Vasorelaxation by New Hybrid Compounds Containing Dihydropyridine and Pinacidil-Like Moieties

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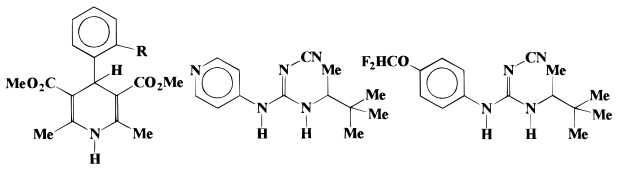
The synthesis and pharmacological properties of a novel type of vasorelaxant hybrid compounds are described. The investigated compounds originate from fluorinated 4-aryl-1,4-dihydropyridines, which are known calcium channel blockers, and/or from fluorinated analogues of pinacidil, which is an opener of ATP-sensitive potassium channels. In particular, we studied the most potent hybrid, 2,6-dimethyl-3,5-dicarbomethoxy-4-(2-difluoromethoxy-5-*N*-(*N*″-cyano-*N*′-1,2,2-trimethyl-propylguanidyl)-phenyl)-1,4-dihydropyridine (**4a**), together with its parent compounds, the dihydropyridine **1b** and the pinacidil analogue **3**. In isolated rat mesenteric arteries, micromolar concentrations of **4a** relaxed contractions exerted by K^+-depolarization or by norepinephrine. The latter effect was sensitive to the potassium channel blocker glibenclamide. Micromolar **4a** also inhibited [^{3}H](+)-isradipine and [^{3}H]P1075 binding to rat cardiac membranes, and it blocked L-type calcium channels expressed in a mammalian cell line. The respective parent compounds **1b** and **3** were always more potent and more selective regarding calcium channel or potassium channel interaction, respectively. In contrast, **4a** combined both effects within the same concentration range, indicating that it may represent a lead structure for a novel class of pharmacological hybrid compounds.

Introduction

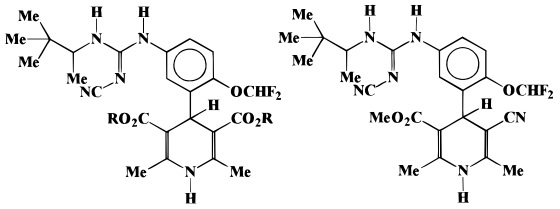
The 4-aryl-1,4-dihydropyridine derivatives (1,4-DHP), for example nifedipine (**1a**) and foridon (**1b**), are effective L-type calcium channel blockers (Scheme 1). They are used widely in medical practice for the treatment of hypertension because of their vasorelaxant properties. A similar effect is exerted by the so-called potassium channel openers, for example, pinacidil (**2**). Several types of openers of ATP-sensitive potassium channels have attracted the attention of medicinal chemists over the past several years. We have previously found a hypotensive effect exhibited by a less toxic fluorine-containing benzene analogue (**3**) of pinacidil that we named flocalin.[1,2] A rationale for combination therapy (as frequently used in hypertensive patients) as well as for the design of hybrid compounds is a common main pharmacological effect (here: vasodilation) and a divergent side effect profile. Furthermore, blockade of L-type calcium channels together with potassium channel activation may even lead to a combined antianginal and antiischemic action, respectively, due to the proposed role of ATP-sensitive potassium channels in cardiac ischemic preconditioning.[3]

In this work we have synthesized hybrid compounds that contain the structural elements of calcium channel modulators and a potassium channel activator−flocalin (**3**). We have obtained two types of preparations (Scheme 2): hybrids of calcium channel blocker and potassium channel activator (**4**), as racematic mixtures, and a hybrid of potassium channel activator and presumed weak calcium channel activator (according to ref 4) containing a cyano group instead of the alkoxycarbonyl group in 5-position of dihydropyridine ring (**5**), which represents a mixture of four stereoisomers. We describe our attempts to elucidate whether the test hybrid compounds type **4** and **5** are active as vasodilators. More specifically, we elucidated whether compound **4a** indeed interacts with L-type calcium channels and whether it behaves like a potassium channel opener.

Scheme 1



Scheme 2



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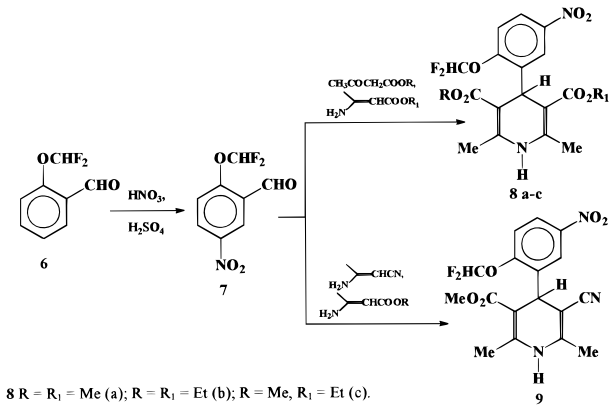
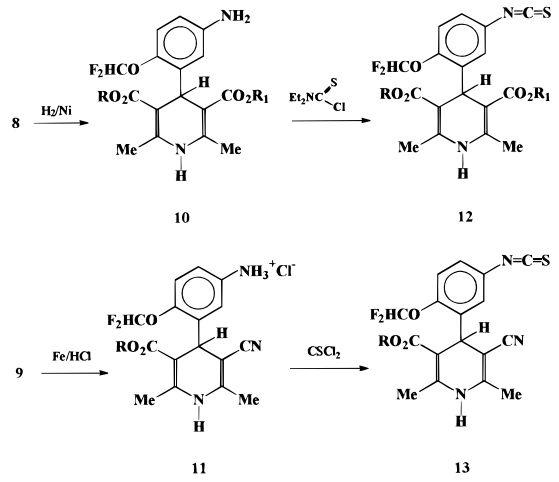
Scheme 3



