Imidazo[2,1-*b*]thiazole System: A Scaffold Endowing Dihydropyridines with Selective Cardiodepressant Activity

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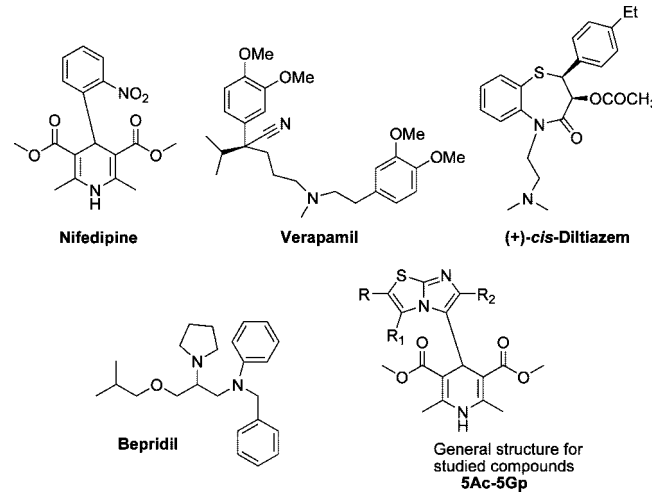
The synthesis, characterization, and functional in vitro assays in cardiac tissues and smooth muscle (vascular and nonvascular) of a number of 4-imidazo[2,1-*b*]thiazole-1,4-dihydropyridines are reported. The binding properties for the novel compounds have been investigated and the interaction with the binding site common to other aryl-dihydropyridines has been demonstrated. Interestingly, the novel 4-aryl-dihydropyridines are L-type calcium channel blockers with a peculiar pharmacological behavior. Indeed, the imidazo[2,1-*b*]thiazole system is found to confer to the dihydropyridine scaffold an inotropic and/or chronotropic cardiovascular activity with a high selectivity toward the nonvascular tissue. Finally, molecular modeling studies were undertaken for the most representative compounds with the aim of describing the binding properties of the new ligands at molecular level and to rationalize the found structure–activity relationship data. Due to the observed pharmacological behavior of our compounds, they might be promising agents for the treatment of specific cardiovascular pathologies such as cardiac hypertrophy and ischemia.

Introduction

Voltage-gated Ca^{2+} channels are heteromultimeric transmembrane proteins that are made of four or five subunits (α_1, α_2, δ, β, γ). Among them, the main α_1 subunit allows the selective passage of Ca^{2+} ions into excitable cells and controls the voltage sensitivity and the gating mechanism.[1] These proteins have a crucial role in a broad range of cellular processes, such as neurotransmitter release, second messenger cascades, cardiac excitation and contraction, and gene regulation supporting learning and memory.[2] Due to their important functions, voltage-gated Ca^{2+} channels have been extensively studied and, to date, different drugs are available that are known to interact with them. Among the others, L-type calcium channel (LTCC[a]) blockers have gained a critical role in the treatment of different cardiovascular pathologies. The above-mentioned drugs can be divided in four structurally different classes: 1,4-dihydropyridines (1,4-DHPs), such as Nifedipine, phenylalkylamines (PAAs), such as Verapamil, 1,5-benzothiazepines (BTZs), such as (+)-*cis*-Diltiazem, and pyrrolidineethanamine, such as Bepridil (Chart 1).

Each class of LTCC blockers exhibits distinct characteristics in their cardiovascular profiles in mediating antihypertensive, antianginal and antiarrhythmic activities.[3] In particular, Verapamil and Diltiazem are essentially nonselective and 1,4-DHPs usually display a vascular selectivity. One obvious reason for the different tissue-selectivity has to be ascribed to the different topographic localization of their binding sites of the channel. It has been demonstrated that these drugs bind at distinct sites within the α_1 subunit of the L-type Ca^{2+} channel (Ca_V1) and they noncompetitively affect each other's binding.[3] Moreover, it can also be argued that the targeted subclass of L-type channel and the state-dependent interactions of LTCC blockers with the protein might have a role in determining tissue selectivity. In addition to the above-described factors, small structural variation on the 1,4-DHPs scaffold led to significant differences in voltage sensitivity of binding, which has a direct influence on the vascular/cardiac selectivity. Interestingly, in our earlier works we demonstrated that compounds such as fluodipine displayed a high cardiac selectivity.[4] The differential tissue-selectivity displayed by LTCC blockers has allowed their employment as "specific probes" for the pharmacological and structural characterization of the Ca_V1 channel.[5] To date, several α_1 subunits

Chart 1



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[a] Abbreviations: LTCC, L-type calcium channel; 1,4-DHPs, 1,4-dihydropyridines; PAAs, phenylalkylamines; BTZs, 1,5-benzothiazepines; GPILSM, guinea-pig ileum longitudinal smooth muscle.

