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Brief Article

Stereochemical Requirements for the Mineralocorticoid Receptor Antagonist Activity of Dihydropyridines

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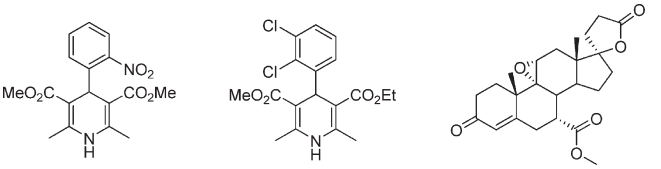
A number of known 1,4-dihydropyridine CCBs were identified as having comparable potency to the steroidal MR antagonist eplerenone. Chiral resolution of mebudipine revealed that MR and CCB activity reside in opposite enantiomers. Small molecule X-ray crystal structures showed that the C4 stereochemistry of optimized selective MR analogues, e.g. **5**, is consistent with MR-active mebudipine. Molecular modeling supports a binding pose consistent with that previously proposed for DHP diesters.

Introduction

It is estimated that almost one in three adults in the U.S. has high blood pressure (BP[a]), putting them at a markedly increased risk of major cardiovascular and renal diseases and shortened life expectancy.[1] Several classes of antihypertensive drugs have been developed; these include diuretics, calcium channel-blockers (CCB), and drugs that target the renin−angiotensin−aldosterone system, including rennin inhibitors, angiotensin converting enzyme inhibitors (ACEi), and angiotensin receptor blockers (ARBs).[2] Aldosterone is a steroid hormone that mediates sodium reabsorption by binding to the mineralocorticoid receptor (MR, NR3C2) a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Abnormal activation of the MR by elevated levels of aldosterone and salt imbalance cause hypertension and other detrimental effects to the cardiovascular system such as heart failure and myocardial fibrosis.[3] Two approaches have been developed to treat this abnormal activation of the MR. One approach involves lowering elevated aldosterone levels using aldosterone synthase inhibitors.[4] Alternatively MR antagonists, such as spironolactone and more recently eplerenone **1**, block MR activation, which results in lowering of blood pressure in hypertensive patients and improves survival in heart failure patients.[5] However, steroidal MR antagonists in general present issues of complex chemical synthesis, undesirable physical properties, and poor selectivity versus other steroid hormone receptors. The latter results in unwanted clinical side effects, for example, gynocomastia in male patients due to spironolactone's poor selectivity against the androgen receptor.[6] Thus, there has been a keen interest in discovering novel classes of nonsteroidal selective MR antagonists in recent years.[7] We previously reported that during screening for MR antagonists a number of 1,4-dihydropyridine (DHP) CCBs were found to inhibit aldosterone-induced activation of a luciferase reporter driven by MR ligand binding domain (LBD).[8] 4-Aryl-1,4-dihydropyridines have also been claimed as inhibiting [^{3}H]aldosterone binding to MR at 10 μM, however no data was disclosed regarding the functional activity of this series.[9]



Nifedipine Felodipine Eplerenone, **1**

Figure 1. DHP diesters with MR antagonist activity comparable to eplerenone.

1,4-DHP CCBs act at the L type voltage dependent calcium channel and are potent peripheral vasodilators which have been extensively studied since their introduction almost 30 years ago.[10] Although the first DHP CCBs developed (e.g., nifedipine, Figure 1) were achiral molecules, chiral DHPs with nonidentical ester groups in the 3- and 5-positions were later developed in search of more potent and longer acting vasodilators (e.g., felodipine, mebudipine **2**) although they are marketed as racemic mixtures. The absolute configuration at the C4 position of enantiomerically pure 1,4-DHP CCBs, such as nicardipine and barnidipine, has been established to be a crucial determinant of CCB activity.[11] These findings prompted us to examine the stereochemical requirements for MR activity in DHPs with the goal of designing potent MR selective inhibitors.

Results and Discussion

Screening of the Pfizer compound collection identified a number of widely used calcium channel antagonist 1,4-DHPs, such as nifedipine and felodipine, as having MR antagonist activity comparable to eplerenone **1** (Figure 1).