Scheme 4



Chemistry

The starting *o*-difluoromethoxybenzaldehyde (**6**) was synthesized by difluoromethylation of salicylic aldehyde.[5] The nitration of **6** afforded 2-difluoromethoxy-5-nitrobenzaldehyde (**7**) in 90% yield. Aldehyde **7** was introduced in modified Hantzsch reaction (Scheme 3) to give 1,4-DHP with alkoxycarbonyl groups in 3,5-positions of dihydropyridine ring (**8a**−**c**) and also 1,4-DHP with one methoxycarbonyl and one cyano group (**9**).

Reduction of nitro group by hydrogen/Raney Ni (for compounds **8**) or by Fe in hydrochloric acid (for compound **9**) led to corresponding amino derivatives **10** or **11** (available in situ) that were converted into isothiocyanates **12** and **13** by treating of diethylthiocarbamoyl chloride or thiophosgen (Scheme 4).

When treated with stoichiometric equivalents of pinacolylamine, isothiocyanates **12** and **13** gave thioureas **14** and **15**, respectively. Thioureas **14** were converted into carbodiimides **16** by action of the system CCl_4−Et_3N−PPh_3, whereas thiourea **15** was dehydrosulfurizated into carbodiimide **17** by mercury oxide. Compounds **4** and **5** were prepared by treatment of carbodiimides **16** and **17** with cyanamide in the presence of diisopropylethylamine (Scheme 5).

Pharmacological Results and Discussion

Vasorelaxation in Mesenteric Arteries. All tested compounds exerted a concentration-dependent relaxation of resistance arteries. The potency, gauged by the log(EC_{50}) value, depended on the precontracting stimulus (Table 1). As expected for a dihydropyridine, **1b** was potent in relaxing K^+-constricted arteries, and significantly less potent in case of norepinephrine contractions. The reverse was true, again as expected, for the potassium channel opener **3**. The hybrid compounds **4a**, **4b**, and **5** were all less potent when tested with K^+-depolarization, indicating weaker calcium channel block compared with **1b**. Compound **5** and its parent dihydropyridine did not show functional evidence of calcium channel agonism ($N = 4$, not shown). Therefore, we focused our attention on the most potent hybrid compound, **4a**. This compound was nearly equally potent ($p > 0.05$) with regard to relaxation of K^+-induced versus norepinephrine-induced contractions, unlike its parent compounds **1b** and **3**. Like the potassium channel opener **3**, relaxation by **4a** of norepinephrine-contracted arteries was sensitive to the blocker of ATP-sensitive potassium channels, glibenclamide ($p < 0.05$). Thus, functional evidence exists that both calcium channel blockade and potassium channel opener action may be present−at pharmacologically active concentrations−in the hybrid **4a**.

Scheme 5

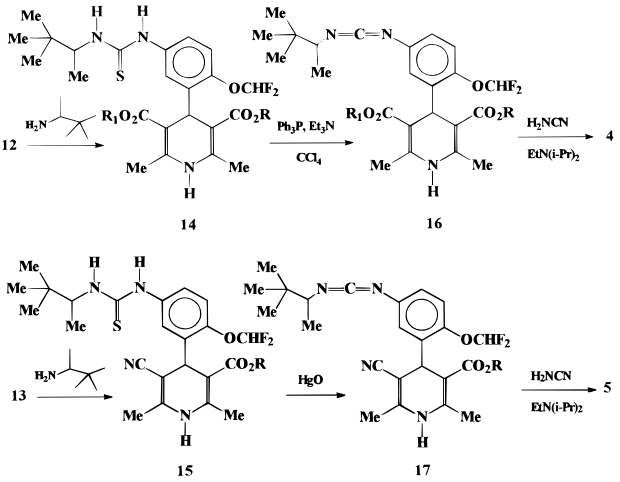


Table 1. Vasorelaxation of Rat Mesenteric Arteries by Test Compounds[a]

compd	contractile stimulus	log (EC_{50}) mean ± SEM	*p* value (vs norepinephrine)	*N*
1b	K^+-depolarization	−8.75 ± 0.13	0.011	6
	norepinephrine (NE)	−8.20 ± 0.14		6
	NE + glibenclamide	−8.44 ± 0.12	ns	6
3	K^+-depolarization	−5.13 ± 0.09	0.00001	5
	norepinephrine (NE)	−6.54 ± 0.13		6
	NE + glibenclamide	−6.12 ± 0.21	0.076	5
4a	K^+-depolarization	−6.43 ± 0.11	ns	10
	norepinephrine (NE)	−6.70 ± 0.12		9
	NE + glibenclamide	−6.20 ± 0.08	0.002	9
4b	K^+-depolarization	−6.02 ± 0.06		6
5	K^+-depolarization	−5.63 ± 0.05		6

[a] Vasorelaxation was measured by cumulative addition of the test compounds after the respective contractile stimulus (second column). Significance was checked (two-tailed *t* test) for a given compound between log(EC_{50}) values obtained with norepinephrine (10^{-5} M) versus K^+-depolarization (120 mM) or versus norepinephrine in the presence of glibenclamide (3×10^{-6} M).