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have been described and the sequence relationship for these channel subtypes has also been disclosed.[6] Recently, the α_1 subunits have been classified in α_{1S} found in skeletal muscle,[7] α_{1C} mainly cardiac,[8] α_{1D} in neurons and heart,[9] and α_{1F} retinal not yet functionally expressed.[10] The classification of the L-type voltage dependent calcium channel family nomenclature was recently revised accordingly by Ertel et al. in $Ca_v1.1$ (α_{1S}) found in skeletal muscle; $Ca_v1.2$ (α_{1C}) found in cardiac, smooth, and endocrine tissues; $Ca_v1.3$ (α_{1D}) found in brain, ear, and endocrine tissues; $Ca_v1.4$ (α_{1F}) found on retina, respectively.[1,6] Unfortunately, to date, a comprehensive description of the sensitivity of the different channel subtypes toward 1,4-DHPs is still unavailable.[11] Indeed, there are subtype-specific differences in ligand recognition properties for the LTCC; these differences are extremely common for a large number of receptor types and subtypes specific agonists and antagonists. From this point of view, the availability of tissue-selective LTCC blockers could facilitate an accurate description of the pharmacological profile of these channels. On the other hand, tissue selective LTCC blockers might also have an enhanced therapeutic utility in specific cardiovascular pathologies. In this context, it is worth noting that the 1,4-DHP nucleus appears to be an interesting structure interacting with a wide variety of channel and receptors and is an example of a "privileged structure": a core structure that by appropriate molecular decoration can be directed to diverse pharmacological tissues.[12] The term "privileged structure" introduced by Evans et al.[13] prompts that adequate modification of such a structure could be a valid route in the search for new LTCC blockers with well-defined channel subtype selectivity. Interestingly, in a previous work promising results were obtained by testing a series of different 4-imidazothiazole-DHPs for their antiarrhythmic, inotropic, and chronotropic properties.[14] Therefore, in the present paper starting from these encouraging results we designed and synthesized a small library of imidazo[2,1-*b*]thiazole derivatives (Chart 1) with the aim of identifying new tissue selective 1,4-DHPs. In particular, taking into account that the double bond at the position 2,3 is necessary for the activity and the 2-methyl derivatives showed antiarrhythmic but not cardiodepressant activity,[14] to define the structure–activity relationship we performed the synthesis of new analogues bearing: unsubstituted at the 2,3-position; a chlorine or a small aliphatic group (methyl, ethyl, prophyl) at the position 2; a methyl at the position 3. In addition, based on reported results[14] to investigate the influence of the substitution at the position 6, we consider the following feature as suitable pharmacophoric groups: chlorine, methyl, trifluoromethyl, unsubstituted/substituted phenyl ring. Moreover, molecular modeling studies were undertaken with the aim of describing the binding properties of the new ligands at molecular level and to rationalize the structure–activity relationship (SAR) data could, in turn, prospectively guide the design of novel analogues endowed of the same pharmacological properties.

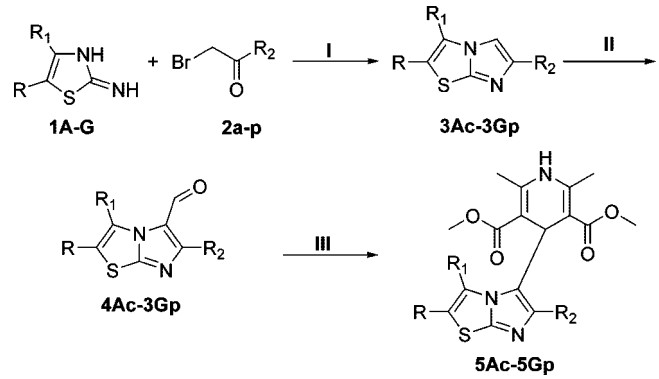
Chemistry

1,4-DHPs **5**, reported in Scheme 1, were synthesized by means of the well-known Hantzsch reaction[15] with the appropriate aldehydes **4**, obtained in turn by means of the Vilsmeier method on the corresponding imidazo[2,1-*b*]thiazoles **3**. The bicyclic heterocycles were obtained by cyclocondensation using the appropriate 2-aminothiazoles **1** and bromoketones **2**.

