Syntheses, Calcium Channel Antagonist and Anticonvulsant Activities of Substituted 1,4-Dihydro-3,5-pyridinedicarboxylates Containing Various 3-Alkyl Ester Substituents

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Summary

A group of 3-alkyl 5-isopropyl 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates **10–20** containing an amine, quaternary ammonium, aryl(heteroaryl)alkenyl, 4-(4-fluorophenyl)-piperazin-1-yl or methoxy moiety in the C-3 alkyl ester R-substituent in combination with a C-4 phenyl ring bearing a 2,3-Cl_2, 3-NO_2, 3-NMe_2, 4-NMe_2 or 3,4,5-$(OMe)_3$ X-substituent were prepared using the Hantzsch 1,4-dihydropyridine reaction. *In vitro* calcium channel antagonist activity (CCA) was determined using a guinea pig ileum longitudinal smooth muscle assay. In the C-4 3-nitrophenyl series of compounds, the C-3 ester R-substituent was a determinant of CCA activity where the relative potency order was $-CH_2CH_2CH{=}C$-(2-methylphenyl)$_2 \geq -CH_2CH_2NMe_2.HCl >$ $-CH_2CH_2CH{=}C$-(3-methyl-2-thienyl)$_2 > -CH_2CH_2{}^+NMe_3\ I^-$. The position and nature of the C-4 phenyl X-substituent, were also determinants of CCA activity where the relative activity order was 3-NMe_2 > 4-NMe_2 > 3,4,5-$(OMe)_3$. Anticonvulsant activities were determined in mice using the subcutaneous metrazol (scMet) and maximal electroshock (MES) screens. The compounds investigated were generally not effective for protecting againist scMet induced seizures, except for **10** {X = 2,3-Cl_2, R = 2-[4-(4-fluorophenyl)piperazin-1-yl]ethyl} and **14a** (X = 3-NMe_2.HCl, R = CH_2CH_2OMe), which exhibited modest activity. Compound **11a** (X = 3-NO_2, R = $-CH_2CH_2NMe_2$.HCl) was the most effective agent in the MES screen. All of the compounds investigated, except for **11b** (X = 3-NO_2, R = $-CH_2CH_2{}^+NMe_3\ I^-$, *K*p = 0.15), are lipophilic with *n*-octanol/aqueous phosphate buffer (pH = 7.4) partition coefficients (*K*p) in the 121–424 range relative to the reference drug nimodipine (*K*p = 187). The structure-activity relationships acquired reinforce the concept that calcium is only one of several factors that are involved in seizure generation.

Introduction

Although the mechanisms responsible for epileptic seizures are not fully elucidated, there is convincing evidence that calcium is involved. A pathological influx of calcium into neurons[1] is most likely associated with neuronal damage in status epilepticus[2]. Furthermore, the calcium channel agonist Bay K 8644, which stimulates the influx of calcium into cells, induces seizures in a dosage dependent manner[3]. These observations prompted investigations to evaluate the potential use of calcium channel antagonists (CCAs) as a novel class of antiepileptic drugs. In this regard, verapamil (**1**) was reported to retard the rate of kindling seizures in rats[4], and flunarizine (**2**)[5] was portrayed as a potential antiepileptic drug for the future. Nimodipine (**3**), a 1,4-dihydropyridine (DHP) CCA, provided protection against seizures induced by maximal electroshock (MES)[6–7], pentylenetetrazole[8–10] and picrotoxin[11] (see Figure 1).

It was therefore of interest, as part of our on-going program to develop calcium channel modulators, to design brain-targeted 1,4-dihydropyridine CCAs as potential anticonvulsant agents. Accordingly, 1,4-DHP compounds possessing a variety of substituents (2,3-Cl_2, 3-NO_2, 3-NMe_2, 4-NMe_2, 3,4,5-OMe_3) on the C-4 phenyl ring system have been investigated to determine structure-activity relationships for/between CCA and anticonvulsant activities. The localization of anticonvulsant drugs in the brain may be limited by either i) their ability to effectively cross the blood brain barrier (BBB), or ii) their rapid egress from brain[12], which would result in a sub-therapeutic brain concentration. To overcome these limitations, lipophilic amine moieties such as 2-[4-(4-fluorophenyl)piperazin-1-yl]ethyl (**10**) and 2-dimethylaminoethyl (**11a**) were attached to the C-3 ester moiety. It was envisaged that these lipophilic moieties may enhance their ability to cross the BBB, and undergo subsequent protonation which would allow binding to a negative domain, to provide an anchor on the extracellular side of the lipid-bilayer, on the 1,4-DHP binding site[13]. Knutsen *et al.* reported that attachment of a lipophilic 4,4-bis(3-methyl-2-thienyl)-3-butenyl (tiagabine, **4**), or 4,4-bis(2-methylphenyl)-3-butenyl, substituent to the N-1 nitrogen atom of nipecotic acid or guvacine provided potent GABA-uptake inhibition agents that possessed *in vivo* efficacy as anticonvulsant agents[14]. It was therefore of interest to determine the effect which attachment of a C-3 4,4-bis(2-methylphenyl)-3-butenyl- (**12**) and 4,4-bis(3-methyl-2-thienyl)-3-butenyl- (**13**) ester substituent has on CCA and anticonvulsant activities.

Chemistry

4,4-Bis(2-methylphenyl)-1-buten-4-ol (**6c**), required for the synthesis of 4,4-bis(2-methylphenyl)-3-butenyl acetoacetate (**7c**), was prepared according to a method previously developed[15], and 2-[4-(4-fluorophenyl)piperazin-1-yl]-ethanol (**6d**, 55% yield), required for the preparation of 2-[4-(4-fluorophenyl)piperazin-1-yl]ethyl acetoacetate (**7d**),

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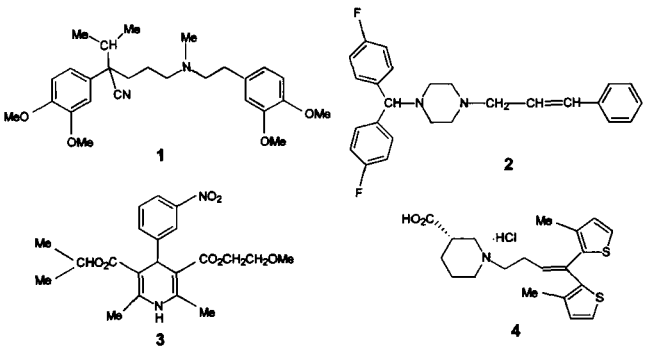
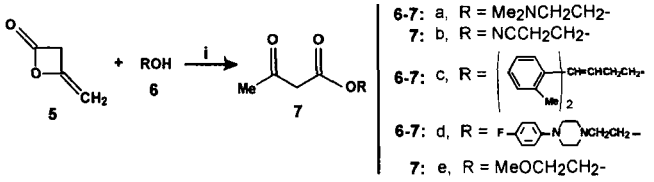


Figure 1. Structures of verapamil (**1**), flunarizine (**2**), nimodipine (**3**), and tiagabine (**4**).

was synthesized by the condensation of 1-(4-fluorophenyl)piperazine with 2-bromoethanol in the presence of Et_3N. The alkyl acetoacetate analogues (**7a, 7c, 7d**),