Binding Experiments. To substantiate the interaction of the test compounds with L-type calcium channels or ATP-sensitive potassium channels respectively, com-

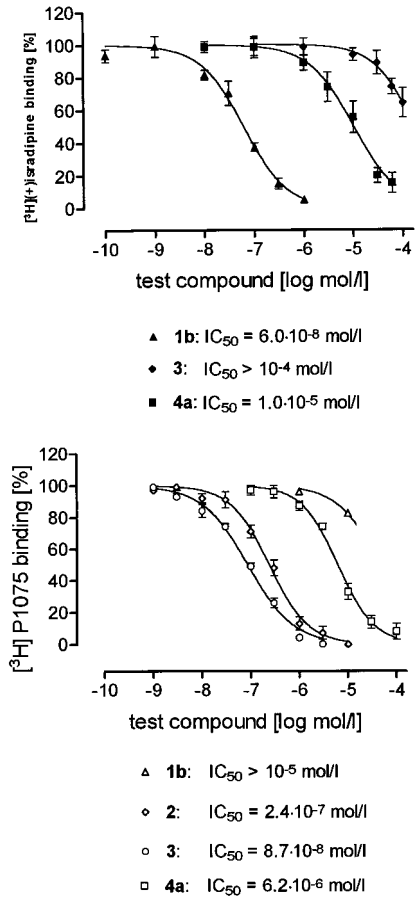


Figure 1. Competition binding experiments in rat cardiac membranes. Test compounds were coincubated with [^{3}H](+)-isradipine (top) as a calcium channel ligand and with [^{3}H]-P1075 as a potassium channel ligand. Data (mean ± SEM) are from $N = 3-5$ independent experiments. IC_{50} values are from nonlinear regression. Slope factors were close to unity.

petition binding assays were performed using classical high-affinity ligands ([^{3}H](+)-isradipine and [^{3}H]P1075, respectively) in rat cardiac membranes (Figure 1). [^{3}H]-(+)-Isradipine binding was inhibited concentration-dependently, with rank order of potency of **1b** ≫ **4a** > **3**. This is the same order of potency as found for vasorelaxation in depolarized arteries, corroborating the idea of calcium channel inhibition for **1b**, the hybrid **4a**, and interestingly for the pinacidil analogue **3**. This is in contrast to the absence of significant calcium channel blockade reported[6] for the prototype pinacidil (**2**). Binding of [^{3}H]P1075 was also inhibited in a competitive, concentration-dependent manner, but the rank order of potency was reversed: **3** ≫ **4a** > **1b**. The novel potassium channel opener **3** was slightly more potent than **2**, included here for comparison. Compound **1b** inhibited [^{3}H]P1075 binding only at very high concentrations. Importantly, the hybrid compound interacts with both binding sites within the same concentration range. These data further validate our assumption that vasorelaxation by **4a** is due to both calcium channel and potassium channel interaction.

Calcium Channel Inhibition. Since inhibition of dihydropyridine binding can be found with blockers and activators of calcium channel, we sought direct evidence for calcium channel block by the hybrid compound **4a**. The cloned α_{1C-b} isoform of the calcium channel pore-forming subunit expressed in arterial smooth muscle[7] was used. As depicted in Figure 2, one drug concentration was checked in each experiment at two holding potentials (−80 mV and −30 mV). We chose equimolar (10^{-5} M) and equieffective (approximately half-maximal block at the −30 mV holding potential: 10^{-8} M **1b**, 10^{-6} M **4a**, and 10^{-5} M **3**) concentrations of **1b**, **3**, and **4a**. As shown in Table 2, all three compounds inhibited calcium channel currents voltage[8]- and concentration-dependently, with a rank order of potency (**1b** ≫ **4a** > **3**) similar to the dihydropyridine binding experiments.

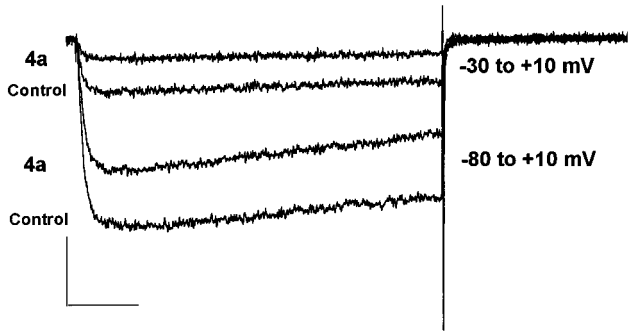


Figure 2. Inhibition of barium currents in CHO cells transfected with the α_{1C-b} calcium channel subunit by **4a**. Cells were depolarized to +10 mV from a holding potential of either −80 mV (bottom traces) or −30 mV (top traces). Data were obtained before and after application of compound **4a** (10^{-5} M) as indicated. Scale bars denote 20 ms and 200 pA, respectively.

