH_2-C_6H_5$), 1.75 (s, 6H, $2CH_3$ on C-2 and C-6), 2.6 (t, 2H, J = 6.9 Hz, CH_2 of CH_2CH_2Ph), 3.52 (t, 1H, J = 7.01 Hz, CH on C-4), 3.75 (s, 6H, CH_3, $COOCH_3$), 4.2 (q, 4H, J = 7.3 Hz, $2CH_2$ of $COOCH_2CH_3$), 4.31 (s, 1H, CH of N–CH–$(COOEt)_2$), 7.3–7.05 (m, 5H, Ar–H of $CH_2CH_2C_6H_5$). Mass m/z: 486.9 $(M)^+$. Anal. Calc. for $C_{26}H_{33}NO_8$: C, 64.05; H, 6.82; N, 2.87. Found: C, 63.99; H, 6.80; N, 2.86.

*5.1.2.10. Diethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-phenethyl pyridine-3,5-dicarboxylate (**7j**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.34 g) of 3-phenylpropionaldehyde were used and obtained the compound as dull yellow crystals with 53% yield; IR (KBr) ν_{max}, cm^{-1}: 3081 (Ar–C–H str), 2996 (Alk, C–H str), 1732 (ester, –C=O str), 1721 (–CH=CH–C=O, C=O str), 1154 (Alph, C–N str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 12H, J = 7.3 Hz, $4CH_3$ of $COOCH_2CH_3$), 1.6 (t, 2H, J = 6.9 Hz, CH_2 of $-CH_2-CH_2-C_6H_5$), 1.75 (s, 6H, $2CH_3$ on C-2 and C-6), 2.6 (t, 2H, J = 6.9 Hz, CH_2 of CH_2CH_2Ph), 3.52 (t, 1H, J = 7.01 Hz, CH on C-4), 4.2 (m, 8H, $4CH_2$ of $COOCH_2CH_3$), 4.31 (s, 1H, CH of N–CH–$(COOEt)_2$), 7.3–7.05 (m, 5H, Ar–H of $CH_2CH_2C_6H_5$). Mass m/z: 514.9 $(M)^+$. Anal. Calc. for $C_{28}H_{37}NO_8$: C, 65.23; H, 7.23; N, 2.72. Found: C, 65.18; H, 7.21; N, 2.73.

*5.1.2.11. Dimethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-styryl pyridine-3,5-dicarboxylate (**7k**).* 0.03 mol (3.2 ml) of methylacetoacetate and 0.01 mol (1.32 g) of cinnamaldehyde were used and obtained the compound as pale white crystals with 50% yield; IR (KBr) ν_{max}, cm^{-1}: 3081 (Ar–C–H str), 3023 (trans-C–H str), 2996 and 2952 (Alk, C–H str), 1738 (ester, –C=O, str), 1723 (C=O str α,β-unsaturated ester), 1648 (trans C=C str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 6H, J = 7.1 Hz, $2CH_3$ of $COOCH_2CH_3$), 2.1 (s, 6H, $2CH_3$ on C-2 and C-6), 4.15 (q, 4H, J = 7.1 Hz of $COOCH_2CH_3$), 4.6 (d, 1H, J = 6.3 Hz, CH on C-4), 5.15 (s, 1H, CH of N–$CH(COOCH)_2$), 6.15 (dd, 1H, J = 12.3 Hz, CH of $-CH=CHC_6H_5$), 6.21 (d, 1H, J = 12.3 Hz, $-CH=CH-C_6H_5$), 7.35–8.10 (m, 5H, Ar–H of $CH=CH-C_6H_5$). Mass m/z: 485.9 $(M+H)^+$. Anal. Calc. for $C_{26}H_{31}NO_8$ found (theoretical); C 63.95 (64.32); H 6.04 (6.44); N 2.79 (2.88); O 27.22 (26.36). Anal. Calc. for $C_{26}H_{31}NO_8$: C, 64.32; H, 6.44; N, 2.88. Found: C, 64.18; H, 6.41; N, 2.87.

