Synthesis, Biological Evaluation, Calcium Channel Antagonist Activity, and Anticonvulsant Activity of Felodipine Coupled to a Dihydropyridine−Pyridinium Salt Redox Chemical Delivery System

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3-(2-Hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**7**) was prepared using a modified Hantzsch reaction, which was then elaborated to 3-[2-[[(1-methyl-1,4-dihydropyrid-3-yl)carbonyl]oxy]ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate [**10**, felodipine−chemical delivery system (CDS)]. The equipotent 3-(2-hydroxyethyl) **7** ($IC_{50} = 3.04 \times 10^{-8}$ M) and felodipine−CDS (**10**, $IC_{50} = 3.10 \times 10^{-8}$ M) were, respectively, 2- and 21-fold less potent calcium channel antagonists than the reference drugs nimodipine ($IC_{50} = 1.49 \times 10^{-8}$ M) and felodipine ($IC_{50} = 1.45 \times 10^{-9}$ M). Compounds **7**, **10**, nimodipine, and felodipine are highly lipophilic (K_p = 236, 366, 187, and 442, respectively). 3-(2-Hydroxyethyl) **7**, felodipine−CDS (**10**), and felodipine provided protection against maximal electroshock-induced seizures in mice but were inactive in the subcutaneous metrazol anticonvulsant screen. *In vitro* incubation studies of felodipine with rat plasma and 20% brain homogenates showed felodipine was very stable in both biological media. Similar incubations of felodipine−CDS showed its rate of biotransformation followed psuedo-first-order kinetics with half-lives of 15.5 h in rat plasma and 1.3 h in 20% rat brain homgenates. *In vivo* biodistribution of felodipine and felodipine−CDS was studied. Uptake of felodipine in brain produced a peak brain concentration of 5 μg/g of brain tissue at 5 min, after which it rapidly egressed from brain resulting in undetectable levels at 60 min. Peak blood concentrations of **10** occurred at about 7 min followed by a rapid decline to a near undetectable concentration by 17 min. The pyridinium salt species **9**, resulting from oxidation of **10**, also reached peak concentrations at about 7 min but it slowly decreased to undetectable concentrations at 2 h. 3-(2-Hydroxyethyl) **7** remained at near undetectable concentrations throughout a 2 h time period. Localization of **10** in brain provided a peak concentration of 4.2 μg/g of brain tissue at 5 min and then decreased to negligible concentrations at 15 min. The concentration of oxidized pyridinium species **9** in brain remained high providing detectable concentrations up to 4 days. In contrast, the concentration of the 3-(2-hydroxyethyl) hydrolysis product **7** in brain remained at very low levels throughout the study. The slow hydrolysis rate of the pyridinium ester **9** to the 3-(2-hydroxyethyl) **7** and the rapid egression of felodipine−CDS from brain are believed to contribute to the moderate anticonvulsant activity exhibited hy the felodipine−CDS (**10**).

Introduction

The incidence of epilepsy worldwide is about 1%,[1] and some 20−30% of epileptic individuals are refractory to drugs currently available for seizure control.[2] The design and development of new drugs with lower toxicity, which act by new mechanisms of action, that are effective for seizure control in refractory epileptics continues to present a challenge.

There is convincing evidence, even though the mechanism(s) of epileptogenesis is not fully understood, that calcium influx is involved in the pathogenesis of seizures. For example, calcium is believed to be one of the mediating currents[3] involved in intrinsic burst-firing activity of neurons, and there are increased calcium deposits in brain tissue following seizure activity.[4,5] Accordingly, calcium channel antagonists (CCAs), which block the L-type calcium channel,[6] have been evaluated in a variety of seizure models.[7,8] The central nervous system (CNS) selective 1,4-dihydropyridine CCA nimodipine (**1**) provided protection against electroshock-,[9] pentylenetetrazole-,[10] and picrotoxin[11]-induced seizures.

Bodor's lipophilic [(1-methyl-1,4-dihydropyrid-3-yl)-carbonyl]oxy(amino) chemical delivery system (CDS) has been investigated extensively as a method to enhance the selective delivery of drugs to the brain.[12] After entry into brain, the CDS moiety is oxidized to a polar pyridinium species that can not egress from the brain which then undergoes ester or amide cleavage to release the active drug and trigonelline. The γ-aminobutyric acid (GABA)−CDS (**3**)[13] and diphenylhydantoin (DPH)−CDS (**4**)[14] provided improved anticonvulsant activity relative to the parent compounds GABA and DPH in animal seizure models.

The CCA felodipine (**2**) is a potent vasodilator that is used clinically as an antihypertensive agent.[15] Due to its significant selectivity toward vascular smooth muscle, relative to myocardial tissue, felodipine, unlike many other CCAs, does not exhibit cardiac side effects.[16] It was therefore of interest to couple felodipine to the Bodor CDS to evaluate its efficacy as an anticonvulsant agent. To be useful as an anticonvulsant agent, the felodipine−CDS should be eliminated rapidly from the systemic circulation to reduce smooth muscle calcium channel antagonist effects that would decrease blood