Table 2. Calcium Channel Inhibition by the Test Compounds[a]

compd	concn (M)	holding potential (mV)	peak current (%) mean × SEM	*N*
	0	−80	71.4 ± 5.0	10
	0	−30	17.2 ± 3.2	10
1b	10^{-8}	−80	47.4 ± 3.5	7
	10^{-8}	−30	7.8 ± 2.0	7
	10^{-5}	−80	9.2 ± 1.4	4
	10^{-5}	−30	0.9 ± 0.3	4
3	10^{-5}	−80	58.2 ± 4.3	6
	10^{-5}	−30	10.8 ± 1.6	6
	10^{-4}	−80	26.7 ± 3.0	6
	10^{-4}	−30	2.4 ± 0.6	6
4a	10^{-6}	−80	60.4 ± 4.7	7
	10^{-6}	−30	9.2 ± 1.2	7
	10^{-5}	−80	38.2 ± 4.6	7
	10^{-5}	−30	2.5 ± 0.9	7

Barium currents were measured in CHO cells transfected with the α_{1C-b} calcium channel subunit. Peak current was measured at the optimum test potential, and initial stable values obtained at a holding potential of −80 mV were set at 100%. Data in the first two data rows are from time-matched drug-free control experiments, indicating slight time-dependent rundown at a holding potential of −80 mV and a pronounced steady-state inactivation at −30 mV.

In summary, the novel hybrid compound **4a** displays the pharmacological properties expected from its ancestor chemical constituents. Like **1b**, it relaxes depolarized arteries, inhibits dihydropyridine binding, and blocks calcium channels. Like **3**, it relaxes agonist-contracted vessels, it acts in a glibenclamide-sensitive manner, and it inhibits binding of a ligand at the potassium channel opener site. Importantly, the two pharmacological effects of **4a** are exerted within the same concentration range. It still has to be tested which stereoisomer(s) possess the pharmacological activities. Whether the hybrid mode of action represents a beneficial therapeutic strategy also remains to be determined.

Experimental Section

All reagents and solvents were of reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries and are given uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian VXR-300 spectrometer, and chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS, 0.00 ppm). Infrared spectra were obtained on a UR-20 infrared spectrophotometer.

2-Difluoromethoxy-5-nitrobenzaldehyde (7). *o*-Difluoromethoxybenzaldehyde (**6**) (17.2 g, 0.1 mol) was added dropwise during 1 h to a stirred mixture of sulfuric acid ($d = 1.84$) (25 mL) and fumic nitric acid ($d = 1.52$) (25 mL) at −10 to −5 °C. The mixture was slowly heated to room temperature and was poured into crushed ice after 1 h. The precipitate was filtered off, washed with water, and dissolved in ether (100 mL). The ether solution was washed with water (50 mL), 5% solution of $NaHCO_3$ (2 × 50 mL), and water (50 mL) and dried with $MgSO_4$. Evaporation of solvent afforded product **7** (19.5 g, 90%): mp 41−43 °C, pure enough for the next synthesis. Recrystallization of a sample from hexane afforded the analytically pure product: mp 45 °C; ^{1}H NMR ($CDCl_3$) δ 7.37 (t, 1H, $J = 72$ Hz), 7.61−7.65; 8.51−8.67 (m, 3H). Anal. ($C_8H_5F_2NO_3$) C, H, N.

2,6-Dimethyl-3,5-dicarbomethoxy-4-(2-difluoromethoxy-5-nitrophenyl)-1,4-dihydropyridine (8a). A mixture of **7** (6.5 g, 0.03 mol), methyl 3-aminocrotonate (3.45 g, 0.03 mol), methyl acetoacetate (3.5 g, 0.03 mol), and methanol (20 mL) was stirred and heated to reflux for 8 h. The mixture was cooled, and after 12 h at 0 °C the yellow crystals formed were filtered off, recrystallized from 2-propanol, and dried to afford the product **7a** (8.5 g, 69%): mp 187−189 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H), 3.60 (s, 6H), 5.41 (s, 1H), 6.08 (s, 1H), 6.67 (t, 1H, $J = 73$ Hz), 7.25−7.38, 8.01−8.25 (m, 3H). Anal. ($C_{18}H_{18}F_2N_2O_7$) C, H, N.

2,6-Dimethyl-3,5-dicarboethoxy-4-(2-difluoromethoxy-5-nitrophenyl)-1,4-dihydropyridine (8b). Prepared from **7** (6.5 g, 0.03 mol), ethyl 3-aminocrotonate (3.9 g, 0.03 mol), and ethyl acetoacetate (3.85 g, 0.03 mol) in ethanol by the procedure described in the preparation of **8a**. Yield 10.24 g (78%): mp 191−192 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 6H, $J = 7$ Hz), 2.37 (s, 6H), 4.07 (q, 4H, $J = 7$ Hz), 5.31 (s, 1H), 6.20 (s, 1H), 6.52 (t, 1H, $J = 72$ Hz), 7.70−7.75, 8.10−8.30 (m, 3H). Anal. ($C_{20}H_{22}F_2N_2O_7$) C, H, N.