Since a number of groups have previously reported on the stereoselectivity of calcium channel antagonism activity for

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[a] Abbreviations: BP, blood pressure; CCB, calcium channel blocker; ACEi, angiotensin converting enzyme inhibitor; ARBs, angiotensin receptor blockers; MR, mineralocorticoid receptor; DHP, 1,4-dihydropyridine; LBD, ligand binding domain; ER, estrogen receptor; PR, progesterone receptor; GR, glucocorticoid receptor; AR, androgen receptor; PK, pharmacokinetic; CL, clearance.

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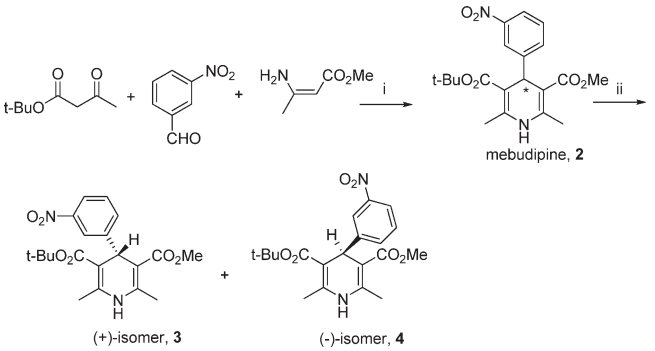
Scheme 1. Synthesis and Resolution of Mebudipine **2**[a]



[a] Reagents: (i) EtOH/piperidine (cat.), reflux, 12 h; (ii) chiral SFC.

Table 1. MR and CCB Activity of Mebudipine and its Stereoisomers[a]

compd	stereoisomer	MRAT IC_{50} (nM)	L-type Ca^{++} channel IC_{50} (nM)
1		135	> 5000
2	racemic	126	> 20
3	(+)-isomer	46	607
4	(−)-isomer	536	> 0.5

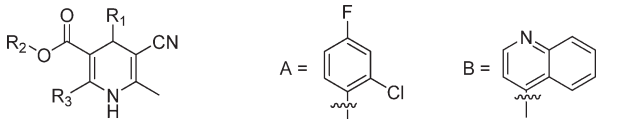
[a] IC_{50} values were obtained through curve-fitting of dose response ($n \geq 3$/concentration,6−10 concentrations) using the 4-parameter logistic model. Standard error of the IC_{50} was generally less than 30%.

DHPs, we were particularly interested in studying the MR activity of asymmetric DHPs where different substitutions of the ester groups render the molecule chiral. We envisioned that the possibility of calcium channel and MR antagonist activity residing in different stereoisomers of an asymmetric DHP would allow for the development of a new class of selective MR antagonists. To investigate the stereochemical origin of the MR activity observed in traditional DHPs, we chose **2**, a newer asymmetric 1,4-DHP that has been reported to have improved pharmacokinetic properties with respect to nifedipine.[12] Compound **2** was prepared by a variation of the Hantzsch synthesis from *tert*-butyl oxobutanoate, (*Z*)-methyl 3-aminobut-2-enoate, and 3-nitrobenzaldehyde as shown in Scheme 1.[13]

The resulting racemic product was then resolved into the corresponding enantiomers, (+)-mebudipine **3** and (−)-mebudipine **4**, using chiral supercritical fluid chromatography (SFC) on an asymmetric resin with a mobile phase containing a mixture of ethanol/CO_2. The ability of racemic **2** and enantiomers **3** and **4** to antagonize MR and L-type calcium channel was investigated (Table 1).

MR inhibition potency was evaluated on aldosterone-induced activation of a luciferase reporter driven by MR ligand binding domain fused to a heterologous DNA binding domain from yeast transcription factor Gal4. A fluorescence-based calcium indicator dye was used to quantify the influx of extracellular calcium through the L-type calcium channel in the A10 cell line of rat thoracic origin. The L-type calcium channel was activated upon depolarization induced by the addition of elevated extracellular potassium, producing an increase in calcium sensitive fluorescence within the cells that was measured on the kinetic plate reader. Enantiomer (−)-**4** exhibited excellent CCB activity and more than 1000 times greater activity than the corresponding (+) isomer **3** in the L-type Ca^{++} channel assay. Previous studies on the CCB antagonist activity of asymmetric DHPs showed the *S*-configuration at the C4 position to be required for activity.[11] On the basis of this precedent, we assigned the absolute configuration of CCB active isomer **4**, (−)-isomer, as *S* (Scheme 1).