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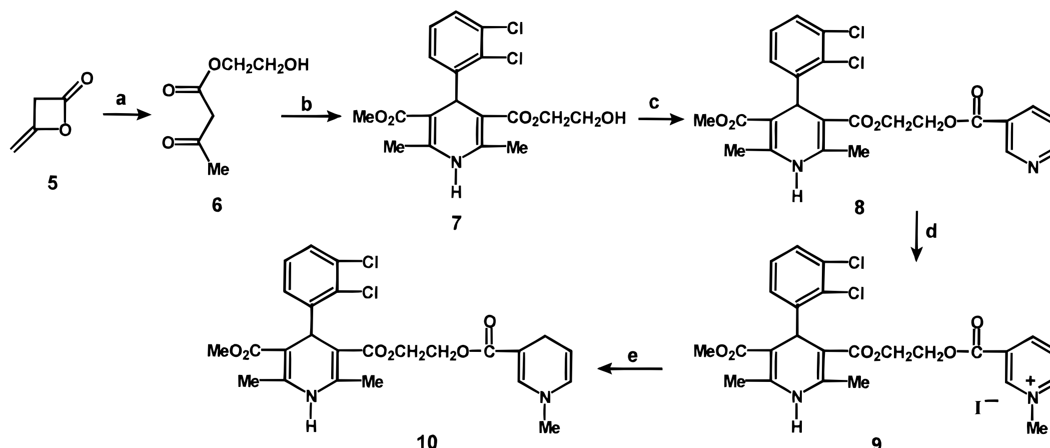
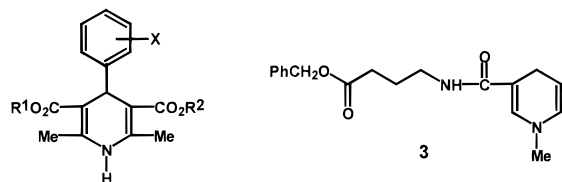
Scheme 1[a]



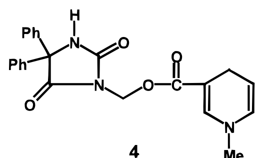
[a] Reagents: (a) $HOCH_2CH_2OH$, Et_3N; (b) 2,3-dichlorobenzaldehyde, $CH_3C(NH_2){=}CHCO_2Me$; (c) nicotinoyl chloride hydrochloride, Et_3N; (d) MeI, acetone; (e) $Na_2S_2O_4$, $NaHCO_3$, H_2O-Et_2O.

pressure. We now report the synthesis, *in vitro* calcium channel antagonist activity, anticonvulsant activity, *in vitro* stability in rat plasma and brain homogenate, *in vivo* rat plasma and brain levels, and brain biotransformation of the felodipine−CDS (**10**).



1; R^1 = *i*-Pr, R^2 = CH_2CH_2OMe, X = 3-NO_2

2; R^1 = Me, R^2 = Et, X = 2,3-Cl_2



Chemistry

The target compound 3-[2-[[(1-methyl-1,4-dihydropyrid-3-yl)carbonyl]oxy]ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**10**) was prepared using a modified Hantzsch reaction.[17] Thus, reaction of diketene (**5**) with ethylene glycol, in the presence of Et_3N used as a catalyst, yielded 2-hydroxyethyl acetoacetate (**6**; 84%). Condensation of **6** with 2,3-dichlorobenzaldehyde and methyl 3-aminocrotonate afforded 3-(2-hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**7**; 48%). Acylation of **7** with nicotinoyl chloride hydrochloride in the presence of Et_3N (used as a catalyst) yielded the corresponding 3-[2-[(3-pyridylcarbonyl)oxy]ethyl] ester **8** (90%), which on quaternization with iodomethane afforded the 3-[2-[[(1-methylpyridinium-3-yl)carbonyl]oxy]ethyl] iodide salt (**9**; 91%). Regioselective reduction of **9** using sodium dithionite under alkaline reaction conditions using an ether-degassed water two-phase solvent system afforded the target compound **10** (57%) as illustrated in Scheme 1. The two-phase solvent system served an important role in the reduction since the 1,4-dihydropyridyl product **10** is extracted into the ether layer immediately upon formation, whereas reduction of the pyridinium salt **9** by sodium dithionite occurs in the water phase.

Table 1. Calcium Channel Antagonist Activity and Partition Coefficient (K_p) Data.

compd	calcium channel antagonist act: IC_{50}, M[a]	partition coefficient (K_p)[b]
7	$3.04 \pm 0.45 \times 10^{-8}$ (3)	236
10	$3.10 \pm 0.54 \times 10^{-8}$ (3)	366
nimodipine	$1.49 \pm 0.08 \times 10^{-8}$ (3)	187
felodipine	$1.45 \pm 0.05 \times 10^{-9}$ (3)	442

[a] The molar concentration of the antagonist test compound causing a 50% decrease in the slow component, or tonic contractile response ($IC_{50} \pm$ 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 curves. The number of experiments is shown in brackets. [b] K_p = concentration of test compound in *n*-octanol/concentration of the test compound in phosphate buffer (pH 7.4).

Results and Discussion

The *in vitro* calcium channel antagonist activities of the felodipine−CDS (**10**), the 3-(2-hydroxyethyl) compound **7** which is a potential biotransformation product of **10**, and the reference drugs nimodipine (**1**)[9−11] and felodipine (**2**) 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−water partition coefficients (K_p) are summarized in Table 1. The equipotent 3-(2-hydroxyethyl) **7** ($IC_{50} = 3.04 \times 10^{-8}$ M) and felodipine−CDS (**10**, $IC_{50} = 3.10 \times 10^{-8}$ M) were 2- and 21-fold less potent calcium channel antagonists than the reference drugs nimodipine ($IC_{50} = 1.49 \times 10^{-8}$ M) and felodipine ($IC_{50} = 1.45 \times 10^{-9}$ M). These results indicate that introduction of the [(1-methyl-1,4-dihydropyrid-3-yl)carbonyl]oxy CDS moiety does not decrease activity relative to the 3-(2-hydroxyethyl) compound **7**. All four compounds, **7**, **10**, nimodipine and felodipine (K_p = 236, 366, 187, and 442, respectively) are highly lipophilic which should allow their facile passage across the blood−brain barrier (BBB).[18]