2,6-Dimethyl-3-carbomethoxy-5-carboethoxy-4-(2-difluoromethoxy-5-nitrophenyl)-1,4-dihydropyridine (8c). Prepared from **7** (2.17 g, 0.01 mol), methyl 3-aminocrotonate (1.15 g, 0.01 mol), and ethyl acetoacetate (1.30 g, 0.01 mol) in methanol (7 mL) by the procedure described in the preparation of **8a** except that the reaction mixture was refluxed for 10 h. Recrystallization of the product from ethanol and then from toluene/hexane 1:1 gave 2 g (46%) of **8c**: mp 153−155 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, $J = 7$ Hz), 2.32 (s, 3H), 2.34 (s, 3H), 3.59 (s, 3H), 4.04 (q, 2H, $J = 7$ Hz), 5.34 (s, 1H), 5.86 (s, 1H), 6.61 (t, 1H, $J = 72$ Hz), 7.10−7.20, 8.05−8.20 (m, 3H). Anal. ($C_{19}H_{20}F_2N_2O_7$) C, H, N.

2,6-Dimethyl-3-carbomethoxy-5-cyano-4-(2-difluoromethoxy-5-nitrophenyl)-1,4-dihydropyridine (9). A mixture of **7** (4.35 g, 0.02 mol), methyl 3-aminocrotonate (2.3 g, 0.02 mol), 3-aminocrotonitrile (1.64 g, 0.02 mol), and acetic acid (15 mL) was stirred and heated to reflux for 2 h. The mixture was cooled, poured into water (150 mL), and stirred vigorously for 0.5 h. The precipitate was filtered off, washed with water, and dissolved in toluene (25 mL). The toluene solution was stirred and heated to reflux with Al_2O_3 (1 g). Absorbent was filtered off, and the hot solution was diluted with hexane (25 mL) and cooled to afford the pure light yellow **8a** (3.94 g, 57%): mp 191−192 °C; ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H), 2.41 (s, 3H), 3.56 (s, 3H), 5.16 (s, 1H), 6.09 (s, 1H), 6.67 (t, 1H, $J = 72$ Hz), 7.50−8.10 (m, 3H); IR (KBr) 1110 (CF), 1715 (C═O), 2210 (CN), 3300 (NH) cm^{-1}. Anal. ($C_{17}H_{15}F_2N_3O_5$) C, H, N.

2,6-Dimethyl-3,5-dicarbomethoxy-4-(2-difluoromethoxy-5-aminophenyl)-1,4-dihydropyridine (10a). A mixture of **8a** (4.12 g, 0.01 mol), Raney nickel (0.5 g, 0.01 mol), and methanol (40 mL) was stirred for 6 h at 50−55 °C under an atmosphere of hydrogen. After filtration the warm solution was concentrated to about 25% of volume. The precipitated (after 12 h at 0 °C) solid was filtered off, washed with cold methanol (5 mL), and dried to afford the analytically pure **10a** (3.43 g, 90%): mp 203−205 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H), 3.50 (s, 6H), 3.54 (s, 2H), 4.96 (s, 1H), 5.62 (s, 1H), 6.59 (t, 1H, $J = 72$ Hz), 6.90−7.20, 7.80−7.90 (m, 3H). Anal. ($C_{18}H_{20}F_2N_2O_5$) C, H, N.

2,6-Dimethyl-3,5-dicarboethoxy-4-(2-difluoromethoxy-5-aminophenyl)-1,4-dihydropyridine (10b). Prepared from **8b** (4.38 g, 0.01 mol) by the procedure described in the preparation of **10a**, except that the reaction was held in ethanol. Yield 3.54 g (87%): mp 147−148 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 6H, $J = 7$ Hz), 2.31 (s, 6H), 3.55 (s, 2H), 4.06 (q, 4H, $J = 7$ Hz), 5.01 (s, 1H), 5.90 (s, 1H), 6.60 (t, 1H, $J = 73$ Hz), 7.05−7.15, 7.90−8.00 (m, 3H). Anal. ($C_{20}H_{24}F_2N_2O_5$) C, H, N.

2,6-Dimethyl-3,5-dicarbomethoxy-4-(2-difluoromethoxy-5-isothiocyanatophenyl)-1,4-dihydropyridine (12a). A mixture of amino derivative **10a** (3.8 g, 0.01 mol), diethylthiocarbamoyl chloride (1.7 g, 0.011 mol), and anhydrous dichloroethane (50 mL) was stirred and heated to reflux for 2 h. After cooling the resulting solution was filtered through short column (5 cm) filled with silica gel (40−100 μm) and then eluted with methylene chloride. Evaporation of solvent and recrystallization from CCl_4 afforded product **12a** (2.76 g, 65%): mp 150−151 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 6H), 3.41 (s, 6H), 5.16 (s, 1H), 5.68 (s, 1H), 6.45 (t, 1H, $J = 72$ Hz), 6.92−7.25 (m, 3H); IR (KBr) 1130 (CF), 1705 (C═O), 2060 (N═C═S), 3300 (NH) cm^{-1}. Anal. ($C_{19}H_{18}F_2N_2O_5S$) C, H, N.

2,6-Dimethyl-3,5-dicarboethoxy-4-(2-difluoromethoxy-5-isothiocyanatophenyl)-1,4-dihydropyridine (12b). Prepared from **10b** (4.1 g, 0.01 mol) by the procedure described in the preparation of **10a**. Yield 2.98 g (66%): mp 166−167 °C; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 6H, $J = 7$ Hz), 2.24 (s, 6H), 4.00 (q, 4H, $J = 7$ Hz), 5.17 (s, 1H), 5.70 (s, 1H), 6.43 (t, 1H, $J = 72$ Hz), 6.90−7.19 (m, 3H); IR (KBr) 1125 (CF), 1710 (C═O), 2050 (N═C═S), 3300 (NH) cm^{-1}. Anal. ($C_{21}H_{22}F_2N_2O_5S$) C, H, N.