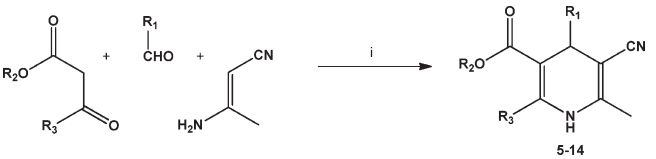
Table 2. MR and CCB Activity of 3-Cyano-DHPs[a]



compd	R_1	R_2	R_3	conf	MR IC_{50} (nM)	L-type Ca^{++} channel IC_{50} (nM)
5	A	t-Bu	Me	racemic	59	> 1550
6	A	t-Bu	Me	*S*	3520	3840
7	A	t-Bu	Me	*R*	3.7	*[b]
8	B	Me	Me	*S*	469	14570
9	B	Me	Me	*R*	77	7730
10	B	Et	Pr	racemic	31	1100
11	B	Et	Pr	*S*	3280	1330
12	B	Et	Pr	*R*	10	2200
13	B	Me	$-CH_2Cl$	*S*	2800	11270
14	B	Me	$-CH_2Cl$	*R*	340	7930
15	A	Me	$-CH_2Cl$	*S*	2200	570
16	A	Me	$-CH_2Cl$	*R*	41	2070

[a] IC_{50} values were obtained through curve-fitting of dose response ($n \geq 3$/concentration,6−10 concentrations) using the 4-parameter logistic model. Standard error of the IC_{50} was generally less than 30%. [b] * not determined.

Scheme 2. Synthesis of 3-Cyano DHPs[a]



[a] Reagents and conditions: (i) EtOH/piperidine (cat.), reflux, 8−12 h

To our pleasant surprise, CCB inactive isomer (+)-**3** (*R*-isomer), showed excellent MR antagonist activity while (−)-**4** was roughly 10 times less potent. These findings indicated that it was possible to design MR selective antagonists with a 1,4-DHP core and prompted us to continue our investigation. In an attempt to improve its metabolic stability, we turned our attention to the replacement of the methyl ester group with a cyano group, which led to very potent MR antagonists (Table 2). Racemic compounds, **5** and **10**, prepared as shown in Scheme 2, exhibited good MR antagonist activity and no CCB activity. Chiral resolution and testing of the corresponding enantiomers confirmed our findings with **3** and **4** isomers, and only one enantiomer of **5** and **10** (**7** and **12**, respectively) exhibited MR antagonist activity. The complete SAR studies for the 5-cyano series will be reported elsewhere.

On the basis of MR potency, a group of enantiomerically pure analogues was selected for evaluation of their selectivity versus other steroidal nuclear hormone receptors, determined in a similar Gal4 cellular assay format (Table 3).

The new cyano ester DHPs were highly selective against the estrogen receptor (ER) and the progesterone receptor (PR) while also exhibiting greater than 20-fold selectivity over the glucocorticoid receptor (GR) and the androgen receptor (AR).

To get an early reading on any potential liabilities of the new scaffold that could hinder further optimization, compounds **7** and **12** were selected for evaluation of their pharmacokinetic parameters in rats. For pharmacokinetic (PK) studies, male Sprague−Dawley rats (n = 2) were dosed

Table 3. Nuclear Receptor Selectivity for Select Enantiomerically Pure DHPs[a]

compd	MR IC_{50} (nM)	GR IC_{50} (nM)	AR IC_{50} (nM)	PR IC_{50} (nM)	ER IC_{50} (nM)
3	46	3260	1270	768	*[b]
7	3.7	1370	246	3520	> 10000
12	10	270	730	1340	> 10000

[a] IC_{50} values were obtained through curve-fitting of dose response ($n \geq 3$/concentration, 6−10 concentrations) using the 4-parameter logistic model. Standard error of the IC_{50} was generally less than 30%. [b] * not determined.