The anticonvulsant activities of 3-(2-hydroxyethyl) **7**, felodipine−CDS (**10**), nimodipine, and felodipine were determined by the U.S. National Institutes of Health, Antiepileptic Drug Development Program.[19,20] Phase 1 identification of anticonvulsant activity in mice, follow-

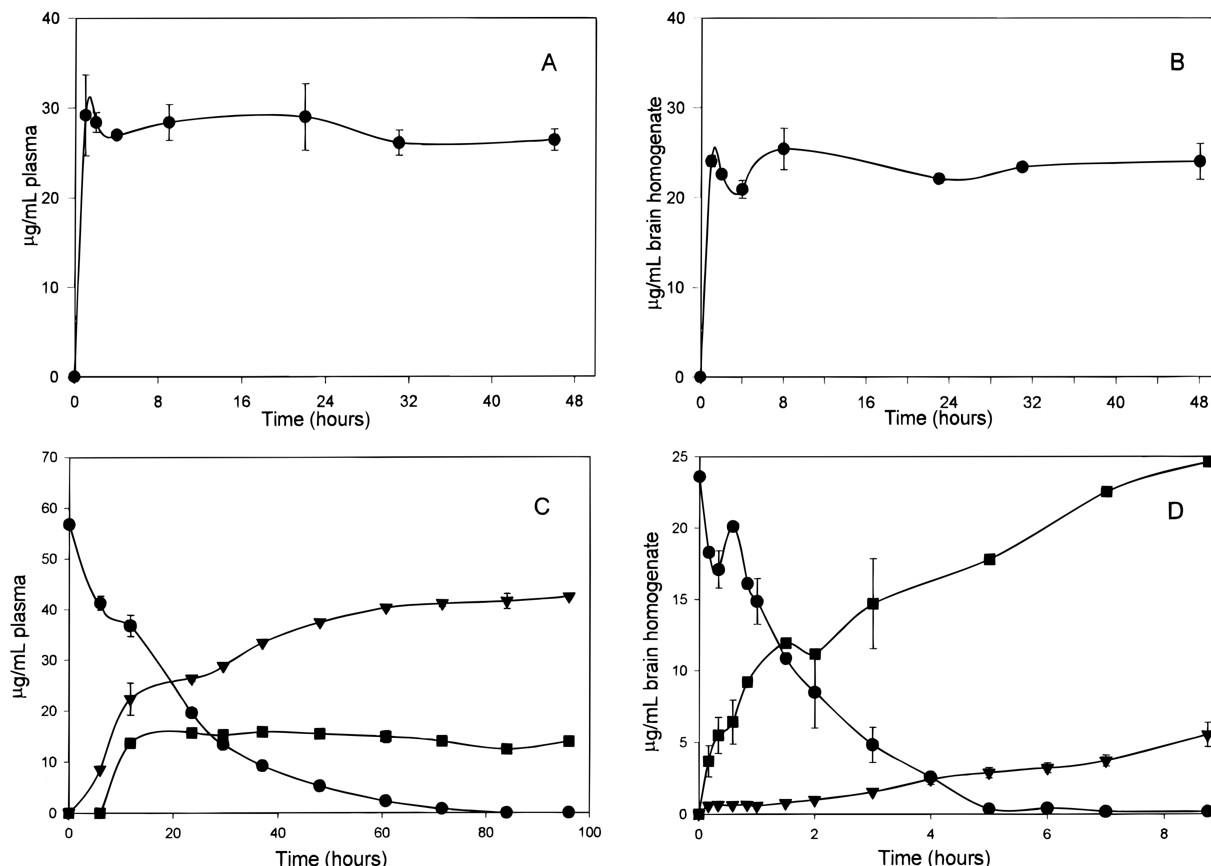


Figure 1. Panel A: concentration of felodipine in rat plasma after incubation at 37 °C. Panel B: concentration of felodipine in 20% rat brain homogenate in phosphate buffer (pH 7.4) after incubation at 37 °C. Panel C: concentration of felodipine−CDS (**10**; ●), 3-(2-hydroxyethyl) **7** (▼), and the pyridinium species **9** (■) after incubation of felodipine−CDS (**10**) with rat plasma. Panel D: concentration of felodipine−CDS (**10**; ●), 3-(2-hydroxyethyl) **7** (▼), and the pyridinium species **9** (■) after incubation of felodipine−CDS (**10**) with 20% rat brain homogenate in phosphate buffer (pH 7.4) at 37 °C.

Table 2. Anticonvulsant Activities in the Subcutaneous Metrazol (scMet) and Maximal Electroshock (MES) Screens

	MES[a]				scMet[a]			
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg	
compd	0.5 h[b]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
7	2/2	ND[c]	ND	ND	1/1	0/1	ND	ND
10	0/3	1/3	0/1	1/1	0/1	0/1	0/1	0/1
nimodipine	0/3	1/3	0/1	1/1	0/1	0/1	0/1	0/1
felodipine	2/3	2/3	1/1	ND	0/1	0/1	0/1	ND

[a] The results are expressed as the number of animals protected/the number of animals tested. The test compound was administered ip to mice using poly(ethylene glycol) or methylcellulose (0.5%, w/v) as the vehicle. [b] Time after test compound administration. [c] ND = not determined.

