Unique Structure−Activity Relationship for 4-Isoxazolyl-1,4-dihydropyridines

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A series of 4-isoxazolyl-1,4-dihydropyridines (IDs) were prepared and characterized, and their interaction with the calcium channel was studied by patch clamp analysis. The structure−activity relationship (SAR) that emerges is distinct from the 4-aryldihydropyridines (DHPs), and affinity increases dramatically at higher holding potentials. Thus, among the 3′-arylisoxazolyl analogues *p*-Br > *p*-Cl ≫ *p*-F, and *p*-Cl > *m*-Cl > *o*-Cl ≫ *o*-MeO. Four of the analogues were examined by single-crystal X-ray diffractometry, and all were found to adopt an O-exo conformation in the solid state. The calculated barrier to rotation, however, suggests that rotation about the juncture between the heterocyclic rings is plausible under physiological conditions. A variable-temperature NMR study confirmed the computation. With Striessnig's computational sequence homologation procedure, a working hypothesis was derived from the data that explains the unique SAR for IDs.

Introduction

Small molecules that selectively modulate ion channels have important therapeutic applications. Calcium channel blockers (CCBs), including the 4-aryl-1,4-dihydropyridines (DHP) exemplified by "nifedipine", have been used in general medical practice worldwide for the treatment of hypertension and vasospastic angina for over 2 decades.[1] Recently, controversy concerning side effects has emerged. The first was an increased incidence of myocardial infarction among patients on a regimen of "nifedipine",[2a,b] which led the National Institutes of Health to issue a warning and to the use of time release formulations. The second was a retrospective correlation of increased incidence of cancer among older patients taking calcium channel blockers.[2c,d] The current evidence on CCBs largely precludes the adverse effects of the magnitude suggested by the scare in the mid-1990s but attests to their safety and efficacy. It is also consistent with the recommendation in the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure that puts β blockers and diuretics as first-line agents for the treatment of uncomplicated hypertension, with DHPs and ACE inhibitors second.[3] Despite a rocky stretch, CCBs retain an important role in hypertension therapy and their improvement remains an important endeavor.

As a consequence, the search for analogues with novel binding properties has attracted renewed interest. Understanding the structure and function of membrane-bound ion channels remains a challenge to chemists, and the design of molecules as ligands to selectively modulate ion channels holds significant therapeutic promise. A recent milestone in this arena is the solved structure of the K^+ channel.[4] The K^+ channel in particular has several general features analogous to the calcium channel, though two major differences arise: (1) At 400 kDa, the calcium channel is much larger than the 77 kDa potassium channel, and (2) while the four motifs comprising the α1 subunit are homologous, they are not identical, complicating diffractometry. The binding site of DHPs to the L-type Ca^{2+} channel has been approached from several directions. Covalent photoaffinity labeling provided the first evidence that the DHP binding site was located on the transmembrane strands IIIS6 and IVS6 and on the S5/S6 helices. Site-directed mutagenesis and chimera studies have been used to define the binding site of the 4-aryl-1,4-dihydropyridines to the calcium channel. Site-directed mutagenesis has been approached from both the perspectives of (1) changing the residues, which then abolish DHP binding,[5] and (2) adding residues that confer DHP binding to a non-L-type channel.[6] On the basis of the latter (or constructive approach), Schleifer performed 3D-QSAR pseudoreceptor modeling based on the nine residues that are essential determinants of DHP binding and common to both antagonist and agonist binding sites. Striessnig displayed the utility of the potassium channel's crystal structure by performing *in silico* site-directed mutagenesis of the 12 critical amino acid residues and modeling interactions of those residues with a DHP to understand binding characteristics of the hypothesized binding domain. This seems to be a usable working mimic of the L-type calcium channel. We have recreated and employed Striessnig's model in order to study these interactions with 4-isoxazolyl-1,4-dihydropyridines (IDs) and interpret structure−activity relationship (SAR) results in order to optimize our picture of the Ca^{2+} channel (vide infra). Our own working hypothesis for the 4-isoxazolyl-1,4-dihydropyridine binding site,[7] developed prior to the reports of site-directed

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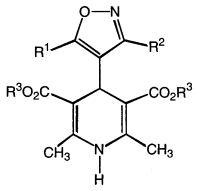
Table 1. Structures of 4-Isoxazolyl-1,4-dihydropyridines (IDs), **1**, and IC_{50} from Patch Clamp Studies



Entry	R^1	R^2	R^3	$IC_{50}(\mu M)$	n_H	Max. Inhib.	Recovery(%)	ref.
1.a.	CH_3	CH_3	Et	8.10±0.96	0.89±0.06	100	87±3	11
1.b.	CH_3	p-F-C_6H_4	CH_3	2.71±0.38	0.98±0.14	93	66±10	10
1.c.	CH_3	p-Cl-C_6H_4	CH_3	1.74±0.53	0.82±0.14	85	64±5	10
1.d.	CH_3	p-Br-C_6H_4	CH_3	0.58±0.09	1.07±0.13	88	60±6	10
1.e.	$(CH_2)_2C_6H_5$	CH_3	Et	1.79±0.07	1.95±0.18	100	46±9	7
1.f.	$(CH_2)_2$m-Br-C_6H_4	CH_3	Et	0.13±0.03	1.31±0.33	100	48±7	7
1.g.	$(CH_2)_2$m-MeO-C_6H_4	CH_3	Et	1.59±0.23	1.31±0.09	100	49±1	7
1.h.	$(CH_2)_2$-2-naphthyl	CH_3	Et	1.18±0.24	1.24±0.10	100	41±8	7
1.i.	$(CH_2)_2$-1-naphthyl	CH_3	Et	0.47±0.15	1.44±0.09	76	43±8	7
1.j.	CH_3	p-Cl-C_6H_4	Et	1.31±0.13	0.83±0.04	100	77±8	this work
1.k.	CH_3	m-Cl-C_6H_4	Et	1.98±0.44	0.91±0.08	100	65±6	this work
1.l.	CH_3	o-Cl-C_6H_4	Et	9.68±3.95	1.00±0.17	70	65±9	this work
1.m.	CH_3	2-MeO-5-Cl-C_6H_3	Et	6.86±2.28	2.09±0.38	33	82±5	this work
1.n.	CH_3	o-MeO-C_6H_4	Et	17.17±4.86	1.57±0.19	55	69±10	this work

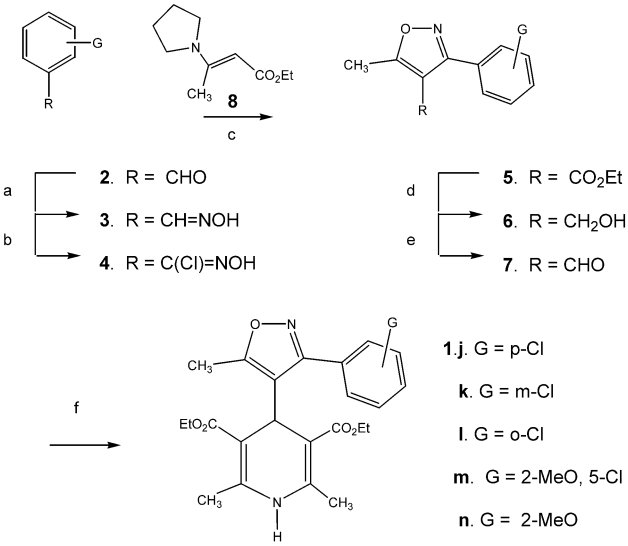
mutagenesis, is similar to the Schleifer pseudoreceptor model in many respects.[8] However, none of the models to date are entirely satisfactory, and the elucidation of how the channel conformational changes relate to function and how subtle structural features might be exploited for therapeutic benefit represents a formidable task.

We herein report the first patch clamp studies of the 4-isoxazolyl-1,4-dihydropyridines, which include a new series of 3-substituted IDs designed to further systematically delineate the SAR of this class of compounds (Table 1) and which has revealed a unique SAR for the 4-IDs. We also report an extension of the model developed by Striessnig to develop a working hypothesis of ID drug−receptor interaction, which provides a reasonable explanation of our experimental SAR observations.