Table 4. Rat Pharmacokinetic Data for **7** and **12**

compd	dose (mpk)	CL (mL/min/kg)	$T_{1/2}$ (h)	V_{dss} (L·kg)
7	2	84.8	1.14	4720
12	2	9.7	6.54	0.42

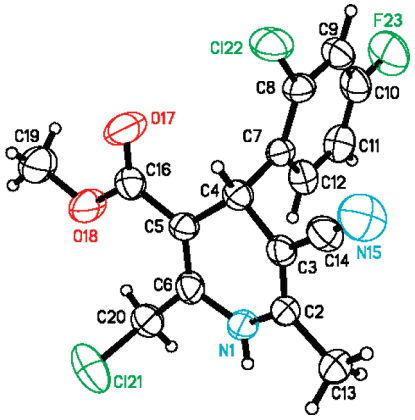


Figure 2. X-ray crystal structure of MR active enantiomer **16**.

intravenously (iv) at 2 mg/kg. Compounds were formulated for iv dosing in 10% ethanol/40% PEG400/50% phosphate buffered saline pH7.4. Plasma samples were analyzed by LC/MS/MS. The PK profiles of compounds **7** and **12** are shown in Table 4. While **7** exhibited a moderate to high clearance (CL), the CL measured for **12** was low and the half-life was adequate for future proof-of-concept study in rats.

To positively establish the configuration at C4 for the MR active 5-cyano DHP series, compound **16** was prepared in racemic form according to the procedure shown in Scheme 2. The enantiomers were resolved by chiral chromatography and tested for MR activity. An X-ray crystal structure of MR-active enantiomer **16** was obtained (Figure 2), and the configuration at C4 was determined to be *R*. Similarly, the structure of a derivative of **14** was solved and the stereochemistry at C4 was again shown to be *R* (see Supporting Information, SI). These results confirmed our initial C4 configuration assignment based on the CCB activities of (+) and (−)-mebudipine.

Induced-fit docking[14] was used to examine potential binding mode differences between the *R* and *S* enantiomers, with similar results being obtained for both the wild-type (2A3I) and S810L (2AA6) MR crystal structures, in each case with helix 12 truncated to allow for active antagonism. For the *R* enantiomers **7** and **12**, the top group of poses consistently favored the binding mode previously hypothesized for DHP diesters.[8] In this pose, the NH of the DHP ring donates a hydrogen to helix 3 Asn 770, the hydrophobic ester group fills the α-face pocket, and the DHP 4-aryl ring resides in the

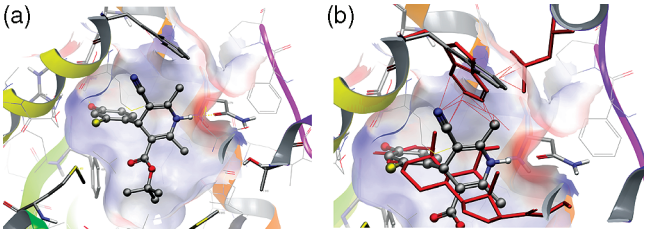


Figure 3. Representative docking pose of MR active DHP **7**. (a) Top induced fit docking pose in wt 2A3I MR-LBD crystal structure with H12 truncated. (b) Comparison with native steroid pose (red), showing induced clash between Trp 806 and helix 12 Leu 960.

A-ring pocket, making a tee interaction with Phe 829 and (in compound **12**) a hydrogen bond to Gln (Figure 3a). The DHP fails to make stabilizing contacts with the steroid D-ring-binding area of helix 11, including Thr 945.[15] Perturbation of Trp 806 by the cyano and methyl groups may also contribute an active antagonism component by inducing close contact with helix 12 residue Leu 960 (Figure 3b). In contrast, the *S* enantiomers **6** and **11** (as well as **4**) typically placed the 4-aryl group in the α-face pocket and occupied the A-ring pocket poorly if at all.

Conclusions

The present study demonstrates that the stereochemical configuration of asymmetric 1,4-DHPs is critical for their CCB or MR antagonist activity. Indeed, while it has been previously reported that CCB active asymmetric DHPs possess the *S* configuration at C4, this study establishes that MR activity requires the *R* configuration at C4. Replacement of the ester group at the C3 position by a cyano group led to a series of very active MR antagonists with no CCB activity. These findings provide important pharmacophore information for the exploration of an attractive target and a drug-like scaffold for the design of novel MR antagonists.