ing intraperitoneal injection of the test compound, was determined against subcutaneous metrazol (scMet)- and maximal electroshock (MES)-induced seizures which are models for absence or petit mal and generalized tonic clonic or grand mal epilepsy, respectively (see Table 2). None of the compounds investigated were effective in protecting mice in the scMet screen. Since many studies[7,21,22] have demonstrated that nimodipine protects against scMet-induced seizures, the inability of the compounds studied to provide protection is likely due to the fact that only one mouse was used in this phase 1 identification screen. In contrast, all compounds provided a greater degree of protection in the MES screen. Thus, felodipine provided better protection than nimodipine in the MES screen for a 100 mg/kg ip dose, a result which parallels their relative calcium channel antagonist potencies (felodipine > nimodipine). The 3-(2-hydroxyethyl) compound **7** protected 2/2 mice at 30 min postdrug administration for a 100 mg/kg ip dose, while the felodipine−CDS protected 1/3 mice (100 mg/kg ip dose) and 1/1 mice (300 mg/kg ip dose) at 4 h postdrug administration. The 3-(2-hydroxyethyl) compound exhibited toxicity in the rotorod test at 30 min following a 300 mg/kg ip dose (lethal to 4/4 mice). In contrast, the felodipine−CDS (**10**) displayed no toxicity at either 30 min (0/8) or 4 h (0/4) after a 100 mg/kg ip dose or 30 min (0/4) for a 300 mg/kg ip dose, but some toxicity (1/2) was observed at 4 h for the 300 mg/kg ip dose.

Since felodipine had a rapid onset of action [2/3 (100 mg/kg) mice were protected at 30 min postdrug administration] and the felodipine−CDS (**10**) had a slow onset of action [1/3 (100 mg/kg) and 1/1 (300 mg/kg) mice were protected at 4 h postdrug administration] in the MES screen, their *in vitro* biological stability and/or biotransformation in rat plasma and brain homogenate warranted investigation. In these studies, felodipine was very stable during incubation with rat plasma (Figure 1, panel A) and 20% rat brain homogenate in phosphate buffer, pH 7.4 (Figure 1, panel B), at 37 °C for times up to 46 h. The high recovery of unchanged felodipine from rat plasma (96%) and brain homogenate (85%) after 48 h incubations indicates the felodipine ester substituents and the 1,4-dihydropyridine ring are remarkably stable under these conditions. Similar *in vitro* incubation studies employing the felodipine−CDS (**10**) showed that

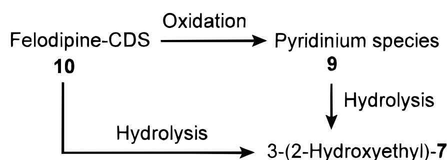


Figure 2. Two putative pathways for the biotransformation of felodipine–CDS (**10**) to the 3-(2-hydroxyethyl) hydrolysis product **7** in rat plasma and 20% brain homogenate in phosphate buffer, pH 7.4.

its rate of biotranformation followed pseudo-first-order kinetics with half-lives of 15.5 h in rat plasma (Figure 1, panel C) and 1.3 h in 20% rat brain homogenate in phosphate buffer, pH 7.4 (Figure 1, panel D). The plasma incubation data show that the concentration of the 3-(2-hydroxyethyl) compound **7** increases rapidly and continuously up to 100 h, whereas the concentration of the pyridinium species **9** increases up to about 30 h, afterwhich it decreases marginally for times up to 100 h. In contrast, incubation with 20% brain homogenate in phosphate buffer resulted in a rapid and continuous increase in the concentration of the oxidized pyridinium species **9** and a very slow increase in the concentration of the 3-(2-hydroxyethyl) ester hydrolysis product **7** for times up to 9 h. The 3-(2-hydroxyethyl) product **7** could be produced by (i) oxidation of the felodipine–CDS (**10**) to a pyridinium species (**9**) and then hydrolysis to **7** and/or (ii) hydrolysis of the felodipine–CDS directly to the 3-(2-hydroxyethyl) product **7** (see Figure 2).

The *in vivo* biodistribution of felodipine (2.5 mg/kg) and feldopine-CDS (15 mg/kg) administered to rats by tail-vein injection was investigated. Uptake of felodipine in brain was rapid, reaching a maximum brain concentration of 5.0 μg/g of brain tissue at 5 min, after which it rapidly egressed from brain resulting in undetectable levels at 60 min (Figure 3, panel A). The high lipophilicity of felodipine ($K_p = 442$) allows for both rapid entry into and egression from the brain. HPLC analysis of blood from this study showed that only one blood sample showed a trace amount (0.1 μg/mL) at 5 min after felodipine administration. No metabolites of felodipine were detected in brain tissue by HPLC analysis. Although there was no detectable level of felodipine in brain at times greater than 1 h, felodipine protected mice (2/3) in the MES-induced seizure screen at 4 h postdrug administration for a 100 mg/kg ip dose. A similar observation has been reported for the relationship between the anticonvulsant effect of nimodipine and its blood and brain levels in mice.[9]

The *in vivo* biodistribution of the felodipine–CDS in rats is summarized for blood (Figure 3, panel B) and brain (Figure 3, panel C). Peak levels of fedodipine–CDS in blood occurred at about 7 min followed by a rapid decline to near undetectable concentrations by 17 min. The pyridinium species **9** also reached peak concentrations at about 7 min, but its level decreased slowly to an undetectable concentration by 2 h. In contrast, the concentration of the 3-(2-hydroxyethyl) compound **7** remained at near undetectable concentrations throughout a 2 h time period. The felodipine-CDS is therefore rapidly cleared from the peripheral circulation which should reduce significant cardiovascular side effects. Localization of the felodipine–CDS in brain rapidly produced a peak concentration of 4.2 μg/g of brain tissue at 5 min postdrug administration. However, its concentration in brain at 15 min postdrug