Pharmacology

Functional Assays. All compounds were checked at increasing doses to evaluate the percent decrease of inotropic and chronotropic effects on guinea pig isolated left atria driven at 1 Hz and on right atria spontaneously beating, respectively. The vasorelaxant activity was tested on K^+-depolarized (80 mM) aortic strips. Details of which have been reported in Supporting Information. For some selected compounds, the investigation was extended on determination of the relaxant activity on nonvascular smooth muscle: K^+-depolarized (80 mM) guinea-pig ileum longitudinal smooth muscle (GPILSM). According to Langendorff, the guinea-pig isolated perfused heart was used to assay the whole cardiac activity of compound **5Cb** and of the reference Nifedipine. Compounds were checked at increasing concentrations to evaluate changes in left ventricular pressure (inotropic activity), heart rate (chronotropic activity), coronary perfusion pressure (coronary activity), and electrocardiogram (ECG) signal.

Scheme 1[a]



[a] Reagents and conditions: (**I**) (a) acetone reflux; (b) hydrochloridric acid 2 N reflux; (**II**) phosphorus oxychloride, *N*,*N*-dimethylformamide reflux; (**III**) methyl acetoacetate, ammonia solution 30%, isopropyl alcohol. For R, R_1, and R_2, see Table 1.

Data were analyzed using Student's *t*-test and are presented as mean ± S.E.M.[16] Because the drugs were added in cumulative manner, the difference between the control and the experimental values at each concentration were tested for a *P* value <0.05. The potency of drugs defined as EC_{50}, EC_{30}, and IC_{50} was evaluated from log concentration–response curves (Probit analysis using Litchfield and Wilcoxon[16] or GraphPad Prism software[17,18]) in the appropriate pharmacological preparations.

Binding Experiments. The compounds were screened for their affinity for calcium channels from guinea pig heart ventricles. Binding site was determined by using a competitive radiometric receptor binding assays. PN200-110, (+)-[5-methyl-^{3}H] was used to label 1,4-DHPs binding site. Binding affinities were expressed as K_i and IC_{50} values (mean ± S.E.M.).[19] Data were analyzed with Student's *t*-test. The criterion for significance was a *P* value of <0.001.

Displacement binding assays were also carried out on intact HEK-293 cells transfected with either the rabbit Cav1.2a ($\alpha 1_{c\text{-}a}$) or the rabbit Cav1.2b ($\alpha 1_{c\text{-}b}$) coding plasmid. Plasmids were a generous gift by Dr. Andrea Welling. PN200-110, (+)-[5-methyl-^{3}H] was used to label 1,4-DHPs binding sites. Binding affinities were expressed as IC_{50} values (mean ± S.E.M.).[19]

Results and Discussion

A-Functional Studies. A small library of 1,4-DHPs bearing in C-4 a different substituted imidazo[2,1-*b*]thiazole system was designed and synthesized (**5Ac**−**5Gq**, Chart 1). All compounds were tested for their cardiovascular profile on guinea pig left atrium driven at 1 Hz and on spontaneously beating right atrium to evaluate their inotropic and chronotropic effects, respectively,

Table 1. New 1,4-Dihydropyridines **5Ac**−**5Gp**[a]