Results and Discussion

A series of 4-isoxazolyl-1,4-dihydropyridines were prepared by nitrile oxide cycloaddition, and after adjustment of the isoxazole C-4 ester to a carbaldehyde functional group, Hantzsch synthesis provided new compounds **1.j**−**1.n** (Scheme 1). Products were characterized, and their interaction with the calcium channel was studied by patch clamp analysis. The α_{1C}, β_{1B}, and α_2-δ calcium channels were transfected transiently into tsa-210 cells and characterized via whole-cell patch clamp recordings, thus allowing us to work with a defined and pure population of channels. The data shown in Figure 1 were performed at a typical neuronal resting potential of −80 mV, but the affinity increased significantly in a dramatic manner at more depolarized holding potentials of −30 mV (data not shown). A representative experiment is illustrated in Figure 1 (inset). The lipophilic derivatives previously examined by radioligand assay (**1.i** and **1.f.**) were the best antagonists in this series, and overall, the radioligand binding previously studied follows a trend similar to that of the patch clamp electrophysiology reported here. The structure−activity relationship that emerges for IDs is distinct in some critical details from the 4-aryldihydropyridines. Regarding state dependence, we suggest that not only do different side groups interact differently with the varying kinetic states of the calcium channel but also within a given state (e.g., open channel block) the blocking affinity should depend on specific interactions between side groups on the drug molecule and certain residues on the channel (vide infra).

Scheme 1. Synthesis of the New 4-Isoxazolyl-1,4-dihydropyridines, **1.j**−**k**[a]



[a] (a) NH_2OH·HCl, EtOH reflux; (b) NCS, DMF, room temp; (c) Et_3N, 8, room temp; (d) $LiAlH_4$, THF 0 °C; (e) PCC, CH_2Cl_2, room temp; (f) $2CH_3C[O]CH_2CO_2Et$, aqueous NH_3, EtOH, reflux.

Among the 3′-arylisoxazolyl analogues, the larger halogens were more active in the series *p*-Br > *p*-Cl ≫ *p*-F (entries **1.b**−**1.d**). The position of a specific substituent is related to the biological activity in the progression *p*-Cl > *m*-Cl > *o*-Cl ≫ *o*-MeO (entries

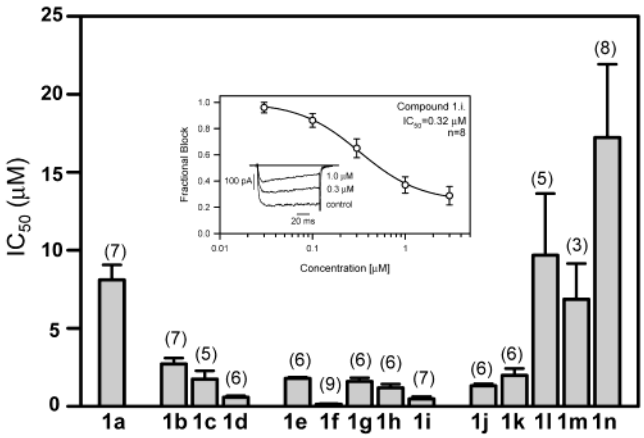


Figure 1. IC_{50} values for L-type calcium channel block obtained with the isoxazolyl DHP series. The experiments were carried out at a holding potential of −80 mV with 5 mM Ba^{2+} as the charge carrier. The IC_{50} values were obtained from fits to individual dose−response curves. The numbers in parentheses reflect the numbers of experiments. Inset: Representative dose−response curve and whole-cell current records obtained with compound **1.i**. The current traces shown were obtained in the absence of drug and after application of 30 nM, 300 nM, 1 μM, and 3 μM **1.i**. The Hill coefficient obtained from the dose−response curve was 1.32, and the IC_{50} was 320 nM. Error bars are standard errors, and eight experiments were included in the figure.

1.j−**1.n**.) (Figure 1); thus, the *ID SAR is essentially the opposite of the well-known DHP SAR for the aryl group*. We felt that this observation required some further examination. An explanation for our newly observed SAR would be more convincing if it supported both the domain interface binding sites proposed by Catterall and the computational model recently reported by Schleifer yet also encompassed the apparent fundamental differences between the typical 4-aryl-DHPs exemplified by "nifedipine" and our new synthetic analogues, specifically **1.j**−**1.n**.

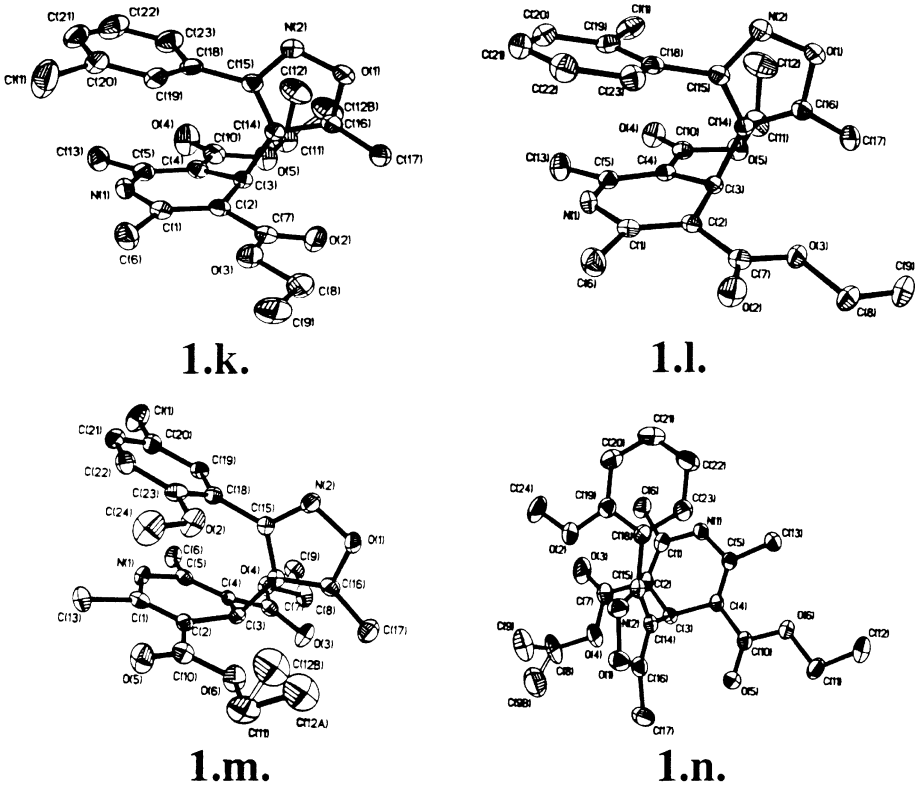
Of the analogues that were examined by single-crystal X-ray diffractometry, all were found to adopt an O-exo conformation in the solid state (Chart 1). The barrier to rotation of **1.l** was estimated using INSIGHT 2000 on a Silicon Graphics Indigo II workstation. The rotational barrier calculations were performed using the torsion force constraint in the Discover module. The bond in question was rotated through 360 intervals (1° increments) using a force constant of 10. After each rotation, the structure was subjected to 1000 steps of minimization, or until an rms value of 0.01 was reached, using the VA09A algorithm. The results of the conformational searches were examined with the Analysis module by constructing a graph of total energy vs dihedral angle. Rotation about three features were considered: (i) the barrier about the ester, (ii) the ring juncture between the heterocyclic rings, and (iii) the ring juncture between the isoxazole and the C-3 aryl moiety. These barriers were calculated to be 6.0, 15.0, and 24.6 kcal/mol, respectively. The magnitude of the barrier to rotation about the heterocyclic ring juncture would suggest that rotation would be plausible under physiological conditions. To experimentally address this point, we examined the variable-temperature (VT) NMR behavior of **1.l**. The signal for the C-2 and C-6 methyl groups of the dihydropyridine, as well as that for the C-3 and C-5 methyl groups of the ethyl esters, reaches coalescence at −53 °C (Figure 2). At temperatures below this, a nuclear Overhauser effect (NOE) is observed between the isoxazole methyl and the DHP C-4 methine and between the phenyl protons and the C-2 and C-6 methyl groups of the DHP, which unequivocally indicates that one low-temperature conformation is similar to that observed in the X-ray. However, a strong NOE is also observed between the isoxazole C-5 methyl and these same C-2 and C-6 methyl groups of the DHP. This is reasonable only if both conformers at the ring juncture are present. At room temperature, the ^{1}H NMR spectrum at 500 Mz exhibits a single signal for the C-2 and C-6 methyls groups of the dihydropyridine, which would only be plausible if the average conformation in solution at this temperature reflected free rotation about the ring juncture between the isoxazole and dihydropyridine moieties. The orthogonal relationship between isoxazole and 3′-aryl observed by crystallography, in contrast, would be expected to be maintained in solution for the ortho-substituted examples and is above the threshold that Ôki has suggested for isolation of atropoisomers.[19]