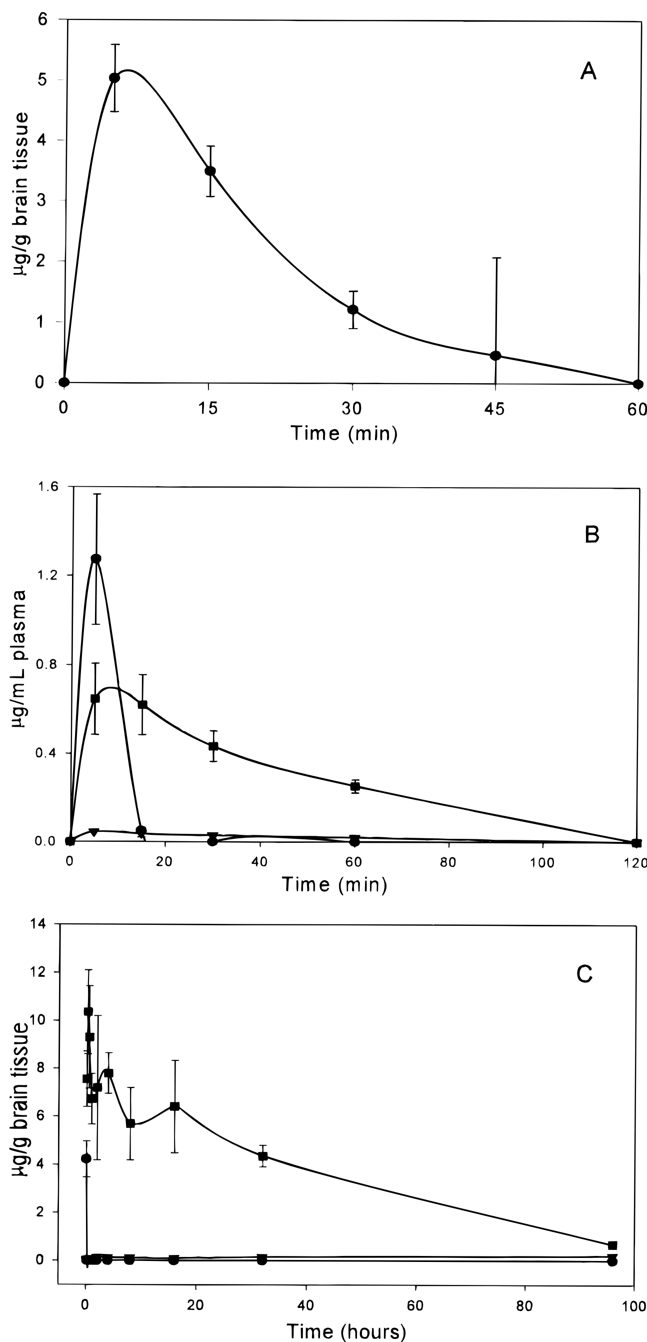


Figure 3. Panel A: concentration of felodipine in rat whole brain tissue after administration of felodipine (2.5 mg/kg tail-vein injection). Panel B: concentration in rat blood of felodipine–CDS (**10**; ●), 3-(2-hydroxyethyl) **7** (▼), and the pyridinium species **9** (■) after administration of felodipine–CDS (15 mg/kg tail-vein injection). Panel C: concentration (μg/mL) in rat whole brain tissue of felodipone-CDS (**10**; ●), 3-(2-hydroxyethyl) **7** (▼), and the pyridinium species **9** (■) after administration of felodipine–CDS (15 mg/kg tail-vein injection).

administration was negligible. The concentration of the oxidized pyridinium species **9** in brain remained high with detectable concentrations up to 4 days postdrug administration. On the other hand, the concentration of the 3-(2-hydroxyethyl) hydrolysis product **7** remained at very low concentrations throughout the study. The rapid decline in brain felodipine–CDS concentrations is likely due to its facile oxidation to the oxidized pyridinium species **9** and rapid egress, like felodipine, from the brain. The low concentration of the 3-(2-

hydroxyethyl) compound **7** in brain suggests that hydrolysis of felodipine–CDS to **7** is not a major biotransformation (in agreement with the *in vitro* incubation study, Figure 1, panel D). It is also possible that the low brain concentration of 3-(2-hydroxyethyl) **7** (K_p = 236) could be due in part to its egress from brain.

Summary

A new felodipine–CDS (**10**) has been evaluated as a potential anticonvulsant agent which provided modest protection against MES-, but not scMet-, induced seizures in mice. These results reinforce the concept that calcium is only one of several factors that are involved in seizure generation.[3] Since the 1,4-dihydropyridines investigated in this study are ineffective in the scMet screen, a role for calcium other than interaction with L-type calcium channels may be pertinent for absence seizures.[23–25] The felodipine–CDS (**10**) enters the brain readily, and it undergoes facile oxidation to a pyridinium species that is retained in brain tissues up to 4 days after drug administration. Further studies will address a method to increase the hydrolysis rate by replacement of the pyridinium ester moiety of **9** [$-CO_2CH_2CH_2O_2C$-(1-methyl-3-pyridiniumyl)] by a more esterase sensitive [$-CO_2CH_2CH_2O_2C$-CH_2O_2C-(1-methyl-3-pyridiniumyl)], or a less hindered trigonelline [$-CO_2CH_2$-CH_2O_2C-CH_2CH_2-(3-amido-1-pyridiniumyl)], moiety. These latter analogs of **9** should undergo a more facile hydrolysis to 3-(2-hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**7**) to increase its efficacy as an anticonvulsant agent.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. IR spectra were recorded using a Nicolet 5DX-FT spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer, and all assignments of exchangeable protons (N*H*, O*H*) were confirmed by addition of D_2O. Analytical HPLC studies were performed using a Water's HPLC system comprised of a Model U6K injector, Model 510 pumps, and a Model 486 variable wavelength UV detector controlled by Millennium 2010 software. Silica gel column chromatography was carried out using Merck ASTM (70–230 mesh) silica gel. Microanalyses were within ±0.4% of theoretical values for all elements listed. Diketene, 2,3-dichlorobenzaldehyde, methyl 3-aminocrotonate, and nicotinoyl chloride hydrochloride were purchased from Aldrich Chemical Co. Felodipine[26] and nimodipine[27] were prepared according to the reported procedures.