cmpd	R	R_1	R_2 (a−r)	formula	MW	yield %	Mp, °C (purif.)
5Ac	H	H	CF_3	$C_{18}H_{16}F_3N_3O_4S$	427.4010	10	222–224 (A)
5Ae	H	H	2-$(CF_3)C_6H_4$	$C_{23}H_{20}F_3N_3O_4S$	491.4867	40	223–227 (A)
5Af	H	H	3-$(CF_3)C_6H_4$	$C_{23}H_{20}F_3N_3O_4S$	491.4867	15	94 (A)
5Ag	H	H	2-$(OCF_3)C_6H_4$	$C_{23}H_{20}F_3N_3O_5S$	507.4861	12	215–216 (D)
5Ah	H	H	3-$(OCF_3)C_6H_4$	$C_{23}H_{20}F_3N_3O_5S$	507.4861	10	171–172 (D)
5Ai	H	H	2-$(OCH_3)C_6H_4$	$C_{23}H_{23}N_3O_5S$	453.5147	10	226–228 (B)
5Aj	H	H	3-$(OCH_3)C_6H_4$	$C_{23}H_{23}N_3O_5S$	453.5147	10	217–220 (B)
5Ak	H	H	2,5-$(OCH_3)_2C_6H_3$	$C_{24}H_{25}N_3O_6S$	483.5408	14	220–222 (A)
5Al	H	H	3,5-$(OCH_3)_2C_6H_3$	$C_{24}H_{25}N_3O_6S$	483.5408	10	218–220 (B)
5Am	H	H	3,4-$(OCH_3)_2C_6H_3$	$C_{24}H_{25}N_3O_6S$	483.5408	10	180–182 (D)
5An	H	H	2,4-$(OCH_3)_2C_6H_3$	$C_{24}H_{25}N_3O_6S$	483.5408	8	189–191 (G)
5Ao	H	H	3,4,5-$(OCH_3)_3C_6H_2$	$C_{25}H_{27}N_3O_7S$	513.5669	10	194–196 (E)
5Ap	H	H	6-(NO_2)2,5-$(OCH_3)_2C_6H_2$	$C_{24}H_{24}N_4O_8S$	528.5385	10	195–197 (C)
5Aq	H	H	4-(NO_2)2,5-$(OCH_3)_2C_6H_2$	$C_{24}H_{24}N_4O_8S$	528.5385	20	196–198 (C)
5Ar	H	H	6-(NO_2)3,4-$(OCH_3)_2C_6H_2$	$C_{24}H_{24}N_4O_8S$	528.5385	15	200–202 (E)
5Ba	Cl	H	CH_3	$C_{17}H_{18}ClN_3O_4S$	395.8637	15	225–228 (B)
5Bb	Cl	H	Cl	$C_{16}H_{15}Cl_2N_3O_4S$	416.2818	16	230–232 (B)
5Bd	Cl	H	C_6H_5	$C_{22}H_{20}ClN_3O_4S$	457.9335	18	200–203 (A)
5Bk	Cl	H	2,5-$(OCH_3)_2C_6H_3$	$C_{24}H_{24}ClN_3O_6S$	517.9857	10	226–228 (B)
5Bp	Cl	H	6-(NO_2)2,5-$(OCH_3)_2C_6H_2$	$C_{24}H_{23}ClN_4O_8S$	562.9833	10	219–221 (B)
5Bq	Cl	H	4-(NO_2)2,5-$(OCH_3)_2C_6H_2$	$C_{24}H_{23}ClN_4O_8S$	562.9833	15	228–231 (B)
5Ca	CH_3	H	CH_3	$C_{18}H_{21}N_3O_4S$	375.4455	24	210–213 (B)
5Cb	CH_3	H	Cl	$C_{17}H_{18}ClN_3O_4S$	395.8637	10	231–235 (E)
5Cc	CH_3	H	CF_3	$C_{18}H_{18}F_3N_3O_4S$	429.4169	18	270–275 (E)
5Ck	CH_3	H	2,5-$(OCH_3)_2C_6H_3$	$C_{25}H_{27}N_3O_6S$	497.5675	11	217–222 (D)
5Cp	CH_3	H	6-(NO_2)2,5-$(OCH_3)_2C_6H_2$	$C_{25}H_{26}N_4O_8S$	542.5652	10	99–105 (A)
5Cq	CH_3	H	4-(NO_2)-2,5-$(OCH_3)_2C_6H_2$	$C_{25}H_{26}N_4O_8S$	542.5652	10	200–203 (E)
5Db	H	CH_3	Cl	$C_{17}H_{18}ClN_3O_4S$	395.8637	10	215–219 (F)
5Dd	H	CH_3	C_6H_5	$C_{23}H_{23}N_3O_4S$	437.5153	8	201–204 (B)
5Eb	CH_3	CH_3	Cl	$C_{18}H_{20}ClN_3O_4S$	409.8903	15	220–223 (F)
5Ed	CH_3	CH_3	C_6H_5	$C_{23}H_{25}N_3O_4S$	439.5312	6	192–194 (A)
5Fk	C_2H_5	H	2,5-$(OCH_3)_2C_6H_3$	$C_{26}H_{29}N_3O_6S$	511.5942	22	210–211 (B)
5Gp	C_3H_7	H	6-(NO_2)2,5-$(OCH_3)_2C_6H_2$	$C_{27}H_{30}N_4O_8S$	570.6185	19	244–248 (B)

[a] Purified by (A) flash chromatography, eluent acetone−petroleum ether, 40–60 °C from 1.9 to 4.6, v/v, to afford an oil that was crystallized from acetone−petroleum ether, 40–60 °C; (B) flash chromatography, eluent acetone−petroleum ether, 40–60 °C from 1.9 to 4.6, v/v, to afford an oil that was crystallized from acetone; (C) flash chromatography, eluent acetone−petroleum ether, 40–60 °C from 1.9 to 4.6, v/v, to afford an oil that was crystallized from methanol−acetone; (D) flash chromatography, eluent acetone−petroleum ether, 40–60 °C from 1.9 to 4.6, v/v, to afford an oil that was triturated with ethanol−hexane, 2:8 v/v; (E) flash chromatography, eluent acetone−petroleum ether, 40–60 °C from 1.9 to 4.6, v/v; (F) flash chromatography, eluent acetone−diethyl ether, 0.5:9.5 v/v; (G) flash chromatography, eluent diethyl ether, to afford an oil that was triturate with acetone.

and on K^+-depolarized (80 mM) guinea pig aortic strips to assess their vasorelaxant activity. Data are collected in Table 2 using Nifedipine as the reference drug. All the synthesized compounds showed a marked selectivity for the cardiac over vascular tissue (Table 2) and, for the tested ones, a selective nonvascular over vascular activity (Table 3). As a matter of fact, none of them possesses a vasorelaxant activity on the K^+-depolarized aortic strips greater than 50% (Table 2), whereas they showed a relaxation activity on K^+-depolarized guinea-pig ileum longitudinal smooth muscle between 51 and 97% (Table 3). In the series, only three (**5Ao**, **5Ca**, and **5Cc**) of 33 compounds synthesized displayed a very low intrinsic activity on all considered parameters.