2,6-Dimethyl-3-carbomethoxy-5-cyano-4-(2-difluoromethoxy-5-isothiocyanatophenyl)-1,4-dihydropyridine (13). To a vigorously stirred mixture of **9** (3.8 g, 0.01 mol), Fe sawdust (4 g, 0.07 mol), acetone (10 mL), and water (10 mL) was added dropwise concentrated ($d = 1.17$) hydrochloric acid (5 mL). The reaction mixture was stirred at 50 °C for 3 h and then filtered, and the solid was washed with water (20 mL). The solution of thiophosgen (1.3 g, 0.011 mol) in methylene chloride (5 mL) was added dropwise at ambient temperature. The resulting precipitate was dissolved in toluene (25 mL). The toluene solution was stirred and heated to reflux with Al_2O_3 (1 g). Absorbent was filtered off, and the hot solution was diluted with hexane (25 mL) and cooled to afford the pure light yellow **13** (2.0 g, 51%): mp 182−183 °C; ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H), 2.38 (s, 3H), 3.58 (s, 3H), 5.20 (s, 1H), 5.90 (s, 1H), 6.70 (t, 1H, $J = 73$ Hz), 7.00−7.90 (m, 3H); IR (KBr) 1120 (CF), 1715 (C═O), 2060 (N═C═S), 2210 (CN), 3300 (NH) cm^{-1}. Anal. ($C_{18}H_{15}F_2N_3O_3S$) C, H, N.

2,6-Dimethyl-3,5-dicarbomethoxy-4-(2-difluoromethoxy-5-*N*-(*N*′-1,2,2-trimethylpropylthiouredyl)phenyl)-1,4-dihydropyridine (14a). To a stirred suspension of **12a** (2.12 g, 5 mmol) in ethanol (10 mL) was added pinacolylamine (0.7 g, 7 mmol) dropwise at ambient temperature. The resultant solution was evaporated in vacuo. Crude product was dissolved in CCl_4 (20 mL), and the solution was stirred vigorously at −10 to −5 °C for 8−10 h until formation of the first crystals, and then it was allowed to stay at 0 °C for 3 days. Filtration and air-drying of the resultant precipitate gave analytically pure **14a** (2.53 g, 97%): mp 185−186 °C; ^{1}H NMR ($CDCl_3$) δ 0.74 (s, 9H), 1.01 (d, 3H, $J = 5$ Hz), 2.23 (s, 6H), 3.53 (s, 6H), 4.30 (m, 1H), 5.23 (s, 1H), 5.61 (d, 1H, $J = 4$ Hz), 5.91 (s, 1H), 6.49 (t, 1H, $J = 74$ Hz), 7.00−7.30 (m, 3H), 7.75 (s, 1H). Anal. ($C_{25}H_{33}F_2N_3O_5S$) C, H, N.

2,6-Dimethyl-3,5-dicarboethoxy-4-(2-difluoromethoxy-5-*N*-(*N*′-1,2,2-trimethylpropylthiouredyl)phenyl)-1,4-dihydropyridine (14b). Prepared from **12b** (2.26 g, 5 mmol) by the procedure described in the preparation of **14a**. Yield 2.54 g (92%): mp 160−161 °C; ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 9H), 1.02 (d, 3H, J = 5 Hz), 1.14 (t, 6H, J = 7 Hz), 2.22 (s, 6H), 4.00 (q, 4H, J = 7 Hz), 4.30 (m, 1H), 5.21 (s, 1H), 5.61 (d, 1H, J = 4 Hz), 5.69 (s, 1H), 6.51 (t, 1H, J = 74 Hz), 6.90−7.20 (m, 3H), 7.40 (s, 1H). Anal. ($C_{27}H_{37}F_2N_3O_5S$) C, H, N.

2,6-Dimethyl-3-carbomethoxy-5-cyano-4-(2-difluoromethoxy-5-*N*-(*N*′-1,2,2-trimethylpropylthiouredyl)phenyl)-1,4-dihydropyridine (15). Prepared from **13** (1.97 g, 5 mmol) by the procedure described in the preparation of **14a**, except that the crystallization time lasted two weeks. Yield 1.13 g (46%): mp171−172 °C; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 9H), 1.00 (d, 3H, J = 5 Hz), 2.29 (s, 6H), 5.24 (m, 1H), 5.32 (s, 1H), 5.65 (d, 1H, J = 4 Hz), 5.72 (s, 1H), 6.51 (t, 1H, J = 74 Hz), 6.90−7.25 (m, 3H), 7.45 (s, 1H). Anal. ($C_{24}H_{30}F_2N_4O_3S$) C, H, N.