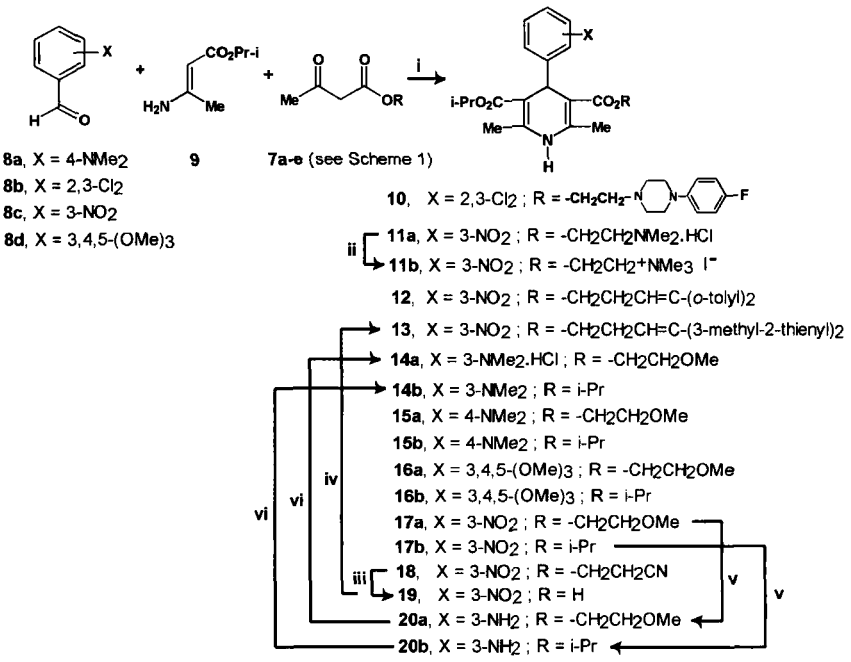
Scheme 1. Reagents and conditions: i, Et_3N, 95 °C, 3 h (Products **7a, 7c, 7d**).

required for the Hantzsch condensation reaction (Scheme 2), were prepared by the Et_3N-catalyzed reaction of diketene (**5**) with the respective alcohol (**6a, 6c** or **6d**) in 84–85% yield as illustrated in Scheme 1. 2-Cyanoethyl acetoacetate (**7b**) was prepared according to the procedure of Ogawa *et al.*[16] and 2-methoxyethyl acetoacetate (**7e**) was purchased from the Aldrich Chemical Co.

The 1,4-DHP compounds (**10, 11a, 12, 15a–b, 16a–b, 17a–b, 18**) were prepared by the Hantzsch reaction. Thus, condensation of the substituted-benzaldehyde (**8a–d**) with an alkyl acetoacetate analogue (**7a–e**) and isopropyl 3-aminocrotonate (**9**) in ethanol yielded the respective 1,4-DHP product in 19–69% yield as illustrated in Scheme 2. Reactions employing the benzaldehyde derivatives **8a** and **8d**, which possess the respective electron-donating 4-dimethylamino and 3,4,5-trimethoxyphenyl substituents require a higher reaction temperature of 100 °C using 2-methoxyethanol as solvent, and a longer reaction time of 48 h (see Scheme 2 legend for reaction conditions). The product yield for these latter reactions are lower (19–42%) relative to those reactions employing benzaldehyde derivatives that possess electron-withdrawing substituents such as **8b–c** (20–69% yield). Reaction of the 2-dimethylaminoethyl ester analogue **11a** with iodomethane afforded the corresponding 2-trimethylammoniumethyl iodide **11b** in 99% yield.

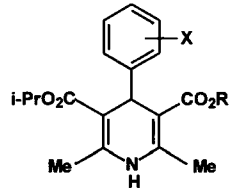
An alternate method was used to synthesize the 4,4-bis(3-methyl-2-thienyl)-3-butenyl ester **13**. Thus, the β-elimination of acrylonitrile from the 2-cyanoethyl ester moiety of **18** using the non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) yielded 3-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate **19** in 66% yield. Subsequent condensation of **19** with 4-bromo-1,1-bis-(3-methyl-2-thienyl)-1-butene in the presence of K_2CO_3 afforded the target compound **13** in 27% yield.

Since 3-dimethylaminobenzaldehyde which may be useful for the Hanzsch synthesis of **14a–b** is not commercially available, an alternative method was employed for their synthesis. Accordingly, hydrogenation of the 3-nitrophenyl compounds **17a–b** using 10% palladium-on-charcoal and H_2 gas at 55 psi afforded the respective 3-aminophenyl derivatives **20a–b**. The subsequent reaction of **20a** and **20b** with formaldehyde and sodium cyanoborohydride in the presence of zinc chloride afforded the respective 3-dimethylaminophenyl products **14a** (44%) and **14b** (50%). This reductive-methylation reaction is a modification of the Escheweiler-Clarke reaction[17] and is suitable for the methylation of primary amines. Sodium cyanoborohydride is a useful and selective reducing agent in this reaction since the electron-withdrawing cyano-group reduces its reactivity[18] thereby preventing any undesired reduction of the 1,4-DHP C-3 and/or C-5 ester moieties.



Scheme 2. Reagents and conditions: i, EtOH, reflux, 16 h (**10, 11a, 12, 17–18**), or 2-methoxyethanol, 100 °C, 48 h (**15a–b, 16a–b**), ii, MeI, acetone, reflux, 24 h; iii, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), MeOH, 25 °C, 24 h; iv, 4-bromo-1,1-bis(3-methyl-2-thienyl)-1-butene, K_2CO_3, DMF, 25 °C, 120 h; v, H_2 gas, 55 psi, 10% Pd-C, EtOH, 25 °C, 2 h; vi, HCHO (37% *w/v*), $NaCNBH_3$, $ZnCl_2$, MeOH, 25 °C, 18 h; HCl, EtOH.

Table 1. Physical, calcium channel antagonist activity and partition coefficients of 3-alkyl 5-isopropyl 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates (**10–16**).