A significant group of compounds (11 of 33) were endowed of negative inotropic selectivity: **5Ae**, **5Ag**, **5Ak**, **5Ap**, **5Ba**, **5Bb**, **5Cb**, **5Cp**, **5Db**, **5Eb**, and **5Gp** with potency between 0.039 and 1.96 μM. Among them, eight compounds (**5Ae**, **5Ag**, **5Ba**, **5Bb**, **5Cb**, **5Cp**, **5Eb**, and **5Gp**) were more potent than the reference Nifedipine [$EC_{50} = 0.026$ μM (c.l. 0.018–0.036)]; in particular, **5Gp** was found to be the most potent as a negative inotropic agent [$EC_{50} = 0.039$ μM (c.l. 0.030–0.051)] even if its efficacy resulted to be lower if compared with that of Nifedipine.

As shown in Figure 1, most of the tested compounds (**5Ac**, **5Af**, **5Ah**, **5Ai**, **5Aj**, **5Am**, **5An**, **5Aq**, **5Ar**, **5Bd**, **5Bk**, **5 Bq**, **5Ck**, **5Cq**, **5Dd**, **5Ed**, and **5Fk**) showed both negative inotropic and chronotropic properties with different selectivity. In particular, different from the reference Nifedipine, they largely showed a negative inotropic over chronotropic selectivity. Inside this group, only two compounds **5Aj** and **5Fk** were revealed to have weak negative chronotropic selectivity. Among these compounds, **5Cq** and **5Aq** are the most potent and selective negative inotropic agents [$EC_{50} = 0.026$ μM (c.l. 0.018–0.036); $EC_{50} = 0.054$ μM (c.l. 0.036–0.079), respectively]. Compounds **5Cq** and **5Aq** are 10- and 5-fold more potent than Nifedipine, respectively, as negative inotropic agents; moreover, they showed a good negative chronotropic/inotropic ratio (81 and 73, respectively). Both **5Aj** and **5Fk** showed a slight negative chronotropic activity with an inotropic/chronotropic ratio of 3. It is well-known that calcium entry blockers, such as Nifedipine, have relevant inhibitory effects on nonvascular smooth muscle.[20] Deeper investigation tested the relaxant activity of some selected compounds on K^+ depolarized (80 mM; GPILSM). Data are collected in Table 3. For all tested compounds, we observed an increased activity in nonvascular with respect to vascular smooth muscle, even if they are, on the whole, less potent than the reference Nifedipine. Compounds **5Ak**, **5Ap**, and **5Gp**, selective negative inotropic compounds, are inactive on guinea pig aortic strips, whereas the potency on nonvascular smooth muscle are in a 11–13 μM range [$IC_{50} = 12.35$ μM (c.l. 8.56–17.80); $IC_{50} = 11.04$ μM (c.l. 8.18–14.90); $IC_{50} = 12.81$ μM (c.l. 10.00–16.41), respectively]. Compound **5Ao** acted selectively on nonvascular smooth muscle with a significant potency [$EC_{50} = 0.55$ μM (0.43–0.69)]. Negative chronotropic selective compound **5Al** [$EC_{30} = 0.86$ μM (c.l. 0.653–0.99)] is 36-fold less potent then Nifedipine, relaxing guinea pig longitudinal smooth muscle. Compound **5Cq** revealed an interesting activity because it was active on left and right atria and on nonvascular