*5.1.2.12. Diethyl 1-(di(ethoxycarbonyl)methyl)-1,4-dihydro-2,6-dimethyl-4-styryl pyridine-3,5-dicarboxylate (**7l**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.32 g) of cinnamaldehyde were used and obtained the compound as white crystals with 55% yield; IR (KBr) ν_{max}, cm^{-1}: 3085 (Ar–C––H str), 3021 (trans C=C str), 2986 and 2962 (Alk, C–H str), 1736 (ester, –C=O str), 1720 (C=O str α,β-unsaturated ester), 1645 (trans C=C str). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 12H, J = 7.3 Hz, $4CH_3$ of $COOCH_2CH_3$), 2.32 (s, 6H, CH_3 on C-2 and C-6), 4.17 (m, 8H, $2CH_2$ of $COOCH_2CH_3$), 4.62 (d, 1H, J = 6.3, CH on C-4), 5.6 (s, 1H, CH of N–$CH(COOEt)_2$), 6.1 (dd, 1H, J = 12.8 Hz, $-CH=CHC_6H_5$), 6.21 (d, 1H, J = 12.8 Hz, $CH=CH-C_6H_5$), 7.35–7.1 (m, 5H, Ar–H of $CH=CH-C_6H_5$). Mass m/z: 512.9 $(M)^+$, 513.9 $(M+H)^+$. Anal. Calc. for $C_{28}H_{35}NO_8$: C, 65.48; H, 6.87; N, 2.73. Found: C, 65.38; H, 6.84; N, 2.72.

*5.1.2.13. Dimethyl 1-(di(ethoxycarbonyl)methyl)-4-(4-nitrostyryl)-1,4-dihydro-2,6-dimethyl pyridine-3,5-dicarboxylate (**7m**).* 0.03 mol (3.2 ml) of methylacetoacetate and 0.01 mol (1.77 g) of 4-nitrocinnamaldehyde were used and obtained the compound as pale yellow crystals with 51% yield. IR (KBr) ν_{max}, cm^{-1}: 3081 (Ar–C–H str), 3023 (tran-C–H str), 2996 and 2952 (Alk, C–H str), 1738 (ester, –C=O, str), 1723 (C=O str, α,β-unsaturated ester), 1648 (trans C=C str), 1433 (N–O asym. str, NO_2), 1349 (N–O sym. str, NO_2). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 12H, J = 7.3 Hz, $2CH_3$ of $COOCH_2CH_3$), 2.32 (s, 6H, CH_3 on C-2 and C-6), 3.75 (s, 6H, CH_3 $COOCH_3$), 4.17 (q, 4H, J = 7.3 Hz, $2CH_2$ of $COOCH_2CH_3$), 4.62 (d, 1H, J = 6.3 Hz, CH on C-4), 5.6 (s, 1H, CH of N–CH $(COOEt)_2$), 6.1 (dd, 1H, J = 14.2 Hz, $-CH=CHC_6H_5$), 6.21 (d, 1H, J = 14.2 Hz, $CH=CH-C_6H_5$), 8.0–7.1 (m, 4H, Ar–H of $C_6H_4NO_2$). Mass m/z: 529.9 $(M)^+$. Anal. Calc. for $C_{26}H_{30}N_2O_{10}$: C, 58.96; H, 5.7; N, 5.28. Found: C, 58.38; H, 5.26; N, 5.27.