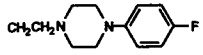


Compd	X	R	Cryst. solvent	mp °C	% Yield	Formula	Anal.[a]	Calcium channel antagonist activity $IC_{50}M$[b]	Partition coeff.[c]
10	2,3-Cl_2	[d]	NR[e]	65–70	48	$C_{30}H_{34}Cl_2FN_3O_4$	C,H,N[f]	$1.59 \pm 0.09 \times 10^{-8}$	265
11a	3-NO_2	-$CH_2CH_2NMe_2$.HCl	CH_2Cl_2-hexane	121–125	61	$C_{22}H_{30}ClN_3O_6$	C,H,N[f]	$6.07 \pm 0.60 \times 10^{-8}$	230
11b	3-NO_2	-$CH_2CH_2NMe_3^+ I^-$	acetone-hexane	178–186	99	$C_{23}H_{32}IN_3O_6$	C,H,N	$1.39 \pm 0.00 \times 10^{-5}$	0.15
12	3-NO_2	-$CH_2CH_2CH{=}CAr_2$[g]	EtOAc-hexane	157–159	49	$C_{36}H_{38}N_2O_6$	C,H,N	$2.25 \pm 0.01 \times 10^{-8}$	385
13	3-NO_2	-$CH_2CH_2CH{=}CHet_2$[h]	*i*-Pr_2O-hexane	105–107	27	$C_{32}H_{34}N_2O_6S_2$	C,H,N	$4.45 \pm 0.28 \times 10^{-6}$	424
14a	3-NMe_2.HCl	-CH_2CH_2OMe	EtOH	209–210	19	$C_{23}H_{33}ClN_2O_5$	C,H,N	$1.96 \pm 0.03 \times 10^{-7}$	137
14b	3-NMe_2	*i*Pr	CH_2Cl_2-hexane	136–139	50	$C_{23}H_{32}N_2O_4$	C,H,N[i]	$3.47 \pm 0.26 \times 10^{-8}$	129
15a	4-NMe_2	-CH_2CH_2OMe	EtOAc-hexane	139–141	25	$C_{23}H_{32}N_2O_5$	C,H,N	$1.33 \pm 0.02 \times 10^{-5}$	121
15b	4-NMe_2	*i*Pr	EtOAc-hexane	135–136	19	$C_{23}H_{32}N_2O_4$	C,H,N	$7.61 \pm 1.50 \times 10^{-6}$	104
16a	3,4,5-$(OMe)_3$	-CH_2CH_2OMe	EtOAc-hexane	119–120	42	$C_{24}H_{33}NO_8$	C,H,N	$2.84 \pm 0.00 \times 10^{-5}$	208
16b	3,4,5-$(OMe)_3$	*i*Pr	EtOAc-hexane	161–162	20	$C_{24}H_{33}NO_7$	C,H,N	$3.09 \pm 0.03 \times 10^{-5}$	259
Nimodipine (3)								$1.49 \pm 0.08 \times 10^{-8}$	187

[a] Microanalytical analyses were within ± 0.4% of theoretical values, unless otherwise indicated.

[b] The molar concentration of antagonist test compound causing a 50% decrease in the slow component, or tonic contractile response, (IC_{50} ± SEM) in guinea pig ileal longitudinal smooth muscle by the muscarinic agonist carbachol (1.6×10^{-7} M) was determined graphically from the dose-response curve ($n = 3$).

[c] The partition coefficient is defined as the concentration of the test compound in *n*-octanol / concentration in an aqueous phosphate buffer at pH = 7.4.

[d] 

[e] NR = Not recrystallized. [f] ½ molecule of water of hydration, [g] Ar = *o*-tolyl, [h] Het = 2-(3-methylthienyl), [i] ¼ molecule of water of hydration.

Table 2. Anticonvulsant test results for 3-alkyl 5-isopropyl 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates (**10–16**).

Compd	MES[a]				scMet[b]				Toxicity Test						Class [c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
10	0/3	ND[e]	0/1	ND	1/1	ND	ND	ND	0/4	1[f]/2	8/8	4/4	4[f]/4	ND	4
11a	3/3	0/3	1/1	ND	1/1[f]	ND	ND	ND	0/4	0/2	7/8	3[f]/4	4[f]/4	ND	1
11b	ND	ND	ND	ND	ND	ND	ND	ND	4[f]/4	ND	8[f]/8	ND	4[f]/4	ND	4
12	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
13	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
14a	0/3	0/3	1/1	1/1	0/1	0/1	0/1	1/1	0/4	0/2	2/8	4/4	4/4	2/2	2
	2/2 (0.25 h)	½ (1 h)													
14b	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	1/2	2
15a	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	2
15b	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
16a	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
16b	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
Nimodipine (3)	0/3	1/3	0/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	2/2	1

[a, b]The results for the MES and scMet seizure tests are expressed as the number of animals protected/the number of animals tested. The test compound was administered ip to mice using either polyethylene glycol (PEG) or methylcellulose (0.5% *w/v*) as the vehicle.
[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg, Class 4 = test compound shows toxicity at a dose equal to or less than 30 mg/kg.
[d] Time after test compound administration. [e] ND = not determined. [f] The test compound caused mortality.

Results and Discussion

The *in vitro* calcium channel antagonist activities of the racemic compounds **10-16**, and the reference drug nimodipine (**3**), were determined using the muscarinic receptor-mediated (carbachol) Ca^{2+}-dependent contraction of guinea pig ileum longitudinal smooth muscle (GPILSM) assay. These results and their *n*-octanol-aqueous phosphate buffer (pH = 7.4) partition coefficients are summarized in Table 1. The most potent calcium channel antagonist compounds **10** {R = 2-[4-(4-fluorophenyl)piperazin-1-yl]ethyl}, **11a** (R = 2-dimethylaminoethyl), **12** [R = 4,4-bis(2-methylphenyl)-3-butenyl], and **14b** (R = *i*-Pr) were approximately equiactive ($IC_{50} = 1.59 \times 10^{-8}$ to 6.07×10^{-8} M range) to the reference drug nimodipine ($IC_{50} = 1.49 \times 10^{-8}$ M). A comparison of the C-4 3-nitrophenyl series of compounds showed the C-3 ester R-substituent was a determinant of activity where the relative potency order was $-CH_2CH_2CH{=}C$-(2-methylphenyl)$_2$ (**12**) ≥ $-CH_2CH_2NMe_2.HCl$ (**11a**) > $-CH_2CH_2CH{=}C$-(3-methyl-2-thienyl)$_2$ (**13**) > $-CH_2CH_2{}^+NMe_3\ I^-$ (**11b**). The significant reduction in potency observed upon elaboration of the C-3 $-CH_2CH_2NMe_2.HCl$ substituent of **11a** ($IC_{50} = 6.07 \times 10^{-8}$ M) to the $-CH_2CH_2{}^+NMe_3\ I^-$ analogue (**11b**) ($IC_{50} = 1.39 \times 10^{-5}$ M) is likely due to the fact that a polymethylene spacer of at least eight carbon atoms [$-CO_2(CH_2)_8{}^+NMe_3\ I^-$] is required for maximal binding to the L-type binding site for charged trimethylammonium alkyl compounds[13]. The position, and nature, of the phenyl X-substituent were also determinants of activity where the relative activity orders were 3-NMe_2.HCl (**14a**) > 4-NMe_2 (**15a**) ≈ 3,4,5-$(OMe)_3$ (**16a**), and 3-NMe_2 (**14b**) > 4-NMe_2 (**15b**) > 3,4,5-$(OMe)_3$ (**16b**). These results (X = 3-NMe_2 > 4-NMe_2) are consistent with the well-documented structure-activity relationship for 1,4-DHPs that C-4 *meta*-X-phenyl > *para*-X-phenyl[19–20]. In the C-4 3-nitrophenyl series of compounds, all agents (**11a, 12, 13**; *K*p = 230, 385, 424, respectively) except for the trimethylammoniumethyl iodide compound (**11b**, *K*p = 0.15) are more lipophilic than the reference drug nimodipine (*K*p = 187). The high lipophilicity of compounds **11a, 12**, and **13** should allow their facile passage across the blood-brain-barrier[21]. In contrast, compounds (**14a–b, 15a–b**) possessing a C-4 phenyl X = 3-NMe_2 or 4-NMe_2 subsituent are less lipophilic (*K*p = 104–137 range) than the corresponding X = 3,4,5-$(OMe)_3$ compounds (**16a–b**, *K*p = 208 and 259, respectively).