Experimental Section

All materials were obtained from commercial sources and used as purchased. Chromatography solvents were HPLC grade and were used without further purification. Thin layer chromatography (TLC) analysis was performed using Merck silica gel 60 F-254 thin layer plates. LC-MS analyses were performed on Mariner TOF from Perseptive Biosystems. The scan range was 100−1000 d. The sample was introduced by flow inject from an Agilent 1100 with 100 μL/min MeOH (10 mM ammonium acetate) into the electrospray source. Preparative reverse phase HPLC was performed on a Gilson 215 liquid handler equipped with a Dynamax Microsorb C18; (300 A) column (41.4 mm × 25 cm, 8 μm) and Gilson 156 variable length UV detector. Analytical chiral chromatography was performed on a OJ-H column (Chiral Technologies, 4.6 mm × 250 mm) eluted with 5% ethanol/hexane. The purity of tested compounds was ≥95% as determined by combustion analysis or by HPLC conducted on an Agilent 1100 system using a reverse phase C8 column with diode array detector. NMR spectra were recorded on a Bruker 400 spectrometer. The signal of the deuterated solvent was used as internal reference. Chemical shifts (d) are given in ppm and are referenced to residual not fully deuterated solvent signal. Coupling constants (*J*) are given in Hz.

General Procedure for the Preparation of 3-Cyanodihydropyridines. *tert*-Butyl 4-(2-Chloro-4-fluorophenyl)-5-cyano-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (5). 4-Chloro-2-fluorobenzaldehyde (1 mmol), 3-aminocrotonitrile (1 mmol), and *tert*-butyl acetoacetate (1 mmol) were dissolved in EtOH (20 mL). Piperidine (cat.) was added, and the reaction mixture was refluxed

gently overnight. The solvent was evaporated under vacuum, and the resulting crude product was purified by preparative HPLC (30−100% CH_3CN/H_2O/0.1% TFA over 40 min at a flow rate of 35 mL/min) to yield 236 mg (65%) of **5** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.18 (s, 9 H), 1.95 (s, 3 H), 2.25 (s, 3 H), 4.99 (s, 1 H), 7.20−7.30 (m, 2 H), 7.38 (dd, J = 8.99, 2.74 Hz, 1 H), 9.11 (s, 1 H). MS (ES) m/z 363 ([M + H]$^+$).

***tert*-Butyl (4*S*)-4-(2-Chloro-4-fluorophenyl)-5-cyano-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (6) and *tert*-Butyl (4*R*)-4-(2-Chloro-4-fluorophenyl)-5-cyano-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (7)** were obtained by chiral chromatography of **5**, same conditions as described for **2**. **6** (white solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 (s, 9 H), 1.87 (s, 3 H), 2.17 (s, 3 H), 4.91 (s, 1 H,) 7.08−7.33 (m, 3 H), 9.02 (s, 1 H); MS (ES) m/z 363 ([M + H]$^+$); >99.5% ee by chiral HPLC. **7** (white solid): MS (ES) m/z 363 ([M + H]$^+$); 99.5% ee by chiral HPLC. Note: optical purity of **6** and **7** was checked from stock solutions at 1, 2, and 4 weeks.