2-Hydroxyethyl Acetoacetate (6). Freshly distilled diketene (8.2 g, 97 mmol) was added dropwise with stirring to a solution of dry ethylene glycol (24 g, 387 mmol) and Et_3N (0.25 mL, 4.6 mmol) at 60 °C with stirring at a rate such that the reaction temperature did not exceed 80 °C. The reaction was allowed to proceed at 95 °C for 3 h, and then the reaction mixture was cooled to 25 °C and placed on the top of a silica gel column. Elution with *n*-hexane–EtOAc (1:2, v/v) gave a bis-*O*-substituted ethylene glycol acetoacetate (0.65 g, 2.8 mmol) which was discarded. Continued elution afforded **6** as a yellow oil (11.9 g, 84%) that was used immediately in the subsequent reaction: IR (film) 3074–3706 (OH), 1760 (CO_2), 1720 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, COC*H*$_3$), 2.90 (br s, 1H, O*H*), 3.49 (s, 2H, COC*H*$_2$CO), 3.78 (t, J = 4.6 Hz, 2H, C*H*$_2$OH), 4.24 (t, J = 4.6 Hz, 2H, CO_2C*H*$_2$).

3-(2-Hydroxyethyl) 5-Methyl 1,4-Dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (7). A solution of **6** (1.5 g, 10.3 mmol), 2,3-dichlorobenzaldehyde (1.8 g, 10.3 mmol), and methyl 3-aminocrotonate (1.18 g, 10.3 mmol) in EtOH (50 mL) was heated at reflux for 16 h. Removal of the solvent *in vacuo* gave a residue which was purified by silica gel column chromatography using *n*-hexane–EtOAc (1:1, v/v) as eluent to afford **7** as pale yellow crystals (2.0 g, 48%) after recrystallization from *n*-hexane–CH_2Cl_2: mp 118–132 °C; IR (KBr) 3336 (br, NH, OH), 1688 (CO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.03 (br s, 1H, O*H*), 2.33 and 2.36 (2 s, 3H each, C-2, C-6 *Me's*), 3.64 (s, 3H, O*Me*), 3.77 (t, J = 4.5 Hz, 2H, C*H*$_2$OH), 4.15–4.18 (m, 2H, CO_2C*H*$_2$), 5.48 (s, 1H, H-4), 5.74 (br s, 1H, N*H*), 7.11 (dd, $J_{4,5} = J_{5,6}$ = 7.8 Hz, 1H, aryl H-5), 7.28 (dd, $J_{5,6}$ = 7.8 Hz, $J_{4,6}$ = 1.4 Hz, 1H, aryl H-6), 7.33 (dd, $J_{4,5}$ = 7.8 Hz, $J_{4,6}$ = 1.4 Hz, 1H, aryl H-4). Anal. ($C_{18}H_{19}Cl_2NO_5$) C, H, N.

3-[2-[(3-Pyridylcarbonyl)oxy]ethyl] 5-Methyl 1,4-Dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (8). A solution of **7** (4.53 g, 11.3 mmol), Et_3N (3.7 g, 37 mmol), and nicotinoyl chloride hydrochloride (3.27 g, 18.4 mmol) in dry CH_2Cl_2 (50 mL) was stirred at 25 °C for 16 h. The reaction mixture was washed with H_2O (3 × 25 mL), and the organic fraction was dried (Na_2SO_4). Removal of solvent from the organic fraction *in vacuo* afforded a yellow foam, which on recrystallization from *n*-hexane–EtOAc gave **8** as a pale yellow crystalline solid (5.14 g, 90%): mp 146–148 °C; IR (KBr) 3345 (NH), 1713 (CO_2), 736 (pyridine CH) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.33 and 2.39 (2 s, 3H each, C-2, C-6 *Me's*), 3.60 (s, 3H, O*Me*), 4.27–4.31 and 4.52–4.68 (2 m, 2H each, O_2CC*H*$_2$C*H*$_2$OCO), 5.46 (s, 1H, H-4), 5.93 (s, 1H, N*H*), 7.04–7.10 (m, 2H, aryl H-5, H-6), 7.36 (dd, $J_{4,5}$ = 7.9 Hz, $J_{4,6}$ = 1.6 Hz, 1H, aryl H-4), 7.77 (dd, $J_{4,5}$ = 7.9 Hz, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-5), 8.61 (dd, $J_{4,5}$ = 7.9 Hz, $J_{2,4}$ = 1.4 Hz, 1H, pyridyl H-4), 8.86 (d, $J_{5,6}$ = 5.0 Hz, 1H, pyridyl H-6), 8.96 (d, $J_{2,4}$ = 1.4 Hz, 1H, pyridyl H-2). Anal. ($C_{24}H_{22}Cl_2N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