The anticonvulsant activities were determined by the U.S. National Institutes of Health, Antiepileptic Drug Development Program. In Phase 1 identification of anticonvulsant activity in mice, test compounds were administered via intraperitoneal injection and challenged by maximal electroshock (MES) and subcutaneous metrazol (scMet) induced seizures[22–23]. Compounds which are effective in these seizure challenges are regarded to be effective for absence or petit mal (scMet), and generalized tonic clonic or grand mal (MES) epilepsy. Toxicity of the test compounds was determined using the rotorod toxicity test[22–23]. The results summarized in Table 2 indicate that none of the compounds investigated protect mice from scMet induced seizures, except for compounds **10** {X = 2,3-Cl_2, R = 2-[4-(4-fluorophenyl)piperazin-1-yl]ethyl} and **14a** (X = 3-NMe_2.HCl, R = $-CH_2CH_2OMe$) which protected 1/1 mice at 0.5 h (100 mg/kg ip dose), and at 4 h (300 mg/kg ip dose) post drug administration, respectively. These results suggest that another type of calcium current, other than the L-type[24] which is modulated by CCAs, may be involved in seizure initiation[25]. A number of compounds **11a** (X = 3-NO_2, R = $-CH_2CH_2NMe_2.HCl$), **14a** (X = 3-NMe_2.HCl, R = $-CH_2CH_2OMe$), **14b** (X = 3-NMe_2, R = *i*-Pr) and **15a** (X = 4-NMe_2, R = $-CH_2CH_2OMe$) protected mice against MES induced seizures. Compound **11a**, which was the most effective in the MES screen, protected 3/3 mice (100 mg/kg ip dose) at 30 min post drug administration, although it was quite toxic. The high toxicity of compounds **10** and **11b** precluded their evaluation in the MES and scMet screens. It is quite possible that this extreme toxicity exhibited by **11b** (X = 3-NO_2, R = $-CH_2CH_2{}^+NMe_3\ I^-$) is due to its acetylcholine-like C-3 ester substituent ($CO_2CH_2CH_2{}^+NMe_3\ I^-$) which may allow it to act as a potent CNS cholinergic agonist. Although compound **12** [X = 3-NO_2, R = $-CH_2CH_2CH{=}C$-(2-methylphenyl)$_2$] exhibited comparable CCA activity to nimodipine and is highly lipophilic (Kp = 385), it was inactive and non-toxic in the MES screen.

The classification of anticonvulsant activity (see Table 2) indicates that the position of the X-substituent on the C-4 phenyl ring is a determinant of anticonvulsant activity for active compounds (**11a, 14a, 14b, 15a**) with an activity profile *meta* > *para* > 3,4,5-$(OMe)_3$ (inactive). These results reinforce the concept that calcium is only one of several factors that are involved in seizure initiation[26].

Acknowledgments

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Experimental

Melting points were determined using a Thomas Hoover capillary apparatus and are uncorrected. IR spectra were acquired using a Nicolet 5DX-FT spectrometer. ^1H NMR spectra were recorded on a Bruker AM-300 spectrometer using $CDCl_3$ or $(CD_3)_2SO$ as solvent with Me_4Si as internal standard. The assignment of exchangeable protons (N*H*, O*H*) was confirmed by the addition of D_2O. Quantitative UV analyses, to determine partition coefficients, were performed using a Philips PU 8700 Series UV/visible spectrophotometer. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). Microanalyses were within ± 0.4% of theoretical values for all elements listed, unless otherwise stated. 1,1-Bis(2-methylphenyl)-1-buten-4-ol (**6c**)[15], 2-cyanoethyl acetoacetate (**7b**)[16] and 4-bromo-1,1-bis(3-methyl-2-thienyl)-1-butene[14] were synthesized according to literature procedures. 2-Methoxyethyl acetoacetate (**7e**), isopropyl 3-aminocrotonate (**9**) and all other reagents used were purchased from the Aldrich Chemical Co.

2-[4-(4-Fluorophenyl)]piperazin-1-yl]ethanol ***6d***

A solution of 1-(4-fluorophenyl)piperazine (4.5 g, 25 mmol), 2-bromoethanol (3.2 g, 25 mmol) and Et_3N (7 ml, 50.2 mmol) in acetone (50 ml) was refluxed for 24 h. The solvent was removed *in vacuo*, the residue obtained was dissolved in CH_2Cl_2 (50 ml) and washed with water (3 × 25 ml). The organic phase was dried (Na_2SO_4), the solvent was removed *in vacuo*, and the residue obtained was purified by silica gel column chromatography using CH_2Cl_2-MeOH (96:4, *v/v*) as eluent to give **6d** as a white foam (3.1 g, 55%).– ^1H NMR ($CDCl_3$): δ 6.86–7.01 (m, 4H, phenyl hydrogens), 3.68 (t, *J* = 5.4 Hz, 2H, CH_2OH), 3.15 (t, *J* = 4.9 Hz, 4H, piperazinyl H-3 and H-5), 2.87 (s, 1H, OH), 2.71 (t, *J* = 4.9 Hz, 4H, piperazinyl H-2 and

H-6), 2.64 (t, J = 5.4 Hz, 2H, CH_2CH_2N). Product **6d** was used immediately for the synthesis of **7d**.

2-Dimethylaminoethyl Acetoacetate **7a**

Freshly distilled diketene (8.4 g, 100 mmol) was added dropwise to a solution of *N,N*-dimethylethanolamine (8.9 g, 100 mmol) and Et_3N (0.5 ml, 9.2 mmol) at 60 °C with stirring. Diketene was added at a rate such that the temperature of the reaction mixture did not exceed 80 °C. After the addition was completed, the reaction was allowed to proceed at 95 °C for an additional 3 h. The reaction mixture was purified by distillation *in vacuo* to yield **7a** as a colourless liquid (14.6 g, 84%), bp 98–99 °C; IR (film): ν = 1745 cm^{-1} (CO_2), 1726 (C=O).– ^1H NMR ($CDCl_3$): δ 4.20 (t, J = 5.7 Hz, 2H, $COOCH_2$), 3.45 (s, 2H, $COCH_2COO$), 2.53 (t, J = 5.7 Hz, 2H, CH_2NMe_2), 2.23 (s, 9H, CH_3CO and NCH_3). Product **7a** was used immediately for the synthesis of **11a**.

4,4-Bis(2-methylphenyl)-3-butenyl Acetoacetate **7c**

The title compound **7c** was prepared according to the procedure used for the preparation of **7a** by reaction of **6c** (1.0 g, 4 mmol), diketene (0.44 g, 5.2 mmol) and Et_3N (0.5 ml, 9.2 mmol). The reaction product was purified by silica gel column chromatography using EtOAc-hexane (1:2, *v/v*) as eluent to afford **7c** as a yellow oil (1.2 g, 84%); IR (film): ν = 1745 cm^{-1} (CO_2), 1721 (C=O).– ^1H NMR ($CDCl_3$): δ 7.08–7.27 (m, 8H, phenyl hydrogens), 5.79 (t, J = 6.9 Hz, 1H, C=C*H*), 4.23 (t, J = 6.9 Hz, 2H, $COOCH_2$), 3.45 (s, 2H, $COCH_2COO$), 2.44 (q, J = 6.9 Hz, 2H, CH_2-CH=C), 2.29 (s, 3H, aryl-CH_3), 2.27 (s, 3H, aryl-CH_3), 2.13 (s, 3H, CH_3CO). Product **7c** was used immediately for the synthesis of **12**.