Subsequent molecular modeling of these compounds showed that a reasonable pocket exists that would accommodate a DHP or ID and provide significant electronic and hydrogen-bonding interactions. Docking calculations were carried out using INSIGHT 2000 on a SGI Indigo II workstation. Calculations utilized the cvff force field. Sequence homologation was performed in accordance with previously published work of Striessnig.[13] The closed conformation of the K^+ channel (KcsA) was used as a scaffold to mimic the Ca^{2+} channel *in silico*. The coordinates for the KcsA crystal structure was downloaded from the Brookhaven Protein Database (accession number 1BL8).[4] Alterations to the KcsA sequence are as follows (numbered according to α1C-II[18]): in IIIS5, Thr-1039, Gln-1043; in IIIS6, Tyr-1152, Ile-1153, Ile-1156, Phe-1158, Phe-1159, Met-1161; in IVS6, Tyr-1463, Met-1464, Ile-1471, Asn-1472.

We considered numerous modes of docking in the putative receptor site. We first considered the lowest energy ID O-exo conformer but reluctantly discarded this notion based on two lines of reasoning: (1) the para halogens, especially the larger ones, would appear to disrupt the N−H hydrogen bonding, a feature critical to all known DHP SAR; (2) none of the binding modes we considered for O-exo IDs provided any reasonable explanation for the lower activity of the *o*-Cl analogue **1.l**. Since our VT NMR data suggested that the O-endo conformers would be reasonably attainable at body temperature, Figure 3 shows two views of molecule **1.l** in this conformation interacting with Striessnig's domain spanning interface of the calcium channel. Note the H bond formed between Gln-1043 and the amino hydrogen, the electronic interactions between Met-1464 and the isoxazole ring, and the $\pi-\pi$ interactions between Tyr-1463 and the C-5′ aryl ring.

Scheme 2 schematically illustrates that meta and para substituents of **1.l** would easily be accommodated within the lipophilic channel as oriented, but the 3′-aryl ortho groups, on being forced from planarity from the isoxazolyl group, would interfere with either the critical Y or M residue of IVS6.[13] It is worthy of note that this

Chart 1. X-ray Crystal Structures for Four IDs[a]



[a] The isoxazolyl- group approximately bisects the 1,4-dihydropyridine ring in each case (87.2−101.8°). All were found to be in the O-exo conformation, that is, presenting the C-3′ aryl group roughly parallel to the 1,4-dihydropyridine ring. This forces the C-3′ aryl to adopt an approximately orthogonal relationship to the isoxazole (88.6−108.4°). The esters were ap/sp for **1.k**, **1.m**, and **1.n** and were sp/sp for **1.l**. Neither $\sum\rho$ nor $\sum\tau$ correlated with IC_{50}.

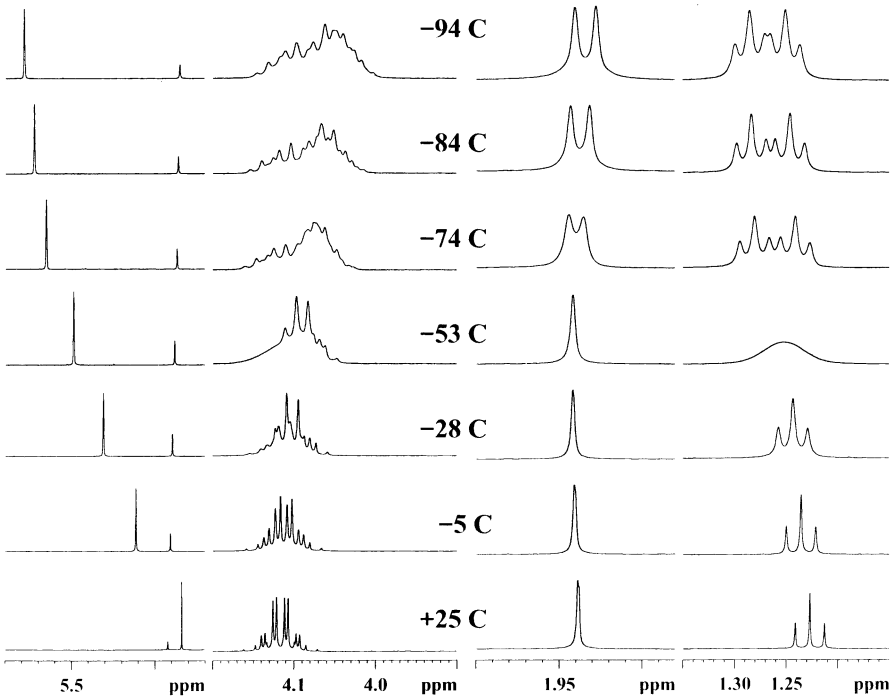


Figure 2. Variable-temperature 500 MHz NMR study of **1.l**.

picture does indeed support both the domain interface spanning picture proposed by Catterall and the computational model recently reported by Schleifer. Figure 3b indicates that in this binding mode, the 4-isoxazolyl is easily accommodated. A superimposed "nifedipine" analogue (not shown) would have ample room for ortho and meta substituents but very limited tolerance for para.

The 4-isoxazolyl-1,4-dihydropyridines reported in this work represent a potentially new series of antihyper-