3-[2-[[(1-Methylpyridinium-3-yl)carbonyl]oxy]ethyl] 5-Methyl 1,4-Dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate Iodide (9). A solution of **8** (0.86 g, 1.7 mmol) and iodomethane (0.53 mL, 8.52 mmol) in dry acetone (50 mL) was refluxed for 16 h. The pale yellow solid which precipitated was filtered, washed with dry diethyl ether (3 × 15 mL), and dried *in vacuo* to afford **9** (1.0 g, 91%): mp 205–207 °C; IR (KBr) 3443 (NH), 1740, 1698, 1641 (CO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.21 and 2.26 (2 s, 3H each, C-2, C-6 *Me's*), 3.45 (s, 3H, O*Me*), 4.24–4.43 (m, 5H, N*Me*, O_2CCH$_2$C*H*$_2$-OCO), 4.51–4.55 (br m, 2H, CO_2C*H*$_2$CH$_2$OCO), 5.30 (s, 1H, H-4), 7.16 (dd, $J_{4,5} = J_{5,6}$ = 7.7 Hz, 1H, aryl H-5), 7.22–7.29 (m, 2H, aryl H-4, H-6), 8.27 (dd, $J_{4,5}$ = 8.2 Hz, $J_{5,6}$ = 6.4 Hz, 1H, pyridinium H-5), 8.73 (d, $J_{4,5}$ = 8.2 Hz, 1H, pyridinium H-4), 9.01 (s, 1H, pyridinium H-2), 9.20 (d, $J_{5,6}$ = 6.4 Hz, 1H, pyridinium H-6), 9.46 (s, 1H, N*H*). Anal. ($C_{25}H_{25}Cl_2IN_2O_6$) C, H, N.

3-[2-[[(1-Methyl-1,4-dihydropyrid-3-yl)carbonyl]oxy]ethyl] 5-Methyl 1,4-Dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (10). Diethyl ether (10 mL) was added to a solution of **9** (0.27 g, 0.421 mmol) and $NaHCO_3$ (0.18 g, 2.1 mmol) in degassed water (10 mL) with stirring under an argon atmosphere. Sodium dithionite (0.37 g, 2.1 mmol) was then added, and the reaction was allowed to proceed at 25 °C with stirring for 1 h. The organic phase was separated, washed with degassed water (3 × 10 mL), and dried (Na_2SO_4), and the solvent was removed *in vacuo*. The yellow solid obtained was recrystallized from MeOH to yield **10** as yellow crystals (0.126 g, 57%): mp 160–165 °C; IR (KBr) 3329 (NH), 1701 (CO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.22 and 2.25 (2 s, 3H each, C-2, C6 *Me's*), 2.87 (br s, 2H, dihydropyridyl H-4), 2.91 (s, 3H, N*Me*), 3.48 (s, 3H, O*Me*), 4.09–4.17 (br m, 4H, CO_2C*H*$_2$C*H*$_2$OCO), 4.67–4.72 (m, 1H, dihydropyridyl H-5), 5.31 (s, 1H, H-4), 5.83 (dd, $J_{4,5}$ = 7.9 Hz, $J_{4,6}$ = 1.4 Hz, 1H, dihydropyridyl H-6), 6.87 (s, 1H, dihydropyridyl H-2), 7.21 (dd, $J_{4,5} = J_{5,6}$ = 7.7 Hz, aryl H-5), 7.28 (dd, $J_{5,6}$ = 7.7 Hz, $J_{4,6}$ = 1.7 Hz, 1H, aryl H-6), 7.36 (dd, $J_{4,5}$ = 7.7 Hz, $J_{4,6}$ = 1.7 Hz, 1H, aryl H-4), 8.98 (s, 1H, N*H*). Anal. ($C_{25}H_{26}Cl_2N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

***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.[28] The IC_{50} value (±SEM, $n = 3$) was determined graphically from the dose–response curve.

Partition Coefficient (K_p) Determination. *n*-Octanol–phosphate buffer K_p values were determined using a modified procedure based on the method of Fujita *et al.*[29] *n*-Octanol and aqueous phosphate buffer (pH 7.4) were mutually saturated by stirring equal volumes of each component at 25 °C for 16 h, and the two layers were separated after standing for 1 h. A standard solution was prepared by dissolving the test compound (7.5–10 mg) in *n*-octanol (5 mL, solution A) which was diluted with *n*-octanol to give five solutions of the desired concentrations. These five solutions were analyzed by UV spectrometry at the λ_{max} of the test compound (345–360 nm) from which an absorbance versus concentration curve was constructed. A mixture of solution A (150 μL), *n*-octanol (4 mL), and phosphate buffer (pH 7.4, 40 mL) was shaken at 25 °C for 1 h, the mixture was allowed to stand for 15 min, and then it was centrifuged at 3600 rpm for 10 min to completely separate the two layers. The concentration of the test compound in the *n*-octanol layer was quantitated by UV spectroscopy as determined from the absorbance versus concentration curve. The amount of test compound in the aqueous phosphate buffer was calculated as the difference between the amount of test compound added minus the amount in the *n*-octanol layer. The partition coefficient was calculated from the equation: K_p = test compound concentration in *n*-octanol/(total concentration–concentration in *n*-octanol layer).

***In Vitro* Incubation Studies with Rat Plasma and Rat Brain Homogenates.** Male Sprague–Dawley rats (obtained from the University of Alberta Animal Unit), 250–300 g in weight, were sacrificed by CO_2 inhalation. Blood samples were obtained by cardiac puncture in heparinized tubes. Plasma was obtained by centrifugation of whole blood (10 mL) at 3000 rpm for 15 min, and the supernatant pale yellow plasma was removed by pipet. The whole brain was removed after incision of the skull, homogenized with phosphate buffer (pH 7.4) (1:4, w/v, brain:phosphate buffer) using a Con-Torque homogenizer (Eberback Co.), and passed through filter paper (Whatman No. 1).