*5.1.2.14. Diethyl 1-(di(ethoxycarbonyl)methyl)-4-(4-nitrostyryl)-1,4-dihydro-2,6-dimethyl pyridine-3,5-dicarboxylate (**7n**).* 0.03 mol (3.8 ml) of ethylacetoacetate and 0.01 mol (1.77 g) of 4-nitrocinnamaldehyde were used and obtained the compound as pale yellow crystals with 53% yield. IR (KBr) ν_{max}, cm^{-1}: 3081 (Ar–C–H str), 3023 (tran-C–H str), 2996 and 2952 (Alk, C–H str), 1738 (ester, –C=O, str), 1723 (C=O str α,β-unsaturated ester), 1648 (trans C=C str), 1433 (N–O asym. str, NO_2), 1349 (N–O sym. str, NO_2). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (t, 12H, J = 7.3 Hz, $4CH_3$ of $COOCH_2CH_3$), 2.32 (s, 6H, CH_3 on C-2 and C-6), 4.17 (m, 8H, $4CH_2$ of $COOCH_2CH_3$), 4.62 (d, 1H, J = 6.3 Hz, CH on C-4), 5.6 (s, 1H, CH of N–CH $(COOEt)_2$), 6.1 (dd, 1H, J = 13.5 Hz, $-CH=CHC_6H_5$), 6.21 (d, 1H, J = 13.5 Hz, $CH=CH-C_6H_5$), 8.0–7.1 (m, 4H, Ar–H of $C_6H_4NO_2$). Mass m/z: 558.9 $(M+H)^+$. Anal. Calc. for $C_{28}H_{34}N_2O_{10}$: C, 60.21; H, 6.14; N, 5.02. Found: C, 60.18; H, 6.04; N, 5.01.

5.2. Pharmacology

Adult male Wistar rats (150–250 g) and Swiss albino mice (18–20 g) 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).

5.2.1. Acute toxicity studies

The acute toxicity was estimated as per OECD-425 guidelines for testing of chemicals acute oral toxicity [20]. The test was used to find the safe dose for compounds **7a**–**7n**. Swiss albino mice (18–20 g) were divided into several groups each containing 5 animals. Drugs were administered by oral route in different concentrations. The animals were observed for their death over a period of seven days.

5.2.2. Evaluation of anticonvulsant activity

5.2.2.1. The subcutaneous pentylenetetrazole seizure test (ScPTZ). This method utilizes a dose of pentylenetetrazole (PTZ) 80 mg/kg in rats that produces clonic seizures. The rats were divided into 16 groups of six rats each. Group 1 was the control group received vehicle (0.5% sodium carboxymethylcellulose); group 2 received (5 mg/kg, i.p) diazepam, groups 3–16 received (10 mg/kg, oral) compounds **7a**–**7n** respectively, which were prepared by suspending in 0.5% sodium carboxymethylcellulose. All the drugs were administered 1 h prior to the PTZ administration and the latency period of the seizures and the mortality were observed [10]. The seizure response was observed for a maximum period of 300sec.

5.2.2.2. The maximal electric shock (MES) test. The anticonvulsant property of the drug in this model was assessed by its ability to protect against maximal electric shock induced convulsions. The rats were divided into 16 groups of six rats each. Group 1 was the control group received vehicle; group 2 received (30 mg/kg, oral) phenytoin, groups 3–16 received (10 mg/kg, oral) compounds **7a**–

7n respectively, which were prepared by suspending in 0.5% sodium carboxymethylcellulose. Maximal electric shock of 150 mA current for 0.2 s was applied through corneal electrodes to induce convulsions in the control, standard and test compounds treated animals. Abolition or reduction in the duration of hind limb extension was considered as the index for anticonvulsant activity [21].

5.2.3. Statistical analysis

All values were expressed as mean value ± SEM. Tests of significance were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test.

5.2.3.1. Calculation of drug-likeness properties. The parameters for drug-likeness were evaluated according to the Lipinski's rule-of-five, using the Supercomputing Facility for Bioinformatics & Computational Biology-IIT Delhi [13] and TPSA values from Molinspiration online property calculator tool kit [22]. 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 %HIA, Caco2 and MDCK cell permeabilities, plasma protein binding and blood–brain barrier penetration values were obtained from ADME calculator [24].

Appendix A. Supplementary data

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2013.12.001. These data include MOL files and InChiKeys of the most important compounds described in this article.

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