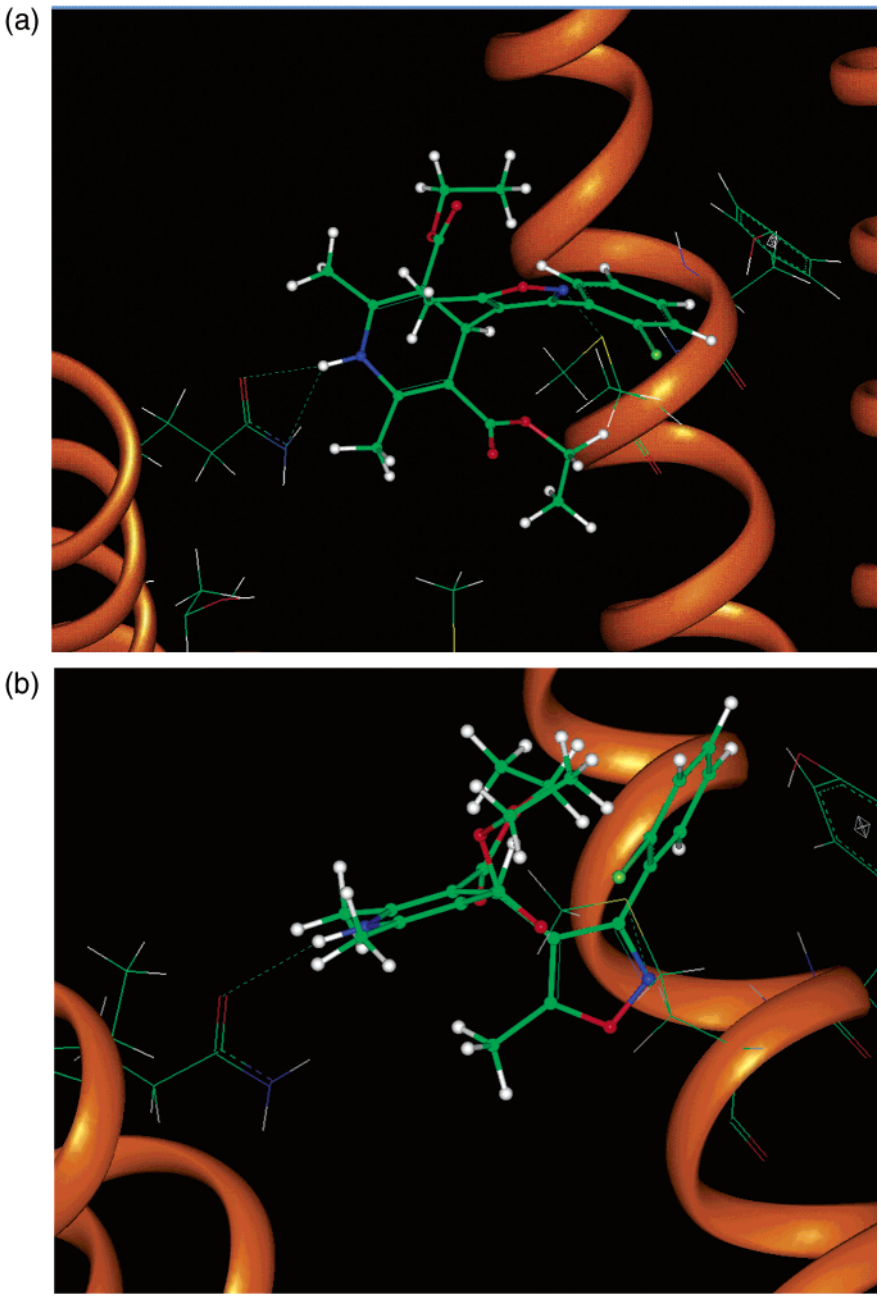


Figure 3. SGI InsightII docking study of **1.l** with the binding pocket of the calcium channel, constructed according to Striessnig (ref 13). (a) This view best illustrates the 4-aryl moiety of **1.l** flanked by M and Y. (b) The isoxazole bears no "para" group and thus is a good bioisostere for the *o*-nitrobenzene group of "nifedipine". In this view, it is clear that a superimposed para-substitiuted 4-aryl (not shown) would not easily be accommodated.

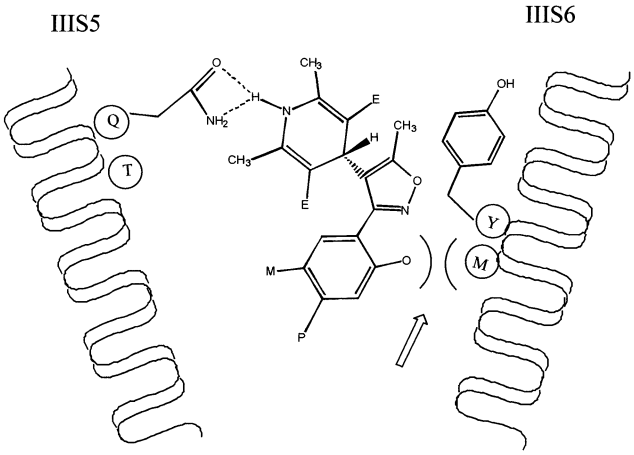
tensives given their unique structure−activity relationship, which in turn probably gives rise to their previously observed lack of negative ionotropic activity,[12] which points to their potential advantage in circumventing the serious issue of myocardial infarction associated with the 4-aryl DHPs. Furthermore, we report a working hypothesis that reconciles some aspects of known SAR of classic DHPs, with certain apparent discrepancies exhibited by our own new synthetic IDs. There are numerous important issues to be explained further; prominent among them are the role of *all* of the essential binding amino acid residues and the explanation of the formidable changes in conformation of the channel during opening and closing. We present this drug−receptor picture as a starting point for the elucidation and, hopefully, eventual better understanding of the DHP sensitive L-type calcium channel.

Our future research will explore these hypotheses, and we will report on our progress in due course.

Experimental Section

All reagents were purchased from Aldrich Chemical Co. and purified by recrystallization or distillation, as appropriate, before use. All chromatography solvents were distilled prior to use. Radial chromatography was performed on a Harrison Associates chromatotron, on silica gel, unless otherwise noted. Flash chromatography was performed using Baker flash gel, and in-house compressed air. NMR spectra were recorded on Bruker AC 200 (200 MHz ^{1}H, 50 MHz ^{13}C) and IBM Bruker AF300 (300 MHz ^{1}H, 75 MHz ^{13}C) spectrometers at ambient temperature in $CDCl_3$ unless otherwise noted. Chemical shifts

Scheme 2. Schematic Representation of the Domain Interface Model for the DHP Binding Site of Catterall[5,6] in Which the 4-Isoxazolyl-DHP Conformationally Adapts To Orient the Lipophilic Aryl Moiety toward the Intracellular Pore of the Ca^{2+} Channel[a]



[a] The orientation shown schematically below is based on the DHP binding model of Schliefer,[8] and the approximate 40° tilt of the strands is based on the potassium channel structure of MacKinnon.[4]

are reported downfield from an internal tetramethylsilane (TMS) standard. Mass spectra were obtained on a VG Micromass 70/70 HS mass spectrometer using electron impact (EI) or chemical ionization (CI) as indicated. Combustion analyses were performed by Desert Analytics, Tucson, AZ.

Synthesis of C-3 Substituted Isoxazole Dihydropyridines. Preparation of Oximes 3: 2-Chlorobenzaldehyde Oxime, 3.l. To a stirred mixture of 2-chlorobenzaldehyde, **2.l** (7.24 g, 51.5 mmol), in 13 mL of water, 13 mL of 95% ethanol, and 25 g of ice was added hydroxylamine hydrochloride (3.90 g, 56 mmol). Next, 5 mL of 50% NaOH was added, turning the mixture strongly alkaline as indicated by litmus paper. The mixture was stirred for 1 h, acidified with concentrated HC1 (to strongly acidic as indicated by litmus paper), and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with water (2 × 50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to give a white solid: yield 7.70 g (96%, crude), mp 70−74 °C. ^{1}H NMR: δ 9.10 (bs, 1H, NOH), 8.57 (s, 1H, CHNOH), 7.81−7.23 (m, 4H, aryl). Oxime obtained in this manner was sufficiently pure for use in the next step. The other oximes were prepared using the same method.

3-Chlorobenzaldehyde Oxime, 3.k. Yield 69% (crude), mp 62−66 °C (lit.[14] mp 68−69.5 °C). ^{1}H NMR: δ 9.12 (br. s, 1 H), 8.11 (s, 1 H), 7.77−7.44 (m, 4H).

2−Methoxybenzaldehyde Oxime, 3.n. Yield 100% (crude), mp 90−91.5 °C (lit.[14] mp 91.5−93 °C). ^{1} H NMR: δ 9.81 (bs, 1 H), 8.47 (s, 1 H), 7.65−6.88 (m, 4H), 3.85 (s, 3H).

3-Methoxybenzaldehyde Oxime. Yellow liquid. Yield 100% (crude). ^{1}H NMR: δ 8.14 (s, 1 H), 7.32−6.92 (m, 4H), 3.80 (s, 3H).

4-Methoxybenzaldehyde Oxime. Dark-orange liquid. Yield 100% (crude). ^{1}H NMR: δ 9.36 (bs, 1H), 8.12 (s,1 H), 7.50 (d, 2H), 6.90 (d, 2H), 3.74 (s, 3H).

Preparation of Hydroximinoyl Chlorides, **4. 2-Chlorobenzohydroximinoyl Chloride, 4.l.** To a stirred solution of the oxime (7.27 g, 46.7 mmol) in 40 mL of DMF was added one-fifth (6.24 g, 46.7 mmol) of *N*-chlorosuccinimide (NCS). After 10 min, the temperature of the solution did not rise, so a small amount of HCl(g) was added using an H_2SO_4/NaCl generator. The temperature of the solution rose to 35 °C and was kept below this by periodic ice bath cooling. The rest of the NCS was added slowly in portions, and the solution was stirred overnight. The solution was then poured into 250 mL of ice−water and extracted with ethyl ether (3 × 100 mL). The ether extracts were washed with water (2 × 100 mL), filtered, dried over anhydrous sodium sulfate, and concentrated in vacuo to give a yellow oil, 8.32 g (94%). ^{1}H NMR: δ 6.99−7.59 (m, 4H). The other hydroximinoyl chlorides were prepared in a similar manner, with stirring for varying lengths of time, and were pure enough to carry on to the next step.

3-Chlorobenzohydroximinoyl Chloride, **4.k.** Yield 90%, mp 65−67 °C (lit.[14] mp 65−67 °C). ^{1}H NMR: δ 8.87 (bs, 1 H), 7.85−7.38 (m, 4H).

In the case of the methoxy analogues, no entraining HCl(g) was added.