2,6-Dimethyl-3,5-dicarbomethoxy-4-(2-difluoromethoxy-5-*N*-(*N*″-cyano-*N*′-1,2,2-trimethylpropylguanidyl)phenyl)-1,4-dihydropyridine (4a). A mixture of **14a** (2.1 g, 4 mmol), triphenylphosphine (1.2 g, 5 mmol), triethylamine (1.5 mL), and CCl_4 (20 mL) was stirred and heated to reflux for 8 h and then evaporated to an oil. The oil was chromatographed on a column of silica gel (40−100 μm) in CCl_4 to afford **16a** pure enough for the next synthesis. It was mixed with cyanamide (0.84 g, 20 mmol), diisopropylethylamine (0.26 g, 2 mmol), and hexane (2 mL), stirred for 5 h, and allowed to stay for 12 h at ambient temperature. The resulting solid was recrystallized from methanol to afford **4a** (1.0 g, 48%): mp 211−212 °C; ^{1}H NMR (acetone-d_6) δ 0.87 (s, 9H), 1.08 (d, 3H, J = 5 Hz), 2.30 (s, 6H), 3.56 (s, 6H), 3.85 (m, 1H), 5.32 (s, 1H), 6.93 (t, 1H, J = 74 Hz), 7.06−7.36 (m, 3H), 7.39 (d, 1H, J = 4 Hz), 8.06 (s, 1H), 8.18 (s, 1H). Anal. ($C_{26}H_{33}F_2N_5O_5$) C, H, N.

2,6-Dimethyl-3,5-dicarboethoxy-4-(2-difluoromethoxy-5-*N*-(*N*″-cyano-*N*′-1,2,2-trimethylpropylguanidyl)phenyl)-1,4-dihydropyridine (4b). Prepared from **14b** (2.2 g, 4 mmol) by the procedure described in the preparation of **4a**. Yield 0.98 g (44%): mp 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 9H), 1.01 (d, 3H, J = 5 Hz), 1.15 (t, 6H, J = 7 Hz), 2.24 (s, 6H), 4.00 (q, 4H, J = 7 Hz), 4.33 (m, 1H), 5.24 (s, 1H), 5.65 (d, J = 4 Hz, 1H), 5.75 (s, 1H), 6.50 (t, 1H, J = 73 Hz), 6.95−7.27 (m, 3H), 7.41 (s, 1H). Anal. ($C_{28}H_{37}F_2N_5O_5$) C, H, N.

2,6-Dimethyl-3-carbomethoxy-5-cyano-4-(2-difluoromethoxy-5-*N*-(*N*″-cyano-*N*′-1,2,2-trimethylpropylguanidyl)phenyl)-1,4-dihydropyridine (5). A mixture of **15** (1.0 g, 2 mmol), yellow HgO (1.3 g, 5 mmol), and toluene (40 mL) was boiled with simultaneous azeotrope distillation for 3 h while the reaction mixture was concentrated to about 25% of volume. After cooling, the mixture was filtered through a short column (1 cm) filled with silica gel (40−100 μm), eluted with toluene (10 mL), and evaporated to afford **16**. Further procedure described in the preparation of **4a** afforded **5** (0.20 g, 20%): mp 235−236 °C; ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 9H), 1.05 (d, 3H, J = Hz), 2.25 (s, 6H), 3.45 (s, 3H), 4.30 (m, 1H), 5.22 (s, 1H), 5.65 (d, 1H, J = 5 Hz), 5.80 (s, 1H), 6.51 (t, 1H, J = 74 Hz), 6.97−7.20 (m, 3H), 7.38 (s, 1H); IR (KBr) 1110 (CF), 1710 (C=O), 2200−2210 (CN), 3300 (NH) cm^{-1}. Anal. ($C_{25}H_{30}F_2N_6O_3$) C, H, N.

Drug Effects in Mesenteric Arteries. Small mesenteric arteries (third branch) were isolated from adult Wistar rats and mounted in a small vessel myograph[9] as described.[10] An isometric force of ~2 mm segments was recorded online (MacLab/4e, MacIntosh Perfoma 6200) at a resting length of 90% of the value obtained at a transmural pressure of 100 mmHg. The solution was gassed (95% O_2/5% CO_2) and maintained at 37 °C. The composition (mM) was NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $NaHCO_3$ 12, $MgCl_2$ 1.1, NaH_2PO_4 0.11, glucose 5.5. Vessels were first precontracted briefly by K^+-depolarization (KCl raised to 120 mM, with corresponding reduction of NaCl) or by norepinephrine (NE, 10^{-5} M, given at a slightly elevated KCl of 10 mM). The integrity of the endothelium was checked by applying carbachol (10^{-5} M). After washout and complete relaxation, the vessel was exposed to the other contractile stimulus (K^+-depolarization, or norepinephrine as described) for 1 h to check the stability of the preparation. After washout, arteries were allowed to recover for about 20 min, before the same long-term stimulus (K^+-depolarization or norepinephrine, respectively) was repeated for a second time. After contraction reached a stable value, test compounds were added cumulatively (usually eight concentrations at half-logarithmic intervals) to obtain a complete concentration−response curve. In some experiments, the concentration−response curve was done in the presence of glibenclamide (3×10^{-6} M). Under all conditions, the test compounds exerted a complete or near-complete (>90%) vasorelaxation in the investigated concentration range. We therefore analyzed the concentration−response curve using a logistic equation with two free parameters, i.e., $Y = 100\%/\{1 + (X/EC_{50})^H\}$, where Y is percent relaxation, X is the concentration, EC_{50} is the concentration causing half-maximal relaxation, and H is a slope factor. EC_{50} values were determined in every experiment. Data are given as mean log(EC_{50}) values.