Methyl (4*S*)-5-Cyano-2,6-dimethyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (8) and Methyl (4*R*)-5-Cyano-2,6-dimethyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (9). Following the general procedure, quinoline-4-carbaldehyde was reacted with 3-aminocrotonitrile and methyl acetoacetate to generate the corresponding cyanodihydropyridine as a solid (60% yield), which was then subjected to chiral separation of the enantiomers on a OD-H column (Chiral Technologies, 21 mm × 250 mm), eluted with 10% ethanol/CO_2, 70 mL/min to yield each enantiomer. **8** (solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.97 (s, 3 H), 2.33 (s, 3 H), 3.25 (s, 3 H), 5.45 (s, 1 H), 7.30 (d, J = 4.56 Hz, 1 H), 7.63 (ddd, J = 8.46, 6.85, 1.34 Hz, 1 H), 7.74 (ddd, J = 8.32, 6.98, 1.34 Hz, 1 H), 7.99 (d, J = 8.32 Hz, 1 H), 8.40 (d, J = 8.32 Hz, 1 H), 8.84 (d, J = 4.57 Hz, 1 H), 9.34 (s, 1 H); HRMS ($C_{19}H_{17}N_3O_2$ + H$^+$) calcd 320.1934, found 320.1389; >99.5% ee by chiral HPLC. **9** (solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.97 (s, 3 H), 2.33 (s, 3 H), 3.25 (s, 3 H), 5.45 (s, 1 H), 7.29 (d, J = 4.56 Hz, 1 H), 7.62 (ddd, J = 8.39, 6.91, 1.34 Hz, 1 H), 7.74 (ddd, J = 8.39, 6.91, 1.34 Hz, 1 H), 7.99 (dd, J = 8.46, 0.94 Hz, 1 H), 8.40 (d, J = 8.32 Hz, 1 H), 8.83 (d, J = 4.56 Hz, 1 H), 9.33 (s, 1 H); HRMS ($C_{19}H_{17}N_3O_2$ + H$^+$) calcd 320.1394, found 320.1393; >99.5% ee by chiral HPLC.

Ethyl 5-Cyano-6-methyl-2-propyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (10). Following the general procedure, quinoline-4-carbaldehyde was reacted with 3-aminocrotonitrile and ethyl acetoacetate to yield **10** as a yellow solid (72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.67 (t, J = 7.03 Hz, 3 H), 0.98 (t, J = 7.42 Hz, 3 H), 1.59−1.69 (m, 2 H), 2.00 (s, 3 H), 2.63−2.74 (m, 2 H), 3.67−3.75 (m, J = 10.57, 7.02, 3.71 Hz, 2 H), 5.49 (s, 1 H), 7.32 (d, J = 4.69 Hz, 1 H), 7.64 (t, J = 7.81 Hz, 1 H), 7.76 (t, J = 7.03 Hz, 1 H), 8.02 (d, J = 7.42 Hz, 1 H), 8.43 (d, J = 8.20 Hz, 1 H), 8.87 (d, J = 4.69 Hz, 1 H), 9.31 (s, 1 H). Anal. ($C_{22}H_{23}N_3O_2$) C, H, N.

Ethyl 5-Cyano-6-methyl-2-propyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (11) and Ethyl 5-Cyano-6-methyl-2-propyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (12) were obtained by chiral separation of **10** under same conditions for the preparation of **8** and **9**. **11** (yellow solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.62 (t, J = 7.03 Hz, 3 H), 0.94 (t, J = 7.33 Hz, 3 H), 1.44−1.62 (m, 2 H), 1.96 (s, 3 H), 2.55−2.77 (m, 2 H), 3.66 (m, 2 H), 5.44 (s, 1 H), 7.27 (d, J = 4.49 Hz, 1 H), 7.60 (dd, J = 15.24, 1.17 Hz, 1 H), 7.72 (t, J = 7.62 Hz, 1 H), 7.97 (d, J = 8.40 Hz, 1 H), 8.38 (d, J = 8.40 Hz, 1 H), 8.82 (d, J = 4.69 Hz, 1 H), 9.26 (s, 1 H); (ES) m/z 362 ([M + H]$^+$); >99.5% ee by chiral HPLC. **12** (yellow solid): ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.62 (t, J = 7.13 Hz, 3 H), 0.94 (t, J = 7.33 Hz, 3 H), 1.49−1.73 (m, 2 H), 1.96 (s, 3 H), 2.51−2.74 (m, 2 H), 3.27 (s, 3 H) 3.66 (m, 2 H), 5.44 (s, 1 H), 7.27 (d, J = 4.69 Hz, 1 H), 7.60 (ddd, J = 8.50, 6.93, 1.37 Hz, 1 H), 7.72 (ddd, J = 8.35, 6.89, 1.37 Hz, 1 H), 7.97 (dt, J = 8.45, 0.76 Hz, 1 H), 8.38 (dt, J = 8.64, 0.66 Hz, 1 H), 8.82 (d, J = 4.49 Hz, 1 H), 9.26 (s, 1 H); MS (ES) m/z 362 ([M + H]$^+$); >99.5% ee by chiral HPLC.