2-[4-(4-Fluorophenyl)piperazin-1-yl]ethyl Acetoacetate **7d**

The title compound **7d** was prepared according to the procedure used for the preparation of **7a** by reaction of **6d** (2.4 g, 10 mmol), diketene (0.84 g, 10 mmol) and Et_3N (0.5 ml, 9.2 mmol). The reaction product was purified by silica gel column chromatography using CH_2Cl_2-MeOH (96:4, *v/v*) as eluent to afford **7d** as a yellow oil (2.6 g, 85%); IR (film): ν =1776 cm^{-1} (CO_2), 1720 (C=O), 1229 (C-F).– ^1H NMR ($CDCl_3$): δ 6.82–6.97 (m, 4H, phenyl hydrogens), 4.30 (t, J = 5.8 Hz, 2H, $COOCH_2$), 3.47 (s, 2H, $COCH_2COO$), 3.09 (t, J = 4.9 Hz, 4H, piperazinyl H-3, H-5), 2.63–2.73 (m, 6H, $COOCH_2CH_2$ and piperazinyl H-2, H-6), 2.27 (s, 3H, CH_3CO). Product **7d** was used immediately for the synthesis of **10**.

3-[4,4-Bis(2-methylphenyl)-3-butenyl] 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate **12**.
General Method for the Synthesis of 3-Alkyl 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-aryl-3,5-pyridinedicarboxylate analogues ***10, 11a, 15a-b, 16a-b, 17a-b, 18***

A solution of 3-nitrobenzaldehyde **8c** (0.49 g, 3.24 mmol), 4,4-*bis*(2-methylphenyl)-3-butenyl acetoacetate **7c** (1.09 g, 3.24 mmol) and isopropyl 3-aminocrotonate **9** (0.46 g, 3.24 mmol) in 95% EtOH (80 ml) was refluxed for 16 h. The solvent was removed *in vacuo*, and the residue obtained was purified by silica gel column chromatography using EtOAc-hexane (1:4, *v/v*) as eluent. Recrystallization of the product from CH_2Cl_2-hexane afforded **12** as a yellow crystalline solid (1.0 g, 49%); mp 157–159 °C; IR (KBr): ν = 3353 cm^{-1} (NH), 1696, 1664 (C=O), 1532, 1359 (NO_2).– ^1H NMR ($CDCl_3$): δ 8.10 (t, J = 1.8 Hz, 1H, nitrophenyl H-2), 7.92 (d, J = 8.0 Hz, 1H, nitrophenyl H-4), 7.60 (d, J = 8.0 Hz, 1H, nitrophenyl H-6), 7.22 (t, J = 8.0 Hz, 1H, nitrophenyl H-5), 7.02–7.20 (m, 8H, *o*-tolyl hydrogens), 5.62–5.67 (m, 2H, N*H*, C*H*=C), 5.06 (s, 1H, H-4), 4.95 (septet, 1H, J = 6.2 Hz, C*H*Me_2), 4.10 (t, J = 6.6 Hz, 2H, $COOCH_2$), 2.30–2.42 (m, 8H, C-2 and C-6 CH_3 and CH_2-CH=), 2.20 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.08 (d, J = 6.2 Hz, 3H, $CHCH_3$).

Compounds **10**, **11a**, **15a–b**, **16a–b**, **17a–b** and **18** were prepared, using the same procedure used to prepare **12**, by condensation of a substituted-benzaldehyde (**8a–d**), isopropyl 3-aminocrotonate (**9**) and an acetoacetate derivative (**7a–e**) as illustrated in Scheme 2.

3-[2-[4-(4-Fluorophenyl)piperazin-1-yl]ethyl] 5-Isopropyl 4-(2,3-Dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate **10**

Product **10** was purified by silica gel column chromatography using EtOAc-hexane (1:1, *v/v*) as eluent; IR (KBr): ν = 3472 cm^{-1} (NH), 1700 (C=O).– ^1H NMR ($CDCl_3$): δ 7.33 (dd, J = 7.9, J = 1.6 Hz, 1H, dichlorophenyl H-4), 7.25 (dd, J = 7.9, J = 1.6 Hz, 1H, dichlorophenyl H-6), 7.08 (t, J = 7.9 Hz, 1H, dichlorophenyl H-5), 6.84–7.00 (m, 4H, fluorophenyl hydrogens), 5.64 (s, 1H, N*H*), 5.45 (s, 1H, H-4), 4.99 (septet, J = 6.3 Hz, 1H, C*H*Me_2), 4.10–4.29 (m, 2H, $COOCH_2$), 3.07 (br t, J = 4.6 Hz, 4H, piperazinyl H-3 and H-5), 2.50–2.64 (m, 6H, piperazinyl H-2, H-6 and CH_2CH_2N), 2.32 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.3 Hz, 3H, $CHCH_3$), 1.06 (d, J = 6.3 Hz, 3H, $CHCH_3$).

3-[2-(Dimethylamino)ethyl] 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride **11a**

The free base of **11a** was purified by silica gel column chromatography using CH_2Cl_2-MeOH (19:1, *v/v*) as eluent. A solution of this free base in EtOH (20 ml), precooled to 5 °C, was treated with a saturated solution of HCl in EtOH (2 ml). Removal of the solvent *in vacuo* and recrystallization of the solid obtained from CH_2Cl_2-hexane afforded **11a** as a yellow crystalline solid; IR (KBr): ν = 3435 cm^{-1} (NH), 1697 (C=O), 1533, 1350 (NO_2).– ^1H NMR ($CDCl_3$): δ 12.95 (br s, 1H, N^+HMe_2), 8.09 (s, 1H, aryl H-2), 8.02 (d, J = 7.9 Hz, 1H, aryl H-4), 7.64 (d, J = 7.9 Hz, 1H, aryl H-6), 7.42 (t, J = 7.9 Hz, 1H, aryl H-5), 6.33 (s, 1H, N*H*), 5.05 (s, 1H, H-4), 4.99 (septet, J = 6.3 Hz, 1H, C*H*Me_2), 4.59 (m, 2H, $COOCH_2$), 3.26 (m, 2H, $CH_2N^+Me_2$), 2.74 and 2.73 (two s, 3H each, N^+CH_3), 2.43 and 2.37 (two s, 3H each, C-2, C-6 CH_3), 1.27 (d, J = 6.3 Hz, 3H, $CHCH_3$), 1.15 (d, J = 6.3 Hz, 3H, $CHCH_3$).