2-Methoxybenzohydroximinoyl Chloride, **4.n.** Yield 78%, mp 135−139 °C (lit.[14] mp 112−112.5 °C, so possibly a syn−anti mixture). ^{1} H NMR: δ 9.61 (bs, 1 H), 7.58−6.73 (m, 4H), 3.89 (s, 3H), 3.42 (s, <1 H), 3.32 (s, <1 H).

4-Methoxybenzohydroximinoyl Chloride. Yield 98%, mp 64−73 °C (lit.[30] mp 87.5−88.5 °C). ^{1} H NMR: δ 9.69 (bs, 1 H), 7.72 (d,2H), 6.91 (d,2H), 3.78 (s,3H).

Nitrile Oxide Cycloaddition. Preparation of C-3 Substituted Isoxazole Esters 5. Ethyl 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylate, 5.l. To a stirred solution of the enamine of ethyl acetoacetate (7.61 g, 41.4 mmol) and triethylamine (3.5 mL) in absolute ethanol (60 mL) at O °C under nitrogen was added a solution of 2-chlorobenzohydroximinoyl chloride (7.89 g, 41.5 mmol) in absolute ethanol (65 mL) dropwise via an addition funnel. The resulting yellow solution was warmed to room temperature and stirred overnight. The ethanol was then removed under vacuum. The residue was then taken up in ethyl ether (150 mL), and this solution was washed with 1 M HCl (75 mL) and water (3 × 50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to give a dark-orange liquid that was distilled using the Kugelrohr apparatus (0.04 mmHg, oven temp 100 °C) to give the ester as a yellow liquid, 8.27 g (75%). Esters prepared in this manner were of sufficient purity to carry on to the next step. An analytical sample was obtained by radial chromatography (2 mm silica plate; eluent, 97% hexane, 2% CH_2Cl_2, 1% EtOAc; R_f = 0.41). ^{1}H NMR: δ 7.38−7.17 (m, 4H, aryl), 4.02 (q, 2H, $CO_2CH_2CH_3$), 2.62 (s, 3H, CH_3), 0.95 (t, 3H, $CO_2CH_2CH_3$). ^{13}C NMR: δ 174.9, 161.3, 160.5, 133.89, 130.7, 130.5, 129.0, 128.5, 126.3, 109.6, 60.3, 13.4, 12.9. IR (NaCl, $CDC1_3$) cm^{-1}: 3130, 3040, 2960, 2915, 2880, 1700, 1585, 1420, 1300, 1240, 1150, 1090, 1050, 1020, 900. MS (CI) *m/z*: 266 (M + 1). Anal. Calcd for $C_{13}H_{12}ClNO_3$: C, 58.77; H, 4.5 5; N, 5.27. Found: C, 58.82; H, 4.41; N, 5.09.

The other esters were prepared in the same manner.

Ethyl 3-(3-Chlorophenyl)-5-methylisoxazole-4-carboxylate, 5.k. Yield 75%, mp 45−46 °C. ^{1}H NMR: δ 7.65−7−33 (m,4H), 4.25 (q,2H), 2.75 (s,3H), 1.25 (s,3H). ^{13}C NMR: δ 176.1, 161.6, 161.2, 133.7, 130.1, 129.7, 129.5, 129.2, 127.5, 108.3, 60.8, 13.8, 13.5. IR (NaCl, $CDC1_3$) cm^{-1}: 2980, 2910, 1715, 1595, 1565, 1435, 1310, 1250, 1145, 1105, 915. MS (El) *m/z*: 265 (M^+). Anal. Calcd for $C_{13}H_{12}ClNO_3$: C, 58.77; H, 4.55; N, 5.27. Found: C, 58.95; H, 4.49; N, 5.13.

Ethyl 3(4-Chlorophenyl)-5-methylisoxazole-4-carboxylate, 5.j. Yield 74% crude, mp 53−57 °C (lit.[15] mp 59−600 °C). ^{1}H NMR: δ 7.52 (d, 2H), 7.40 (d,2H), 4,22 (q,2H), 2,71 (s,3H), 1.22 (s,3H).

Ethyl 3-(2-Methoxyphenyl)-5-methylisoxazole-4-carboxylate, 5.n. Yield 51%, mp 70−76 °C. ^{1}H NMR: δ 7.43−7.39 (m, 2H, aryl), 7.04−6.92 (m, 2H, aryl), 4.13 (q, 2H, $CO_2CH_2CH_3$), 3.75 (s, 3H, OCH_3), 2.69 (s, 3H, CH_3), 1.09 (t, 3H, $CO_2CH_2CH_3$).

Ethyl 3-(4-Methoxyphenyl)-5-methylisoxazole-4-carboxylate. Yield 66%, mp 58−64 °C. ^{1} H NMR: δ 7.57 (dq 2H), 6.93 (dq 2H), 4.23, (qq 2H), 3.83 (s, 3H), 2.70 (s, 3H), 1.24 (t, 3H).

Preparation of C-3 Substituted Isoxaxole Alcohols 6. 3-(2-Chlorophenyl)-5-methylisoxazole-4-carbinol, 6.l. A solution of the isoxazole ester (5.31 g, 20 mmol) in 125 mL of THF (freshly distilled over Na/benzophenone) was stirred under nitrogen at room temperature in a 250 mL three-neck round-bottom flask fitted with a reflux condenser. $LiA1H_4$ (0.76 g, 20 mmol) was added in portions via a dry addition tube. The resulting mixture was stirred overnight and warmed to

room temperature. Sodium sulfate decahydrate (6.40 g, 20 mmol) and 50 mL of THF were added, and the mixture was stirred for 1 h. It was then filtered through Celite and concentrated in vacuo to give the isoxazole alcohol as a honey-like material, 3.93 g (93%). An analytical sample was obtained by radial chromatography (2 mm silica gel plate; eluent, 70% hexane, 15% CH_2C1_2, 15% EtOAc; TLC $R_f = 0.14$). ^{1}H NMR: δ 7.50−7.31 (m, 4H, aryl), 4.36 (s,2H, CH_2OH), 2.47 (s, 3H, CH_3), 2.32 (bs, 1H, OH). ^{13}C NMR: δ 168.2, 161.0, 133.3, 131.7, 130.9, 129.8, 128.3, 126.9, 114.6, 53.9, 11.3. IR (NaCI, $CDC1_3$) cm^{-1}: 3370, 3130, 3040, 2900, 2860, 1605, 1430, 1365, 1270, 1240, 1195, 1115, 1080, 1050, 1020, 1005, 895. All other isoxazole alcohols were prepared in the same manner and were sufficiently pure to carry on to the next step.

3-(3-Chlorophenyl)-5-methylisoxazole-4-carbinol, 6.k. Yield 81%, mp 63−66 °C. ^{1}H NMR: δ 7.78 (m, 1H, aryl), 7.65 (m, 1 H), 7.39−7.33 (m, 2 H), 4.48 (s, 2 H), 3.22 (bs, 1 H), 2.40 (s, 3 H). ^{13}C NMR: δ 168.9, 161.49, 134.79, 130.79, 130.19, 129.8, 128.29, 126.49, 112.99, 53.29, 11.0. IR (NaCl, $CDC1_3$) cm^{-1}: 3450, 30509, 29109, 2860, 1605, 1585, 15509 1420, 13809, 1360, 1270, 1250, 1200, 1120, 10859, 10709, 900.

3-(4-Chlorophenyl)-5-methylisoxazole-4-carbinol, 6.j. Yellow solid, 98% yield, mp 68−71 °C. (lit.[16] mp 70−71 °C). ^{1}H NMR: δ 7.73 (d, 2H), 7.41 (dq 2H), 4.52 (s, 2H), 2.47 (s, 3H).

3-(2-Methoxyphenyl)-5-methylisoxazole-4-carbinol, 6.n. Yellow oil, 87% yield. ^{1}H NMR: δ 7.47−7.36 (m, 2H, aryl), 7.06−6.98 (m, 2H, aryl), 4.30 (s, 2H, CH_2OH), 3.79 (s, 3H, OCH3), 2.67 (bs, 1H, OH), 2.45 (s, 3H, CH_3). ^{13}C NMR: δ 167.49 160.29 156.59 130.9, 120.8, 117.8, 114.69 111.1, 55.4, 53.7, 10.8. IR (NaCl, $CDC1_3$) cm^{-1}: 3390, 2935, 2915, 2860, 2815, 1610, 1590, 1570, 1500, 1450, 1430, 1280, 1250, 1200, 1170, 1150, 1095, 1040, 900.