Felodipine (120 μL of a 12.0 mg/5 mL solution in EtOH) was added to three sample tubes containing rat plasma (2 mL) or brain homogenate (2 mL), and the mixture was shaken in a water bath at 37 °C. At selected time intervals (0.08, 0.25, 0.5, 0.75, 1, 2, 3, 6, 22, 31, 46, 117, and 119 h), a 100 μL aliquot was withdrawn from each tube which was thoroughly mixed with ice-cold MeCN (400 μL). These solutions were stored at −20 °C in the dark prior to quantitative HPLC analysis. Similar *in vitro* incubation studies were performed using felodipine–CDS [40 μL of a standard solution of **10** (14.2 mg/5 mL of DMSO)] with either rat plasma or brain homogenate (2 mL). Aliquots (100 μL) were withdrawn at selected time intervals (6, 12, 24, 37, 48, 60, 72, 84, and 96 h for plasma incubations; 0.17, 0.3, 0.58, 0.83, 1, 2, 3, 4, 5, 6, 7, and 8 h for brain homogenate incubations), thoroughly mixed with ice-cold MeCN (400 μL), and stored at −20 °C in the dark prior to quantitative HPLC analysis.

Anticonvulsant Screens. The subcutaneous metrazol (scMet)- and maximal electroshock (MES)-induced seizure screens were performed by the Anticonvulsant Development Program, Epilepsy Branch, NINCDS, Bethesda, MD, using the procedures previously reported.[30] Briefly, the scMet seizure threshold test was performed by administering 85 mg/kg 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.

***In Vivo* Biodistribution Studies.** A 2.5 mg/kg dose of felodipine [prepared by dissolution of felodipine in DMSO:0.9%, w/v, saline (85:15, v/v) to provide a final concentration in the 14–15 mg/mL range] was administered to temporarily restrained male Sprague–Dawley rats (250–300 g in weight) by tail-vein injection. The rats were then placed in a cage with free access to food and water. Three rats were sacrificed by CO_2 inhalation at each time point (5, 15, 30, 45, 60, and 90 min) after dosing. Whole blood samples were collected by cardiac puncture into heparinized tubes. A blood aliquot (1 mL) was withdrawn from each tube and then added to a solution of MeCN–DMSO (2 mL of 94:6, v/v), this sample was stirred (1 min) and then centrifuged at 4000 rpm for 10 min, and an aliquot of the supernatant liquid (0.5 mL) was stored at −20 °C in the dark prior to quantitative HPLC analysis.

Rat whole brains were also removed from the same animals as used above, the brain tissue was washed with saline (20 mL of 0.9%, w/v), dried on a filter paper, and homogenized in a tube containing MeCN–DMSO (94:6, v/v) (1:2, w/v, brain homogenate:organic solvent). The homogenate was centrifuged at 4000 rpm for 10 min, and the supernatant liquid was transferred (0.5 mL) to a sample tube and stored at −20 °C in the dark prior to HPLC analysis.

Felodipine–CDS [**10**; prepared as a solution in DMSO:0.9%, w/v, saline (85:15, v/v) to provide a test drug concentration of 25–26 mg/mL] was administered to rats (15 mg/kg dose) by tail-vein injection. Three rats were sacrificed for each selected time point (0.08, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 96 h postdrug administration). Blood and brain samples were collected and processed as described above for similar studies described for felodipine.

HPLC Assays. 1. Felodipine. A solution of the internal standard **7** was prepared (7.5 mg/5 mL of EtOH, solution A). A stock solution of felodipine was prepared (12.0 mg/5 mL of EtOH, solution B) from which five standard solutions (C–G) were made by delivering 16, 32, 48, 64, and 80 μL, respectively, of solution B to five separate flasks each containing the internal standard (solution A). The volume of each flask was adjusted to 10 mL with EtOH, and an HPLC linear calibration curve for felodipine (correlation coefficient 0.9974) was prepared by injecting each standard solution C–G (15 μL) with MeCN–H_2O (1:1, v/v) as the mobile phase at a flow rate of 1 mL/min using a Water 3.9 × 150 mm reverse phase C-18 column with UV detection at 350 nm. The retention times of the internal standard **7** and felodipine were 2.7 and 10.0 min, respectively.

The rat plasma and brain homogenate from the *in vitro* felodipine incubation studies were analyzed by quantitative HPLC analysis. Internal standard (1 μL of solution A) was added to each sample (500 μL), the resulting solution was centrifuged at 15 000 rpm for 5 min, and an aliquot (15 μL) of the supernatant liquid was removed for HPLC analysis using the HPLC conditions used to construct the calibration curve. Concentrations were determined using Water Millennium 2010 software.

2. Compounds 7, 9, and 10. A solution of the internal standard 3-(2-methoxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**11**) was prepared (1.1 mg/5 mL of DMSO, solution A). Stock solutions containing **7** (7.5 mg), **9** (11.2 mg), and **10** (11.0 mg) were prepared by dissolution in DMSO (5 mL) and adjusting the volume to 10.0 mL with DMSO (solution B for each compound). Five standard solutions (C–G) were prepared for each compound by delivering 10, 20, 30, 40, and 50 μL of solution B to five volumetric flasks each containing solution A (100 μL), and the volume of each flask was adjusted to 5.0 mL using DMSO. HPLC calibration curves were constructed for each compound by injecting each solution C–G (15 μL) using a Water 3.9 × 150 mm reverse phase C-18 column with UV detection at 350 nm. HPLC conditions: mobile phase of MeCN–phosphate buffer (NaH_2PO_4, 0.015 M; pH 7.4) using gradient elution starting from 38% MeCN and progressing to 100% MeCN over 20 min, starting with a flow rate of 1 mL/min from start to 2 min and then changing linearly to a flow rate of 5 mL/min over 18 min. The retention times for **7**, **9**, internal standard **11**, and **10** were 5.8, 7.3, 9.8, and 10.6 min, respectively. The linear calibration curves prepared in this way had correlation coefficients of 0.9974–0.9997.

The HPLC analysis protocol used to quantitate the rat plasma and brain homogenate samples from the *in vivo* studies following tail-vein injection of **10** was similar to the procedure described for the felodipine assay, except that internal standard (10 μL of solution A above) was added to an aliquot of each sample (500 μL) prior to HPLC analysis.

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