Table 2. Cardiovascular Activity of Compounds **5Ac**−**5Gp**

	% decrease (M ± SEM)		EC_{50} of inotropic negative activity		EC_{30} of chronotropic negative activity		vasorelaxant activity
cmpd	negative inotropic activity[a]	negative chronotropic activity[b]	EC_{50}[c] (μM)	95% conf lim ($\times 10^{-6}$)	EC_{30}[c] (μM)	95% conf lim ($\times 10^{-6}$)	activity[d] (M ± SEM)
5Ac	90 ± 3.8[f]	54 ± 2.7[g]	0.071	0.021–0.14	0.57	0.22–0.87	34 ± 2.1[i]
5Ae	56 ± 2.4[g]	25 ± 1.8	0.093	0.068–0.12			24 ± 1.4
5Af	89 ± 3.3[h]	78 ± 2.5	0.31	0.22–0.42	1.90	1.75–2.03	27 ± 1.3[h]
5Ag	67 ± 2.0[i]	47 ± 1.4	0.075	0.050–0.093			47 ± 1.8
5Ah	86 ± 0.7[h]	69 ± 4.3[i]	0.83	0.60–1.04	1.18	0.83–1.36	26 ± 1.3
5Ai	54 ± 1.1	95 ± 3.7[l]	1.97	1.71–2.31	1.99	1.65–2.24	28 ± 1.7
5Aj	90 ± 3.7	94 ± 2.0[i]	1.90	1.65–2.27	0.55	0.34–0.78	48 ± 1.7[f]
5Ak	92 ± 3.4[h]	31 ± 2.1	0.44	0.29–0.65			24 ± 1.4
5Al	26 ± 1.3	90 ± 0.5[i]			0.86	0.65–0.99	40 ± 2.4[i]
5Am	61 ± 2.7	80 ± 1.9[h]	2.64	2.03–3.01	1.44	1.39–1.77	15 ± 0.9[h]
5An	71 ± 2.8[h]	55 ± 2.5	1.24	0.93–1.48	5.88	4.93–6.12	42 ± 3.1
5Ao	42 ± 0.2[g]	25 ± 1.5[i]					44 ± 3.7
5Ap	71 ± 0.9[f]	12 ± 0.9[l]	0.36	0.25–0.51			17 ± 0.9
5Aq	54 ± 3.4[i]	67 ± 2.3	0.054	0.036–0.079	3.97	3.45–4.18	35 ± 2.9[h]
5Ar	91 ± 1.4[h]	90 ± 0.8[h]	1.34	0.94–1.88	6.97	6.03–7.24	35 ± 1.7
5Ba	63 ± 4.7[h]	30 ± 2.4	0.13	0.079–0.20			24 ± 1.8[h]
5Bb	77 ± 2.9[h]	37 ± 3.5	0.18	0.13–0.23			34 ± 2.9[h]
5Bd	58 ± 4.1	87 ± 3.4	1.96	1.67–2.35	2.14	1.78–2.43	22 ± 1.3[h]
5Bk	55 ± 2.3	84 ± 2.7[l]	0.90	0.56–1.44	0.36	0.095–0.58	11 ± 1.0
5Bp	42 ± 2.1[g]	64 ± 3.6[l]			11.16	8.71–13.43	32 ± 2.2
5Bq	58 ± 3.2[f]	90 ± 2.4[i]	0.36	0.22–0.59	0.31	0.27–0.62	19 ± 1.4[f]
5Ca	43 ± 3.3[j]	33 ± 2.6[h]					38 ± 1.5
5Cb	63 ± 2.7[g]	22 ± 0.7	0.056	0.041–0.076			19 ± 1.3[h]
5Cc	46 ± 1.3[g]	36 ± 1.7					28 ± 1.5[h]
5Ck	78 ± 0.9[h]	58 ± 3.4	1.43	1.02–1.94	1.36	1.03–1.75	15 ± 0.9
5Cp	71 ± 0.1[i]	47 ± 1.2[l]	0.093	0.063–0.14			27 ± 1.9
5Cq	75 ± 3.6[i]	87 ± 5.3	0.026	0.018–0.036	2.11	1.86–2.35	32 ± 2.5
5Db	79 ± 3.1[g]	39 ± 1.5	0.103	0.08–0.15			47 ± 1.1[h]
5Dd	59 ± 4.2	88 ± 3.6[i]	0.83	0.55–1.21	0.64	0.23–0.91	30 ± 1.6[h]
5Eb	73 ± 3.4[i]	29 ± 1.9[i]	0.081	0.056–0.11			29 ± 1.7[f]
5Ed	76 ± 3.4	94 ± 2.4[i]	0.59	0.43–0.81	0.33	0.25–0.56	12 ± 1.1
5Fk	72 ± 3.6[h]	92 ± 2.7	2.24	1.85–2.43	0.73	0.52–0.97	29 ± 2.4[h]
5Gp	55 ± 1.6[h]	25 ± 1.3[i]	0.039	0.030–0.051			5 ± 0.3
Nif[e]	97 ± 2[f]	85 ± 4.2[k]	0.26	0.19–0.36	0.025	0.019–0.031	82 ± 1.3[g]