Methyl (4*S*)-2-(Chloromethyl)-5-cyano-6-methyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (13) and Methyl (4*R*)-2-(Chloromethyl)-5-cyano-6-methyl-4-quinolin-4-yl-1,4-dihydropyridine-3-carboxylate (14). To a solution of quinoline-4-carbaldehyde (4 mmol) in 2-propanol was added methyl 4-chloroacetoacetate (4 mmol), acetic acid (20 mg), and piperidine (35 mg). The reaction was stirred at room temperature (rt) under nitrogen for 20 h. 3-Aminocrotonitrile (4 mmol) was added, and the resulting mixture was stirred at rt for another 20 h. Concentrated hydrochloric acid (0.25 mL) was then added and the stirring continued for 2 h. The reaction mixture was concentrated in vacuo, and the resulting solid (50% yield) was then subjected to chiral separation of the enantiomers under the same conditions described for **8** and **9**. **13** and **14** were characterized as the HCl salt. **13**: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.00 (s, 3 H), 3.28 (s, 3 H), 4.74 (d, J = 11.00 Hz, 1 H), 4.78 (d, J = 11.00 Hz, 1 H), 5.75 (s, 1 H), 7.67 (d, J = 5.10 Hz, 1 H), 7.87 (t, J = 7.92 Hz, 1 H), 8.02 (t, J = 7.52 Hz, 1 H), 8.21 (d, J = 8.59 Hz, 1 H), 8.67 (d, J = 8.86 Hz, 1 H), 9.12 (d, J = 5.37 Hz, 1 H), 9.96 (s, 1 H); HRMS ($C_{19}H_{16}ClN_3O_2$ + H$^+$) calcd 354.1004, found 354.0972; >99.5% ee by chiral HPLC. **14**: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.00 (s, 3 H), 3.28 (s, 3 H), 4.74 (d, J = 11.00 Hz, 1 H), 4.79 (d, J = 11.01 Hz, 1 H), 5.77 (s, 1 H), 7.71 (d, J = 5.37 Hz, 1 H), 7.90 (ddd, J = 8.46, 7.11, 1.07 Hz, 1 H), 8.05 (ddd, J = 8.32, 7.11, 0.94 Hz, 1 H), 8.26 (d, J = 8.05 Hz, 1 H), 8.69 (d, J = 8.59 Hz, 1 H), 9.15 (d, J = 5.37 Hz, 1 H), 10.01 (s, 1 H); HRMS ($C_{19}H_{16}ClN_3O_2$ + H$^+$) calcd 354.1004, found 354.0974; >99.5% ee by chiral HPLC.

Methyl (4*S*)-(2-Chloro-4-fluorophenyl)-2-(chloromethyl)-5-cyano-6-methyl-1,4-dihydropyridine-3-carboxylate (15) and Methyl (4*R*)-(2-Chloro-4-fluorophenyl)-2-(chloromethyl)-5-cyano-6-methyl-1,4-dihydropyridine-3-carboxylate (16). Racemic product was prepared following the same procedure for **14** using 4-chloro-2-fluorobenzaldehyde in place of quinoline-4-carbaldehyde to generate methyl 4-(2-chloro-4-fluorophenyl)-2-(chloromethyl)-5-cyano-6-methyl-1,4-dihydropyridine-3-carboxylate as a solid (62% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.01 (s, 3 H), 3.47 (s, 3 H), 4.71 (q, J = 10.83 Hz, 2 H), 5.07 (s, 1 H), 7.22 (m, 1 H), 7.31 (m, 1 H), 7.37 (dd, J = 9.00, 2.55 Hz, 1 H), 9.68 (s, 1 H). HRMS ($C_{16}H_{13}Cl_2FN_2O_2$ + NH_4^+) calcd 372.0676, found 372.0719. Product was then subjected to chiral separation of the enantiomers on an OJ-H column (Chiral Technologies, 30 mm × 250 mm) eluted with 10%*n*-butanol/CO_2, 70 mL/min flow. **15**: MS (ES) m/z 355 ([M + H]$^+$); >99.5% ee by chiral HPLC. **16**: MS (ES) m/z 355 ([M + H]$^+$); >99.5% ee by chiral HPLC.