3-(4-Methoxyphenyl)-5-methylisoxazole-4-carbinol. Yellow solid, 94% yield, mp 123−126 °C. ^{1}H NMR: δ 7.72 (dd, 2H), 6.97 (dd, 2H), 4.53 (s, 2H), 3.82 (s, 3H), 2.43 (s, 3H). ^{13}C NMR (acetone-d_6): δ 168.0, 161.9, 160.9, 129.7, 122.2, 114.2, 113.4, 54.9, 52.7, 10.2. IR (NaCl, $CDC1_3$) cm^{-1}: 3300, 2940, 2900, 1595, 1510, 1450, 1420, 1370, 1285, 1240, 1170, 1125, 1100, 1025, 900.

Preparation of C-3 Substituted Isoxazole Aldehydes 7. 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxaldehyde, 7.l. To a stirred solution of pyridinium chlorochromate (PCC) (6.90 g, 32.0 mmol, 2 equiv) and anhydrous magnesium sulfate (10 g) in 100 mL of CH_2C1_2 was added a solution of the isoxazole alcohol (3.58 g, 16.0 mmol) in 75 mL of CH_2Cl_2. Upon this addition, the color of the solution turned immediately from orange to brown. The solution was stirred for 2 h and then taken up in 200 mL of ethyl ether, filtered through a 3 in. pad of silica gel in a sintered glass funnel, and concentrated in vacuo to give a green viscous liquid that was Kugelrohr distilled (0.07 mmHg, oven temp 100−110 °C) to give a yellow waxy solid, 2.76 g (82%). An analytical sample was obtained by radial chromatography (2 mm silica gel plate; eluent, 70% hexane, 15% CH_2C1_2, 15% EtOAc; TLC $R_f = 0.17$), giving a white solid, mp 62−66 °C. ^{1} H NMR: δ 9.71 (s, 1H, CHO), 7.57−7.37 (m, 4H, aryl), 2.80 (s, 3H, CH_3). ^{13}C NMR: δ 184.9, 175.5, 160.6, 133.5, 131.7, 131.6, 129.0, 127.2, 126.6, 115.8, 13.0. IR (NaCl, $CDC1_3$) cm^{-1}: 3075, 2840, 2760, 1675, 1575, 1430, 1305, 1365, 1270, 1235, 1040, 1015, 955, 890. All other aldehydes were prepared in the same manner and were of sufficient purity to carry on to the next step.

3-(3-Chlorophenyl)-5-methylisoxazole-4-carboxaldehyde, 7.k. Tan solid (white solid after chromatography), 93% yield, mp 81−82 °C. ^{1}H NMR: δ 9.96 (s, 1 H), 7.72−7.41 (m, 4H), 2.80 (s, 3H). ^{13}C NMR: δ 183.8, 177.3, 160.9, 134.9, 130.6, 130.2, 129.0, 128.9, 127.2, 115.0, 12.9. IR (NaCl, $CDC1_3$) cm^{-1}: 3130, 3040, 2810, 2730, 1670, 1550, 1425, 1370, 1280, 900.

3-(4-Chlorophenyl)-5-methylisoxazole-4-carboxaldehyde, 7.j. Yellow solid, 50% yield, mp 94−96 °C (lit.[16] mp 95 °C). ^{1} H NMR: δ 9.85 (s, I H), 7.58 (dd, 2H), 7.37 (dd, 2H), 2.67 (s, 3H).

3-(2-Methoxyphenyl)-5-methylisoxazole-4-carboxaldehyde, 7.n. Yellow solid (white solid after chromatography), 92% yield, mp 85−86 °C. ^{1}H NMR: δ 9.66 (s, 1H, CHO), 7.52 (t, 2H, aryl), 7.06 (d, 1H, aryl), 7.00 (d, 1H, aryl), 3.81 (s, 3H, OCH_3), 2.75 (s, 3H, CH_3). ^{13}C NMR: δ 185. 6, 174.5, 160.1, 156.8, 132.0, 130.9, 121.1, 116.1, 115.7, 111.1, 55.4, 12−9. IR (NaCl, $CDC1_3$) cm^{-1}: 2900, 2820, 1685, 1590, 1510, 1470, 1440, 1385, 1295, 1270, 1250, 1185, 1165, 1110, 1050, 1025, 975, 910.

3-(4-Methoxyphenyl)-5-methylisoxazole-4-carboxaldehyde. Tan solid (white solid after chromatography), 55% yield, mp 59−63 °C. ^{1} H NMR: δ 9.96 (s, 1 H), 7.66 (d, 2H), 7.03 (d, 2H), 3.87 (s, 3H), 2.76 (s, 3H). ^{13}C NMR: δ 184.6, 176.8, 161.8, 161.4, 130.4, 119.3, 115.1, 114.4, 55.4, 13.0. IR (NaCl, $CDC1_3$) cm^{-1}: 3030, 2980, 2940, 2920, 2820, 1670, 1595, 1560, 1510, 1420, 1285, 1170, 1100, 1020, 965, 900.

Preparation of C-3 Arylisoxazolyl-1,4- dihydropyridines 1. All new isoxazole dihydropyridines were prepared in a manner similar to that described in detail below for **1.l**. For the 4-chloro, dimethyl ester **1.c**, methyl acetoacetate was used instead of ethyl acetoacetate.

Dimethyl 2,6-Dimethyl-(5-methyl-3-[*p*-chlorophenyl]-isoxazol-4-yl)-1,4-dihydropyridine-3.5-dicarboxylate. 1.c. Fluffy, pale-yellow crystals, 57% yield, mp 161−162 °C. ^{1}H NMR: δ 7.42−7.23 (m, 4H), 5.45 (bs, 1 H), 4.99 (s, 1 H), 3.48 (s, 3H), 2.39 (s, 6H, CO_2CH_3), 2.01 (s,6H). ^{13}C NMR: δ 167.6, 166.8, 162.4, 144.1, 134.8, 130.9, 130.8, 129.6, 128.0, 127.9, 119.7, 101.0, 50.9, 29.3, 19.1, 11.2. IR (NaCl, $CDC1_3$) cm^{-1}: 3420, 3125, 2970, 2930, 1675, 1600, 1460, 1420, 1290, 1270, 1205, 1180, 1130, 1090, 1040, 1020, 900. MS (FAB) *m*/*z*: 416 (M^+), 418 (M + 2). Anal. Calcd for $C_{21}H_{21}ClN_2O_5$: C, 60.51; H, 5.08; N, 6.72. Found: C, 60.55; H, 4.87; N, 6.61.

Diethyl 2,6-Dimethyl-(5-methyl-3-[*o*-chlorophenyl]isoxazol-4-yl)-l,4-dihydropyridine-3.5-dicarboxylate, 1.l. A stirred solution of the isoxazole aldehyde **7.l** (2.50 g, 11.28 mmol), ethyl acetoacetate (2.94 g, 22.56 mmol, 2 equiv), 4 mL of NH_3(aq), and 35 mL of 95% ethanol was refluxed for ca. 48 h. It was then concentrated under vacuum to give a yellow solid that was recrystallized from absolute ethanol (rinsing the crystals with cold ethyl ether) to give the dihydropyridine **1.l** as pale-yellow cubic crystals: 2.26 g (45%), mp 169−170 °C. ^{1}H NMR: δ 7.20 (m, 4H, aryl), 5.96 (bs, 1H, NH), 4.93 (s, 1H, methine), 4.11 (qd, 4H, $CO_2CH_2CH_3$), 2.58 (s, 3H, CH3), 1.87 (s, 6H, CH3), 1.24 (t, 6H, $CO_2CH_2CH_3$). ^{13}C NMR: δ 167.2, 165.29, 161.3, 145.0, 134.6, 131.7, 130.0, 129.7, 128.8, 125.6, 118.8, 99.6, 59.4, 29.1, 18.8, 14.3, 11.3. IR (NaC1, $CDC1_3$) cm^{-1}: 3320, 3090, 2980, 2930, 2900, 1650, 1450, 1380, 1325, 1300, 1275, 1210, 1170, 1110, 1055, 1025, 910. MS (EI) 444 (M^+). Anal. Calcd for $C_{23}H_{25}ClN_2O_5$: C, 62.09; H, 5.66; N, 6.36. Found: C, 62.25; H, 5.58; N, 6.30.