[a] Decrease in developed tension in isolated guinea-pig left atrium at 10^{-4} M, expressed as percent changes from the control ($n = 5$–6). The left atria were driven at 1 Hz. 10^{-4} M gave the maximum effect for most compounds. [b] Decrease in atrial rate on guinea-pig spontaneously beating isolated right atrium at 10^{-5} M, expressed as percent changes from the control ($n = 7$–8). Pretreatment heart rate ranged from 165 to 190 beats/min. 10^{-5} M gave the maximum effect for most compounds. [c] Calculated from log concentration–response curves (Probit analysis by Litchfield and Wilcoxon with $n = 6$–7).[16] When the maximum effect was <50%, the EC_{50} ino., EC_{30} chrono., IC_{50} values were not calculated. [d] Percent inhibition of calcium-induced contraction on K^+-depolarized guinea-pig aortic strip at 10^{-4} M ($n = 5$–6). 10^{-4} M gave the maximum effect for most compounds. [e] $IC_{50} = 0.009$ μM (c.l. 0.003–0.02). [f] At the 10^{-5} M. [g] At the 10^{-6} M. [h] At the 5×10^{-5} M. [i] At the 5×10^{-6} M. [j] At the 5×10^{-7} M. [k] At the 10^{-7} M. [l] At the 10^{-4} M.

Table 3. Relaxant Activity of Some Compounds and Nifedipine on K^+-Depolarized Guinea Pig Ileum Longitudinal Smooth Muscle

cmpd	activity[a] (M ± SEM)	IC_{50}[b] (μM)	95% conf lim ($\times 10^{-6}$)
5Ae	92 ± 1.5	1.66	1.36–2.05
5Ag	81 ± 3.2[c]	1.95	1.54–2.47
5Aj	51 ± 2.4[d]	0.083	0.066–0.103
5Ak	51 ± 0.6	12.35	8.56–17.80
5Al	89 ± 3.5[e]	0.055	0.043–0.070
5Ao	64 ± 2.6[f]	0.55	0.43–0.69
5Ap	86 ± 5.2[g]	11.04	8.18–14.90
5Aq	97 ± 1.4	2.56	1.90–3.11
5Cb	93 ± 0.5	2.06	1.55–2.73
5Cq	94 ± 3.5[f]	0.24	0.19–0.29
5Gp	89 ± 2.4[g]	12.81	10.00–16.41
Nif	70 ± 0.36[h]	0.0015	0.0011–0.0022

[a] Percent inhibition of calcium-induced contraction on K^+-depolarized (80 mM) guinea-pig longitudinal smooth muscle at 10^{-5} M. The 10^{-5} M concentration gave the maximum effect for most compounds. [b] Calculated from log concentration–response curves (Probit analysis according to Litchfield and Wilcoxon[16] with $n = 6$–8). When the maximum effect was <50%, the IC_{50} values were not calculated. [c] At 5×10^{-6} M. [d] At 10^{-7} M. [e] At 5×10^{-7} M. [f] At 10^{-6} M. [g] At 10^{-4} M. [h] At 5×10^{-9} M.

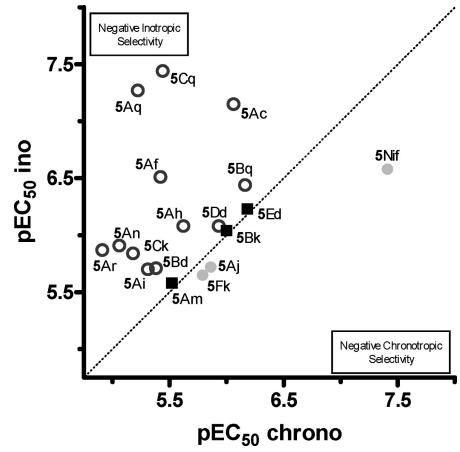


Figure 1. Correlation between negative chronotropic and inotropic potencies (pEC_{50}) for some of the new synthesized 1,4-DHPs. (○) Compounds with selective negative inotropic potency. (●) Compounds with selective negative chronotropic potency. (■) Compounds without selectivity between negative inotropic and chronotropic activities.

smooth muscle with a good selectivity between the three parameters. The peculiar cardiovascular profile of **5Cb** was more deeply investigated by comparing the negative inotropic and chronotropic activities, coronary relaxation, and electrocardiogram (ECG) effects with those of Nifedipine on isolated perfused heart according to Langendorff (Table 4). In perfused Langendorff models, 1,4-DHPs produce ECG alterations such as prolongation of ventricular repolarization time (QT interval), and at high concentrations, they might prolong the atrioven-

Table 4. Cardiac Parameters of **5Cb** and Nifedipine Evaluated in the Isolated Perfused Guinea Pig Spontaneously Beating Heart

cmpd		% change[a]
Nif	+(dP/dt)max[b]	−96 ± 3[g]
	HR[c]	−60 ± 2.3[g]
	CPP[d]	−36 ± 3.9[h]
	PR[e]	+66 ± 2[h]
	QT[f]	+37 ± 2[g]
5Cb	+(dP/dt)max	−35 ± 1.5
	HR	+6 ± 0.5
	CPP	+14 ± 0.9
	PR	+61 ± 3.1
	QT	−16 ± 1.1