3-(2-Methoxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(4-dimethylaminophenyl)-3,5-pyridinedicarboxylate **15a**

The product was purified by silica gel column chromatography using EtOAc-hexane (1:1, *v/v*) as eluent to yield **15a** as a yellow crystalline solid; IR (KBr): ν = 3351 cm^{-1} (NH), 1695, 1651 (C=O), 1111 (C-O-C).– ^1H NMR ($CDCl_3$): δ 7.16 (d, J = 8.7 Hz, 2H, aryl H-2, H-6), 6.61 (d, J = 8.7 Hz, 2H, aryl H-3, H-5), 5.56 (br s, 1H, N*H*), 4.95 (septet, J = 6.2 Hz, 1H, C*H*Me_2), 4.90 (s, 1H, H-4), 4.19 (t, J = 4.9 Hz, 2H, $COOCH_2$), 3.58 (t, J = 4.9 Hz, 2H, CH_2OMe), 3.38 (s, 3H, OCH_3), 2.89 (s, 6H, NCH_3), 2.33 and 2.32 (two s, 3H each, C-2 and C-6 CH_3), 1.25 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.15 (d, J = 6.2 Hz, 3H, $CHCH_3$).

3,5-Diisopropyl 1,4-Dihydro-2,6-dimethyl-4-(4-dimethylaminophenyl)-3,5-pyridinedicarboxylate **15b**

The reaction product was recrystallized from EtOAc-hexane; IR (KBr): ν = 3394 cm^{-1} (NH), 1691 (C=O).– ^1H NMR ($CDCl_3$): δ 7.16 (d, J = 7.8 Hz, 2H, aryl H-2, H-6), 6.62 (d, J = 7.8 Hz, 2H, aryl H-3, H-5), 5.47 (br s, 1H, N*H*), 4.97 (septet, J = 6.0 Hz, 2H, C*H*Me_2), 4.88 (s, 1H, H-4), 2.90 (s, 6H, NCH_3), 2.33 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.0 Hz, 6H, $CHCH_3$), 1.16 (d, J = 6.0 Hz, 6H, $CHCH_3$).

3-(2-Methoxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)-3,5-pyridinedicarboxylate **16a**

The product was purified by silica gel column chromatography using EtOAc-hexane (1:1, *v/v*) as eluent to afford **16a** as yellow crystals; IR (KBr): ν = 3335 cm^{-1} (NH), 1689 (C=O), 1111 (C-O-C).– ^1H NMR ($CDCl_3$) δ 6.54 (s, 2H, aryl H-2, H-6), 5.57 (br s, 1H, N*H*), 4.97–5.04 (m, 2H, C*H*Me_2 , H-4), 4.23 (t, J = 4.8 Hz, 2H, $COOCH_2$), 3.81 and 3.80 (two s, 9H total, aryl OCH_3), 3.59 (t, J = 4.8 Hz, 2H, CH_2OMe), 3.35 (s, 3H, $CH_2CH_2OCH_3$), 2.45 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.16 (d, J = 6.2 Hz, 3H, $CHCH_3$).

3,5-Diisopropyl 1,4-Dihydro-2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)-3,5-pyridinedicarboxylate **16b**

The product was purified by silica gel column chromatography using EtOAc-hexane (1:1, *v/v*) as eluent to afford **16b** as yellow crystals; IR (KBr): ν = 3361 cm^{-1} (NH), 1695 (C=O).– ^1H NMR ($CDCl_3$): δ 6.53 (s, 2H, aryl H-2, H-6), 5.53 (br s, 1H, N*H*), 4.95–5.04 (m, 3H, C*H*Me_2 and H-4), 3.81

and 3.79 (two s, 9H total, OCH_3), 2.35 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.3 Hz, 6H, $CHCH_3$), 1.18 (d, J = 6.3 Hz, 6H, $CHCH_3$).

3-(2-Methoxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate ***17a***

The product was purified by silica gel column chromatography using EtOAc-hexane (1:3, *v/v*) as eluent prior to recrystallization from EtOAc-hexane to yield **17a** as yellow crystals (69%); mp 124–125 °C (Lit. mp 125–126 °C)[28]; IR (KBr): ν = 3312 cm^{-1} (NH), 1696 (C=O), 1532, 1352 (NO_2).– ^{1}H NMR ($CDCl_3$): δ 8.14 (t, J = 1.2 Hz, 1H, aryl H-2), 8.01 (ddd, J = 9.0, J = 1.2, J = 1.2 Hz, 1H, aryl H-4), 7.67 (ddd, J = 9.0, J = 1.2, J = 1.2 Hz, 1H, aryl H-6), 7.38 (t, J = 9.0 Hz, 1H, aryl H-5), 5.68 (br s, 1H, N*H*), 5.10 (s, 1H, H-4), 4.95 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.17 (m, 2H, $COOCH_2$), 3.55 (m, 2H, CH_2OMe), 3.36 (s, 3H, OCH_3), 2.37 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.09 (d, J = 6.2 Hz, 3H, $CHCH_3$). Product **17a** was used immediately for the synthesis of **20a**.

3,5-Diisopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate ***17b***

The product was purified by silica gel column chromatography using EtOAc-hexane (1:2, *v/v*) as eluent prior to recrystallization from CH_2Cl_2-hexane to give **17b** as a yellow solid (64%), mp 130–131 °C; IR (KBr): ν = 3362 cm^{-1} (NH), 1652, 1701 (C=O), 1531, 1348 (NO_2).– ^{1}H NMR ($CDCl_3$): δ 8.14 (t, J = 1.2 Hz, 1H, aryl H-2), 8.00 (ddd, J = 8.0, J = 1.2, J = 1.2 Hz, 1H, aryl H-4), 7.65 (ddd, J = 8.0, J = 1.2, J = 1.2 Hz, 1H, aryl H-6), 7.37 (t, J = 8.0 Hz, 1H, aryl H-5), 5.68 (br s, 1H, N*H*), 5.06 (s, 1H, H-4), 4.95 (septet, J = 6.3 Hz, 2H, C*H*Me$_2$), 2.36 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.3 Hz, 6H, $CHCH_3$), 1.10 (d, J = 6.3 Hz, 6H, $CHCH_3$). Product **17b** was used immediately for the synthesis of **20b**.

3-(2-Cyanoethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate ***18***

The product was purified by silica gel column chromatography using EtOAc-hexane (1:2, *v/v*) as eluent, prior to recrystallization from CH_2Cl_2-hexane, to afford **18** as yellow crystals (26%), mp 132–136 °C; IR (KBr): ν = 3378 cm^{-1} (NH), 2254 (CN), 1647, 1696 (C=O), 1532, 1352 (NO_2).– ^{1}H NMR ($CDCl_3$): δ 8.13 (s, 1H, aryl H-2), 8.03 (d, J = 7.7 Hz, 1H, aryl H-4), 7.68 (d, J = 7.7 Hz, 1H, aryl H-6), 7.41 (t, J = 7.7 Hz, 1H, aryl H-5), 5.76 (br s, 1H, N*H*), 5.09 (s, 1H, H-4), 4.97 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.22–4.31 (m, 2H, $COOCH_2$), 2.65 (t, J = 6.1 Hz, 2H, CH_2CN), 2.40 and 2.38 (two s, 3H each, C-2 and C-6 CH_3), 1.28 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.12 (d, J = 6.2 Hz, 3H, $CHCH_3$). Product **18** was used for the synthesis of compound **19**.

Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate ***19***

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 3.4 g, 22.4 mmol) was added to a solution of **18** (3.1 g, 7.5 mmol) in MeOH (50 ml) and the reaction was allowed to proceed at 25 °C for 48 h with stirring prior to adjustment of the pH to 1 using 2N HCl. The resulting yellow solid was filtered, washed successively with water (3 × 35 ml) and ether (3 × 25 ml), and the pale yellow product **19** was dried *in vacuo* (1.77 g, 66%); mp 165–169 °C (dec); IR (KBr): ν = 2154–3846 cm^{-1} (COOH), 3364 (NH), 1699 (C=O, acid), 1679 (C=O, ester), 1528, 1349 (NO_2).– ^{1}H NMR [$(CD_3)_2SO$]: δ 11.82 (br s, 1H, COO*H*), 8.89 (s, 1H, N*H*), 7.99–8.02 (m, 2H, aryl H-4 and H-2), 7.52–7.61 (m, 2H, aryl H-6, H-5), 4.94 (s, 1H, H-4), 4.82 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 2.28 and 2.27 (two s, 3H each, C-2 and C-6 CH_3), 1.19 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.04 (d, J = 6.2 Hz, 3H, $CHCH_3$). Product **19** was used for the synthesis of compound **13**.

3-[4,4-Bis-(3-methyl-2-thienyl)-3-butenyl] 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate ***13***

A solution of **19** (1.6 g, 4.4 mmol), 4-bromo-1,1-bis(3-methyl-2-thienyl)-1-butene (1.7 g, 5.2 mmol) and K_2CO_3 (1.23 g, 8.8 mmol) in dry DMF (20 ml) was stirred at 25 °C for 120 h. HCl (6N) was then added until the pH of the solution was 1 during which unreacted **19** (0.5 g) precipitated. After filtration, the solvent was partially removed *in vacuo* to remove as much DMF as possible. The residue obtained was dissolved in ethyl acetate (20 ml) and washed with water (3 × 25 ml). After drying the organic layer (Na_2SO_4), the solvent was removed *in vacuo* and the residue obtained was eluted from a silica gel column using ethyl acetate-hexane (2:5, *v/v*) as eluent. Unreacted 4-bromo-1,1-bis(3-methyl-2-thienyl)-1-butene (0.8 g) eluted first. The product **13** eluted next as a yellow foam that was recrystallized from isopropyl ether-hexane as a light yellow crystalline solid which turned to a purple black colour on standing (0.72 g, 27%), mp 105–107 °C; IR (KBr): ν = 3376 cm^{-1} (NH), 1691 (C=O), 1528, 1349 (NO_2), 712 (CH, thiophene).– ^{1}H NMR ($CDCl_3$): δ 8.13 (t, J = 1.6 Hz, 1H, aryl H-2), 7.94 (dd, J = 7.9, J = 1.6 Hz, 1H, aryl H-4), 7.64 (d, J = 7.9 Hz, 1H, aryl H-6), 7.28 (t, J = 7.9 Hz, 1H, aryl H-5), 7.22 (d, J = 5.1 Hz, 1H, thienyl H-5), 7.06 (d, J = 5.1 Hz, 1H, thienyl H-5), 6.85 (d, J = 5.1 Hz, 1H, thienyl H-4), 6.76 (d, J = 5.1 Hz, 1H, thienyl H-4), 6.06 (s, 1H, N*H*), 5.93 (t, J = 6.8 Hz, 1H, C*H*=C), 5.08 (s, 1H, H-4), 4.95 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.14 (t, J = 6.8 Hz, 2H, $COOCH_2CH_2$), 2.44 (q, J = 6.8 Hz, 2H, CH_2-C=), 2.36 and 2.34 (two s, 3H each, C-2 and C-6 CH_3), 2.03 and 1.95 (two s, 3H each, thienyl CH_3), 1.25 (d, J = 6.3 Hz, 3H, $CHCH_3$), 1.10 (d, J = 6.3 Hz, 3H, $CHCH_3$).

3-(2-Methoxyethyl) 5-Isopropyl 4-(3-Aminophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate ***20a****, and the Related Compound* ***20b***

A solution of **17a** (0.41 g, 0.98 mmol) in EtOH (30 ml) was added to a pressure bottle containing 10% palladium-on-carbon (0.1 g). This solution and its contents was shaken under an atmosphere of hydrogen gas at 55 psi for 2 h. Filtration of the palladium catalyst, and evaporation of the solvent *in vacuo* afforded **20a** as a grey coloured oil (0.37 g, 95%); IR (film): ν = 3338 cm^{-1} (NH), 1648 (C=O).– ^{1}H NMR ($CDCl_3$): δ 6.98 (t, J = 7.7 Hz, 1H, aryl H-5), 6.70 (d, J = 7.7 Hz, 1H, aryl H-6), 6.65 (t, J = 1.9 Hz, 1H, aryl H-2), 6.46 (dd, J = 7.7, J = 1.9 Hz, 1H, aryl H-4), 5.70 (br s, 1H, dihydropyridyl N*H*), 4.94–4.98 (m, 2H, C*H*Me$_2$ and H-4), 4.10–4.26 (m, 2H, $COOCH_2$), 3.53–3.59 (m, 4H, CH_2OMe and NH_2), 3.37 (s, 3H, OCH_3), 2.31 (s, 6H, C-2 and C-6 CH_3), 1.24 (d, J = 6.3 Hz, 3H, $CHCH_3$), 1.14 (d, J = 6.3 Hz, 6H, $CHCH_3$). Product **20a** was used immediately for the synthesis of compound **14a**.

3,5-Diisopropyl 4-(3-Aminophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate ***20b***

Product **20b** which was prepared starting from **17b**, using the method described above for the synthesis of **20a** from **17a**, was isolated as a yellow foam (99%); IR (KBr): ν = 3343 cm^{-1} (NH), 1645 (C=O).– ^{1}H NMR ($CDCl_3$): δ 6.98 (t, J = 7.7 Hz, 1H, aryl H-5), 6.70 (d, J = 7.7 Hz, 1H, aryl H-6), 6.62 (t, J = 1.7 Hz, 1H, aryl H-2), 6.46 (dd, J = 7.7, J = 1.7 Hz, 1H, aryl H-4), 5.59 (s, 1H, dihydropyridyl N*H*), 4.90–5.59 (m, 3H, C*H*Me$_2$ and H-4), 3.30–3.70 (br s, 2H, NH_2), 2.31 (s, 6H, C-2 and C-6 CH_3), 1.25 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.15 (d, J = 6.2 Hz, 3H, $CHCH_3$). Product **20b** was used immediately for the synthesis of compound **14b**.