Binding Experiments. Binding studies were performed using homogenates from rat cardiac ventricle prepared as previously described.[10,11] As a calcium channel ligand, [^{3}H](+)-isradipine (NEN) was used; and for studying the potassium channel opener site, [^{3}H]P1075 (Amersham) was employed. Equilibrium binding was measured after incubating the radioligand together with the indicated concentrations of test compounds for 1 h at 32 °C (50 mM Tris·HCl, pH 7.4) or at 37 °C (in mM: NaCl 139, KCl 5, $MgCl_2$ 25, $CaCl_2$ 1.25, HEPES 20, pH 7.4, supplemented with creatine phosphokinase 50 u/mL, creatine phosphate 20 mM, and Na_2ATP 3 mM) for calcium channel and potassium channel binding, respectively. Separation of bound and free ligand was accomplished by rapid filtration (rinsing with ice cold H_2O, or Tris buffer, respectively), and bound radioactivity was determined in triplicate assays by liquid scintillation counting. Nonspecific binding was determined in excess of unlabeled ligand (10^{-5} M of nitrendipine or of unlabeled P1075, respectively). Data are given as percentages of specific binding (amounting to >90% of total [^{3}H](+)-isradipine binding, and 56 ± 2% of total [^{3}H]P1075 binding, respectively).

Electrophysiology. Calcium channel currents were measured in the Chinese hamster ovary (CHO) cell line stably transfected with the pore-forming α_{1C-b} subunit cloned from rabbit lung.[7] Cells were grown in 35 mm polystyrene dishes serving as a bath chambers. They were used 1−3 days after plating. Patch clamp measurements of whole-cell barium currents (Axopatch 1B, Axon) were done at room temperature, using borosilicate pipets containing (mM) CsCl 120, $MgCl_2$ 3, MgATP 5, EGTA 10, and HEPES 5, at pH 7.4. The bath contained (mM) NaCl 120, $BaCl_2$ 10.8, $MgCl_2$ 1, CsCl 5.4, glucose 10, and HEPES 10 at pH 7.4. Test pulses of 100 ms duration were delivered at 10 s intervals. The experimental protocol was as follows: a current−voltage relationship was constructed using a holding potential of −80 mV. The test voltage eliciting maximum currents (+10 to +30 mV) was then used throughout. When stable currents were recorded (= 100%) using this protocol (−80 mV to test potential), the holding potential was switched to −30 mV. Then a compound was added until a steady-state effect was obtained. Finally, the holding potential was returned to −80 mV. Drug-free control experiments were done using the same time schedule and voltage protocol.

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References

(1) Petko, K. I.; Rechitskii, A. N.; Maletina, I. I.; Yagupolskii, L. M. Fluorine-containing aryl-cyanoguanidines − New drug pinacidile analogues. *Ukr. Khim. Zhurn.* **1996**, *62*, N11, 54−61.

(2) Maletina, I. I.; Petko, K. I.; Shavaran, S. S.; Klebanov, B. M.; Lozinski, M. O.; Yagupolskii, L. M. Synthesis and cardiovascular effects of fluorine-containing arylcyanoguanidines (pinacidil analogues). *Khim.-Farm. Zhurn.* **1997**, *31*, N6, 11−13.
(3) Szewczyk, A.; Marban, E. Mitochondria: a new target for K^+ channel openers? *Trends Pharmacol. Sci.* **1999**, *20*, 157−161.
(4) Kastron, V. V.; Duburs, G. Ya.; Vitolin′, R. O.; Skrastin'sh, I. P.; Kimenis, A. A. Synthesis and pharmacological activity of 4-(*o*-difluoromethoxyphenyl)-1,4-dihydropyridines. *Khim.-Farm. Zhurn.* **1985**, *19*, N5, 545−549.
(5) Shelyazhenko, S. V.; Fialkov, Yu. A.; Yagupolskii, L. M. Synthesis and reactions of difluoromethoxy- and difluorochloromethoxy derivatives of benzene. *Zhurn. Org. Khim.* **1992**, *28*, N8, 1652−1659.
(6) Meisheri, K. D.; Swirtz, M. A.; Purohit, S. R.; Cipkus-Dubay, L. A.; Khan, S. A.; Oleynek, J. J. Characterization of K^+ channel-dependent as well as -independent components of pinacidil-induced vasodilation. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 492−499.
(7) Bosse, E.; Bottlender, R.; Kleppisch, T.; Hescheler, J.; Welling, A.; Hofmann, F.; Flockerzi, V. Stable and functional expression of the calcium channel alpha$_1$ subunit from smooth muscle in a somatic cell line. *EMBO J.* **1992**, *11*, 2033−2038.
(8) Bean, B. P. Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6388−6392.
(9) Mulvany, M. J.; Halpern, W. Contractile properties of small arterial resistance vessels in normotensive and spontaneously hypertensive rats. *Circ. Res.* **1977**, *41*, 19−26.
(10) Herzig, S.; Jischa, J.; Beinhauer, A.; Geirhos, B.; Tacke, K.; Hempelmann, R. G. Quinidine-induced potentiation of cardiovascular effects of nitrendipine: functional aspects and possible molecular mechanisms. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1995**, *351*, 636−643.
(11) Löffler-Walz, C.; Quast, U. Binding of K_{ATP} channel modulators in rat cardiac membranes. *Br. J. Pharmacol.* **1998**, *123*, 1395−1402.

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