Single Crystal X-ray Analysis. A representative crystal of compound **16** was surveyed and a 1 Å data set (maximum sin Θ/λ = 0.5) was collected on a Bruker P4/R diffractometer. Friedel pairs were collected in order to facilitate the determination of the absolute configuration. Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[16] All crystallographic calculations were facilitated by the SHELXTL system.[17] $C_{16}H_{13}N_2O_2FCl_2$; FW = 355.18; monoclinic; space group $P2(1)$; unit cell dimensions: a = 12.1090(10) Å, b = 9.0150(10) Å, c = 15.5900(10) Å; volume = 1628.0(2) Å^3; Z = 4; D calcd = 1.449 mg/m^3; absorption coefficient = 3.779 mm^{-1}; F(000) = 728; GOF on $F2$ = 0.988; final R indices [$I > 2\sigma(I)$]; R_1 = 0.0374, wR_2 = 0.0992. The refined structure was plotted using the SHELXTL plotting package (Figure 2). The absolute configuration was determined by the method of Flack.[18] Coordinates, anisotropic temperature factors, distances, and angles are available as SI (Tables S1−S5).

Cell-Based Gal4 Response Element-Controlled Luciferase Reporter Assays. MR Luciferase Reporter Antagonist Assay. HUH7 human hepatocyte cells were maintained in RPMI 1640 plus 10% FBS and transfected with Gal4-MRLBD construct and a luciferase reporter under Gal4 control. After transfection, compounds were added in RPMI 1640 media plus 10% heat-inactivated and charcoal dextran stripped FBS (Hyclone) with and without 1 nM aldosterone. Cells were

harvested 20 h later for reporter activity as described in ref 7. Selectivity against other steroid receptors were assayed in the same manner using Gal4-driven luciferase as reporter.

L Type Calcium Channel Assay. This assay utilizes the thoracic rat cell line A10 and a calcium sensing fluorescence dye run on a kinetic plate reader. The A10 cells were plated into a Biocoat 384-well black-walled, clear bottom plate at 1500 cells per well. Cells were cultured for 48 h prior to running the experiment. One hour before running, the media was removed from the cells, and they were loaded with 50 μL of no wash calcium dye Calcium 4 (Molecular Devices R-8141) following the manufacturers instructions. The plate was then loaded onto the FLIPR^{TETRA} plate reader (Molecular Devices) along with the compound plate and a stimulus plate (384 well Greiner) containing 140 mM KCl to depolarize the cells and activate the L type calcium channel. After establishing a baseline read, 25 μL of compound from the compound plate (source 1) was dispensed into each well of the cell plate. Ten minutes later, 25 μL of the depolarizing KCl solution (source 2) was added to each well (final $[K^+]$ = 45 mM). The peak of the depolarizing response after the second addition (stimulus) was used to produce a concentration response curve.

Docking Studies. Molecular modeling was conducted using the Schrodinger Suite 2009 (Schrodinger Suite 2009 Induced Fit Docking protocol, Glide version 5.5, Prime version 2.1, Jaguar version 7.6; Schrodinger LLC, New York, NY). The wild type MR and S810L docking structures were prepared from the 1.95 Å MR structure with bound corticosterone (PDB: 2A3I)[19] and the 1.95 Å S810L crystal structure with bound progesterone (PDB: 2AA6),[15] respectively by removal of waters, truncation of helix 12 (AF-2) to the C-terminus (residues 958−984), and capping of Pro 957 as the *N*-methylacetamide. Dihydropyridine ligands **7** and **12** were geometry optimized using Jaguar (B3LYP/6-31G**//B3LYP/6-31G**) to ensure appropriate dihydropyridine ring geometries; the corresponding enantiomers **6** and **11** were obtained by chiral inversion. The induced fit docking protocol[14] was used to dock the dihydropyridine ligands (**3**, **4**, **6**, **7**, **11**, **12**, and *R*- and *S*-mebudipine) into the truncated receptor models, using the default induced fit docking protocol parameters with the exception of truncating the side chain of Met 845 during initial Glide docking and using XP scoring for the redock step. No hydrogen bonding or other constraints were used. Pictures were generated using Maestro 9.0.111 (Schrodinger LLC).

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Supporting Information Available: X-ray data for compound **16** and methyl thio ether derivative of compound **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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