3-(2-Methoxyethyl) 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate Hydrochloride ***14a*** *and the Related Compound* ***14b***

A procedure for the reductive-methylation of primary amines reported by Kim *et al.*[27] was used for the preparation of **14a**. To a stirred solution of **20a** (2.7 g, 7.0 mmol) and 37% *w/v* formaldehyde (1.7 ml, 21.0 mmol) in MeOH (25 ml) was added a suspension of sodium cyanoborohydride (0.44 g, 7.0 mmol) and zinc chloride (0.48 g, 3.5 mmol) in MeOH (25 ml). The reaction was allowed to proceed at 25 °C for 18 h before addition of NaOH (50 ml of 0.2N). The organic solvent was removed *in vacuo* before extracting the aqueous residue with EtOAc (3 × 25 ml). The combined ethyl acetate extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue obtained was purified by silica gel column chromatography using EtOAc-hexane (1:2, *v/v*) as eluent to afford the free base of **14a** as a yellow oil. This oil was then dissolved in EtOH (50 ml) and a saturated solution of HCl in EtOH (40 ml) was added at 0 °C. This solution was stirred for 15 min, the solvent was removed *in vacuo*, and the residue obtained was recrystallized from EtOH to yield **14a** as a white crystalline solid (0.62 g, 19%); mp 209–210 °C (dec); IR (KBr): ν = 3471, 3198 cm^{-1} (NH), 1689 (C=O).– ^{1}H NMR [$(CD_3)_2SO$]: δ 8.96 (s, 1H, N*H*), 6.80–7.64 (br m, 4H, phenyl hydrogens), 4.87 (s, 1H, H-4), 4.81 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.04–4.14

(m, 2H, $COOCH_2$), 3.52 (t, J = 4.7 Hz, 2H, CH_2CH_2OMe), 3.26 (s, 3H, OCH_3), 3.01 (s, 6H, N^+CH_3), 2.27 and 2.26 (two s, 3H each, C-2 and C-6 CH_3), 1.18 (d, J = 6.2 Hz, 3H, $CHCH_3$), 1.05 (d, J = 6.2 Hz, 3H, $CHCH_3$).

3,5-Diisopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate ***14b***

The product **14b** (free base), prepared starting from **20b** using the same procedure used for the synthesis of **14a** from **20a** above, was purified by silica gel column chromatography using EtOAc-hexane (1:2, *v/v*) as eluent prior to recrystallization from CH_2Cl_2-hexane; IR (KBr): ν = 3356 cm^{-1} (NH), 1692, 1648 (C=O).– ^1H NMR ($CDCl_3$): δ 7.09 (t, J = 7.9 Hz, 1H, aryl H-5), 6.77 (br m, 1H, aryl H-6), 6.68 (br m, 1H, aryl H-2), 6.55 (br m, 1H, aryl H-4), 5.53 (br s, 1H, N*H*), 4.97 (m, 3H, two C*H*Me$_2$ and H-4), 2.91 (s, 6H, NCH_3), 2.33 (s, 6H, C-2 and C-6 CH_3), 1.25 (d, J = 6.2 Hz, 6H, $CHCH_3$), 1.16 (d, J = 6.2 Hz, 6H, $CHCH_3$).

3-[2-(Trimethylammonium)ethyl] 5-Isopropyl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate Iodide ***11b***

A solution of the free base **11a** (2.3 g, 5.4 mmol) and iodomethane (1.32 ml, 9.3 mmol) in acetone (30 ml) was refluxed for 16 h. The yellow residue which was obtained after removing the solvent was recrystallized from acetone-hexane to give **11b** as a bright yellow crystalline solid (3.4 g, 99%), mp 178–186 °C; IR (KBr): ν = 3335 cm^{-1} (NH), 1694 (C=O), 1526, 1351 (NO_2).– ^1H NMR [$(CD_3)_2SO$]: δ 8.93 (s, 1H, N*H*), 8.05 (t, J = 1.2 Hz, 1H, aryl H-2), 7.99 (dd, J = 7.9, J = 1.2 Hz, 1H, aryl H-4), 7.64 (d, J = 7.9 Hz, 1H, aryl H-6), 7.48 (t, J = 7.9 Hz, 1H, aryl H-5), 5.00 (s, 1H, H-4), 4.93 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.42–4.52 (m, 2H, $COOCH_2$), 3.66–3.76 (m, 2H, $CH_2N^+Me_3$), 3.13 (s, 9H, $N^+(CH_3)_3$), 2.39 (s, 3H, C-2 CH_3), 2.30 (s, 3H, C-6 CH_3), 1.24 (d, J = 6.3 Hz, 3H, $CHCH_3$), 1.15 (d, J = 6.3 Hz, 3H, $CHCH_3$).

Determination of Partition Coefficients (Kp)

n-Octanol-phosphate buffer partition coefficient (*K*p) values were determined using a modified procedure based on the method of Fujita *et al.*[29] *n*-Octanol (500 ml) was purified by successive washing with dilute H_2SO_4 (3 × 30 ml of 1N), NaOH (3 × 30 ml of 1N) and water (3 × 100 ml) prior to distillation *in vacuo* at 80 °C (3 mm Hg) .

n-Octanol and aqueous phosphate buffer (pH = 7.4) were mutually saturated, before use for *K*p determinations, by stirring equal volumes of each component at 25 °C for 16 h. After standing for 1 h, the two layers were separated.

Standard solutions were prepared by dissolving an accurately weight amount (7.5–10 mg) of the test compound in 5 ml of *n*-octanol, except for **11b** which was dissolved in the phosphate buffer, (solution A). Known volumes of this solution were then diluted in volumetric flasks to give five standard solutions. Each solution was then analyzed by UV spectrometry and a calibration curve was prepared by plotting absorbance versus concentration. The wavelength selected for UV analyses was determined from the UV spectrum of each test compound (λ_{max} varies from 345–360 nm). A known volume of solution A (150 µl) was then pipetted into a glass tube containing *n*-octanol (4 ml) and phosphate buffer pH = 7.4 (40 ml). The tube with its contents was then shaken for 1 h at 25 °C. After standing for 15 min, the tube was centrifuged for 10 min (3,600 rpm) to completely separate the two layers. The *n*-octanol layer was removed for UV analysis, except for **11b** where the aqueous layer was analyzed. The concentration of the test compound in the *n*-octanol layer was then determined from the calibration curve. The difference in concentration before and after partitioning gives the amount of the test compound that was partitioned in the aqueous buffer. The partition coefficient was calculated from the equation *K*p = concentration of test compound in octanol/concentration of test compound in phosphate buffer.

In Vitro Calcium Channel Antagonist Assay

The calcium channel antagonist activities were determined as the molar concentration of the test compound required to produce 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.67×10^{-7} M) Ca^{2+} dependent contractions (tonic response) of guinea pig ileum longitudinal smooth muscle (GPILSM) using the procedure reported previously[30]. The IC_{50} value (± SEM, n = 3) was determined graphically from the dose-response curve.

Subcutaneous Metrazol and Maximal Electroshock Anticonvulsant Screens

The subcutaneous metrazol (scMet) and maximal electroshock (MES) induced seizure screens were performed by the Anticonvulsant Development Program, Epilepsy Branch, NINCDS, Bethesda using the procedures previously reported[31]. Briefly, the scMet seizure threshold test was performed by administering 85 mg/kg of metrazol as a 0.5% solution in the posterior midline. Protection in this screen was defined as a failure to observe a single episode of clonic spasms of at least 5 s duration during a 30 min period following administration of the test compound. MES seizures were elicited with a 60 cycle ac of 50 mA intensity delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of electrodes. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES screen.

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