Diethyl 2,6-Dimethyl-(5-methyl-3-[*m*-chlorophenyl]-isoxazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylate, 1.k. Greenish cubic crystals, 39% yield, mp 160−161 °C. ^{1} H NMR: δ 7.41−7.2 7 (ms, 4 H), 5.3 6 (bs, 1 H), 5.02 (s, 1 H), 4.17−3.93 (m, 4 H), 2.5 0 (s, 3H), 1.99 (s, 6H), 1.20 (t, 6H). ^{13}C NMR: δ 167.3, 166.1, 162.5, 144.3, 133.5, 132.7, 129.6, 128.9, 128.6, 127.8, 119.5, 100.7, 59.8, 29.3, 19.1, 14.4, 11.4. IR (NaCl, $CDC1_3$) cm^{-1}: 3410, 3310, 3070, 2960, 2910, 2880,1660, 1600, 1550, 1465, 1430, 1370, 1315, 1260, 1200, 1160, 1085, 1040, 1010, 900. MS (EI) *m*/*z*: 444 (M^+) Anal. Calcd for $C_{23}H_{25}ClN_2O_5$: C, 62.09; H, 5.66; N, 6.36. Found: C, 61.99; H, 5.57; N, 6.16.

Diethyl 2,6-Dimethyl-(5-methyl-3-[*p*-chlorophenyl]isoxazol-4-yl)-1,4-dihydropyridine-3.5-dicarboxylate, 1.j. Pale-yellow crystals, 47% yield, mp 149−151 °C. ^{1}H NMR: δ 7.35 (s, 4 H), S. 4 8 (bs, 1 H), 5.0 2 (s, 1 H), 4.13−3.96 (m, 4 H), 2.4 8 (s, 3 H), 1. 9 8 (s, 6H), 1.18 (t, 6H). ^{13}C NMR: δ 166.4, 165.2, 161.6, 143.1, 133.7, 129.7, 128.4, 126.8, 118.7, 99.9, 58.8, 28.3, 18.0, 13.4, 10.4. IR (NaCl, $CDC1_3$) cm^{-1}: 3410. 3300, 3200, 3070, 2960, 2810, 1655, 1595, 1465, 1410, 1365, 1320, 1260, 1200, 1160, 1080, 1040, 1020, 900. MS (EI) *m*/*z*: 445 (M + 1), 447 (M + 3). Anal. Calcd for $C_{23}H_{25}ClN_2O_5$: C, 62.09; H, 5.66; N, 6.36. Found: C, 62.07; H, 5.62; N, 6.28.

Diethyl 2,6-Dimethyl-(5-methyl-3-[-*o*-methoxyphenyl]-isoxazol-4-yl)-1,4-dihydropyridinedicarboxylate, 1.n. Yellow crystals, 35% yield, mp 204−205 °C. ^{1}H NMR: δ 7.28 (td, 1H, aryl), 7.06 (dd, 1H, aryl), 6.87 (q, 2H, aryl), 4.92 (s, 1H,

methine), 4.87 (bs, 1H, NH), 4.17−4.03 (qd, 4H, CO_2CHCHA), 3.65 (s, 3H, OCHA), 2.54 (s, 3H, CH_3), 1.90 (s, 6H, CH_3), 1.25 (t, 6H, $CO_2CH_2QH_3$). ^{13}C NMR (300 MHz, $CDC1_3$): δ 15 167.5, 164.7, 161.2, 157.7, 144.4, 131.5, 130.2, 119.6, 119.5, 119.2, 109.9, 100.2, 59.6, 55.4, 29.4, 19.1, 14.6, 11.5. IR (NaCl, $CDC1_3$) cm^{-1}: 3390, 3250, 3035, 2930, 2880, 2850, 2780, 1640, 1440, 1340, 1275, 1220, 1180, 1140, 1070, 1025, 1000, 880. MS (CI) *m*/*z*: 441 (M + 1). MS (EI) *m*/*z*: 439 (M − 1). Anal. Calcd for $C_{24}H_{28}N_2O_6$: C, 65.44; H, 6.41; N, 6.36. Found: C, 65.58; H, 6.48; N, 6.28.

Ring Chlorination was observed when methoxyaryls were entrained with HCl(g).

5-Chloro-2-methoxybenzohydroximinoyl Chloride 4.m. To a stirred solution of the 2-methoxybenzaldehyde oxime (4.50 g, 29.8 mmol) in 25 mL of DMF was added about one-fifth of (3.98 g, 29.8 mmol) NCS. After 10 min, the temperature of the solution did not rise, so a small amount of HCl(g) from an H_2SO_4/NaCl generator was added, causing the temperature of the solution to rise to 40 °C. The rest of the NCS was added in portions, with intermittant ice bath cooling such that the temperature of the solution was kept below 35 °C. The solution was stirred overnight, then poured into 300 mL of ice−water and extracted with ethyl ether (2 × 100 mL). The combined ether extracts were washed with water (3 × 50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give a yellow solid, 4.38 g (78%), mp 135−139 °C. ^{1}H NMR: δ 9.61 (bs, 1H, NOH), 7.58−6.73 (ms, 3H, aryl), 3.89 (s, 3H, OCH3), 3.42 (s, <1H), 3.32 (s, <1H) (likely a syn−anti mixture).

Ethyl 3-(5-Chloro-2-methoxyphenyl)-5-methylisoxazole-4-carboxylate, 5.m. To a stirred solution of the enamine of ethyl acetoacetate (3.80 g, 20.75 mmol) and 2 mL of triethylamine in 30 mL of absolute ethanol was added a solution of the hydroximinoyl chloride **4.m** (3.85 g, 20.75 mmol) in 30 mL of absolute ethanol dropwise via an addition funnel, with stirring under nitrogen at 0 °C. The orange-brown solution was warmed to room temperature and stirred, followed by reflux for approximately 20 h. It was then concentrated under vacuum, taken up in 100 mL of ethyl ether, washed with 1 M HCI (1 × 75 mL) and water (3 × 50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give a brown semisolid residue that was Kugelrohr distilled to give a light-yellow solid: 3.48 g (64%), mp 79−81 °C. An analytical sample was obtained by radial chromatography (2 mm silica gel plate; eluent, 70% hexane, 15% CH_2C1_2, 15% EtOAc), giving a white solid, mp 88 °C. ^{1} H NMR: δ 7.29 (dd, 1H, aryl), 7.09 (d, 1H, aryl), 6.80 (d, 1H, aryl), 4.15 (qd, 2H, $CO_2CH_2CH_3$), 3.75 (s, 3H, OCH_3), 2.71 (s, 3H, CH3), 1.12 (t, 3H, $CO_2CH_2CH_3$). ^{13}C NMR: δ 174.4, 161.9, 159.1, 156.2, 130.7, 130.1, 125.3, 119.9, 111.8, 110.1, 60.4, 55.7, 13.9, 13.0. IR (NaCl, $CDC1_3$) cm^{-1}: 2960, 2910, 2890, 2820, 1700, 1585, 1490, 1435, 1370, 1275, 1235, 1120, 1090, 1025, 900. MS (EI) *m*/*z*: 295 (M^+), 297 (M + 2). Anal. Calcd for $C_{14}H_{15}NO_4$: C, 56.86; H, 4.77; N, 4.74. Found: C, 56.66; H, 4.66; N, 4.62.

3-(5-Chloro-2-methoxyphenyl)-5-methylisoxazole-4-carbinol, 6.m. To a stirred solution of the isoxazole ester **5.m** (3.29 g, 12.59 mmol) in THF (40 mL, freshly distilled over Na/benzophenone) under nitrogen at 0 °C was added $LiAlH_4$ (500 Mg, 13−18 mmol) in portions via a dry addition funnel. The resulting mixture was warmed to room temperature and stirred overnight. Sodium sulfate decahydrate (5.16 g, 16 mmol) and 40 mL of THF were added, and the mixture was stirred for 2 h. It was then filtered through Celite and concentrated under vacuum to give a thick orange oil, 2.40 g (87%). An analytical sample was obtained by radial chromatography (2 mm silica gel plate; eluent, 90% hexane, 5% CH_2C1_2, 5% EtOAc), giving a yellow liquid. ^{1}H NMR: δ 7.42−7.36 (m, 2H, aryl), 6.97−6.92 (m, 1H, aryl), 4.33 (s, 2H, CH_2OH), 3.81 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3), 2.36 (bs, 1H, OH). ^{13}C NMR: δ 168.0, 159.3, 155.4, 130.9, 130.7, 126.0, 119.6, 114.7, 112.6, 59.1, 53.9, 11.0. IR (NaCl, $CDC1_3$) cm^{-1}: 3360, 3050, 2920, 2820, 1610, 1490, 1445, 1425, 1250, 1235, 1110, 1010, 900.

3-(5-Chloro-2-methoxyphenyl)-5-methylisoxazole-4-carboxaldehyde, 7.m. To a stirred solution of PCC (3.93 g, 18.24 mmol, 2 equiv) and anhydrous magnesium sulfate (15 g) in CH_2C1_2 (100 mL) was added a solution of the isoxazole alcohol **6.m** (2.00 g, 9.12 mmol) in CH_2C1_2 (40 mL). Upon this addition, the solution turned immediately from orange to brown. The mixture was stirred for 2 h and then taken up in ethyl ether (250 mL), filtered through a 3 in. silica gel pad in a sintered glass funnel, and concentrated under vacuum to give a tan solid, 1.71 g (86%). An analytical sample was obtained by radial chromatography (2 mm silica gel plate; eluent, 98% hexane, 2% EtOAc), giving a white solid, mp 124−125 °C. ^{1}H NMR: δ 9.66 (s, 1H, C*H*O), 7.51−6.95 (m, 3H, aryl), 3.81 (s, 3H, OCH_3), 2.76 (s, 3H, CH_3). ^{13}C NMR: δ 184.0, 174.0, 158.0, 154.6, 130.7, 129.6, 125.1, 116.8, 114.8, 111.5, 54.9, 12.0. IR (NaCl, $CDC1_3$) cm^{-1}: 3000, 2950, 2920, 2820, 2725, 1675, 1490, 1450, 1395, 1365, 1270, 1235, 1170, 1110, 1015, 900.

Diethyl 2,6-Dimethyl-(5-methyl-3-(2′methoxy-5′-chloro)phenylisoxazol-4-yl)-1,4-dihydropyridine-3.5-dicarboxylate, 1.m. A stirred solution of the isoxazole aldehyde **7.m** (1.50 g, 6.91 mmol), ethyl acetoacetate (1.80 g, 13.82 mmol, 2 equiv), and NH_3(aq) (3 mL) in 20 mL of 95% ethanol was refluxed for approximately 48 h, upon which time crystallization began to take place. The ethanol was then removed under vacuum, and the solid was recrystallized from absolute ethanol to give a yellow-green powder: 1.18 g (3 9%), mp 248−250 °C. ^{1} H NMR: δ 7.32−6.78 (m, 3H, aryl), 4.93 (s, 1H, methine), 4.71 (br. s, 1H, N*H*), 4.11 (qd, 4H, $CO_2CH_2CH_3$), 3.66 (s, 3H, OCH_3), 2.5 4 (s, 3H, CH_3), 1.99 (s, 6H, CH_3), 1.25 (t, 6H, $CO_2CH_2CH_3$). MS (EI) *m*/*z*: 475 (M^+), 477 (M + 2).

Transient Transfection of Calcium Channel Subunits into tsa-201 Cells. Human embryonic kidney tsa-201 cells were grown in standard DMEM medium, supplemented with 10% fetal bovine serum and 0.5 mg/mL penicillin streptomycin (5% CO_2, 37 °C). Cells were grown to 85% confluency, split with trypsin−EDTA and plated on round glass coverslips at 10% confluency 12 h before transfection. Just prior to transfection, the medium was replaced with fresh DMEM, and a standard calcium phosphate protocol was used to transiently transfect the cells with cDNA constructs encoding for calcium channel α_{1C}, β_{1b}, and α_2-δ subunits and green fluorescent protein as an expression marker (7, 7, 7, and 4 μg, respectively). After 12 h, cells were washed with fresh medium, recover for 12 h and then incubated at 28 °C in 5% CO_2 for 2−4 days prior to recording.

Electrophysiology. Immediately prior to recording, individual coverslips were transferred to a 3.5 cm polystyrene culture dish containing external recording solution comprising 5 mM $BaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 40 mM TEACl, 10 mM glucose, and 87 mM CsCl (pH 7.2). Patch pipets (Sutter borosilicate glass, BF150-86-15) were pulled using a Sutter P-87 microelectrode puller, fire-polished using a Narashige microforge, and showed a typical resistance of about 3−4 MΩ. Recording pipets were filled with 108 mM CsMS, 4 mM $MgCl_2$, 9 mM EGTA, and 9 mM HEPES (pH 7.2). Cells were selected on the basis of their GFP expression as visualized by UV-induced fluorescence. Seals were formed directly in the external recording solution, and after seal rupture, cells were dialyzed for 5−10 min prior to recording. Whole-cell patch clamp recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) linked to a personal computer equipped with pCLAMP v 6.0. Series resistance was compensated by 85% to minimize voltage errors. To assess the DHP block of noninactivated channels, currents were typically elicited from a holding potential of −80 mV to various test potentials using Clampex software (Axon Instruments). To assess inactivated channel block, cells were held at a more depolarized voltage (−30 mV) or steady-state inactivation curves were recorded in the presence and absence of the blockers. Drugs were dissolved in DMSO at 10 mM stock concentrations and diluted into the external recording solution. The drug-containing recording solutions were perfused directly into the vicinity of the cells using a gravity-driven home-built

microperfusion system. Data were usually filtered at 1 kHz and recorded directly into a personal computer.

Data Analysis. Data are analyzed using Clampfit (Axon Instruments) and Sigmaplot 4.0 (Jandel Scientific). Steady-state inactivation curves were fitted with the Boltzman equation

$$I_{peak}\ (\text{normalized}) = \frac{1}{1 + \exp(\frac{V - V_h}{25.6})z}$$

where V and V_h are respectively the conditioning and the half-inactivation potential and z is a slope factor. Current−voltage relations were fitted according to the equation

$$I_{peak} = (V - E_{rev})G\left(\frac{1}{1 + \exp\left(\frac{V_a - V}{S}\right)}\right)$$

where E_{rev} is the reversal potential, V_a is the half-activation potential, G is the maximum slope conductance, and S is a slope factor that is inversely proportional to the effective gating charge.

NMR. The ^{1}H and ^{13}C NMR high-resolution and solid-state spectra were obtained with a Bruker DRX500 spectrometer. The signal assignments were performed on the basis of a series of 2D experiments with z-gradient selection: ^{1}H−^{1}H DQF COSY, ^{1}H−^{13}C HMQC, and ^{1}H−^{13}C HMBC.[17] The low-temperature NOE experiments were performed in the rotating frame (2D ROESY) with a mixing time of 250 ms.[17] The solid-state ^{13}C spectra were obtained under cross-polarization magic angle spinning (CP-MAS) conditions with a Bruker 5 mm CP-MAS probe. The spinning rates were 8 and 14.5 kHz. These spectra were referenced to the external secondary standard (solid adamantane, 38.6 and 28.8 ppm from TMS).

X- ray Crystallography. Data were collected on a Siemens SMART CCD (charge-coupled device) diffractometer equipped with an LT-2 low-temperature apparatus operating at 193 K. A suitable crystal was chosen and mounted on a glass fiber. Data were measured using ω scans of 0.3° per frame for 10 s such that a hemisphere was collected. A total of 1271 frames were collected with a final resolution of 0.90 Å. The first 50 frames were re-collected at the end of the collection to monitor and correct for decay. Cell parameters were retrieved using SMART software and were refined using SAINT software on all reflections. Data reduction was performed using the using SAINT software, which corrects for Lp and decay. Absorption corrections were applied using XEMP supplied by Siemens' SHEXTL-PC software.

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Supporting Information Available: Low-temperature NOE of **1.l**, X-ray crystallographic files for **1.k**, **1.l**, and **1.m** and **1.n**, including crystal data and structure refinement, atomic coordinates, and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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