[a] Percentage change of the basal value at the highest concentration tested (10 μM). Each value corresponds to the mean ± SEM (GraphPad Prism Software,[17,18] n = 4–6). [b] +(dP/dt)max: maximal rate of the rise in left ventricular pressure. [c] HR: heart rate calculated from ECG signal. [d] CPP: coronary perfusion pressure. [e] PR: atrio-ventricular conduction time. [f] QT: ventricular repolarization time. [g] At the 10^{-8} M. [h] At the 10^{-6} M.

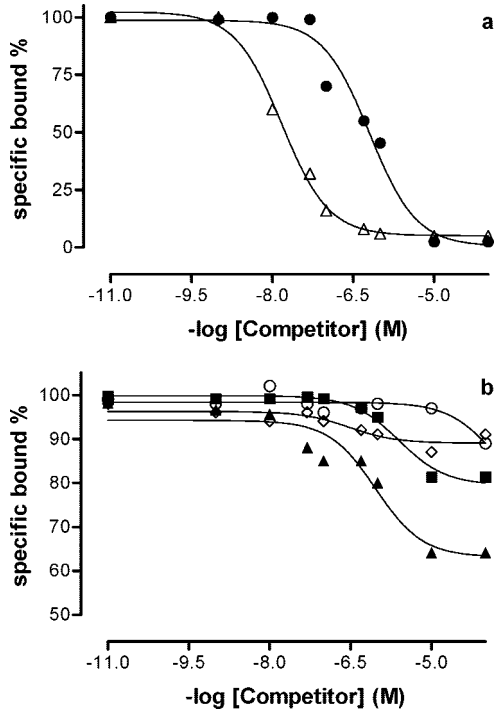


Figure 2. Effect of Nifedipine (△) and **5Al** (●) (**a**) and **5Ac** (■), **5Ag** (▲), **5Cb** (◇), and **5Cq** (○) (**b**) on PN 200-110, (+)-[5-methyl-^{3}H] binding on membranes prepared from guinea pig ventricular myocardium. Points are the mean from three independent experiments (S.E.M. smaller than the symbols).

tricular conduction time (PR interval).[21,22] In our model, Nifedipine showed to prolong the PR interval only at the highest tested concentrations. This behavior might be related to the depression of the heart rate (HR) and to first-degree atrio-ventricular blocks that occasionally occur. In the same way, **5Cb** prolonged PR interval but slightly reduced QT interval, avoiding arising of the long QT syndrome.

B-Binding Assays. For some selected compounds, binding assays on guinea pig ventricular membranes were carried out to establish their binding affinity to the 1,4-DHP-sensitive site. PN200-110, (+)-[5-methyl-^{3}H] (0.5nM) was incubated with increasing concentrations (0.1−50 μM) of the compounds as described in Supporting Information. Data presented in Figure 2, showed the effects of **5Al (a)** and **5Ac**, **5Ag**, **5Cb**, **5Cq (b)** on PN200-110, (+)-[5-methyl-^{3}H] binding. Compounds affect the binding of PN200-110, (+)-[5-methyl-^{3}H], with different affinities which could be related to their different selectivity on functional studies. As shown in Figure 2a, **5Al** that is endowed with a selective negative chronotropic activity causes a concentration-dependent inhibition of PN200-110, (+)-[5-methyl-^{3}H] binding with pK_i of 6.51, whereas nifedipine pK_i was 8.83. On the contrary, **5Ac** (pK_i = 5.35) and **5Cq** (pK_i < 5), which possess both negative inotropic and chronotropic activity, or **5Ag** (pK_i = 6.8) and **5Cb** (pK_i < 5), which show a selective negative inotropic activity, interact with the 1,4-DHPs binding site with low affinity (Figure 2b). It is worth noting that the discrepancy between functional and binding studies are not unusual and several explanations for this peculiarity are possible: the existence of high and low affinity sites for 1,4-DHPs and their ability to bind other receptors; the affinity of the 1,4-DHPs for the binding site is dependent upon the conformation of the receptor, which is itself dependent on membrane potential; each 1,4-DHP may have unique effects on calcium channel gating.[23] In addition, some 1,4-DHPs have demonstrated activity at a variety of potassium and sodium channels.[24] Of particular interest are 1,4-dihydropyridines that are activators of K^+_{ATP} channels, where they are more active than at Ca^{2+} channels.[25] 1,4-DHPs also display activity at several G-protein-coupled receptors and this is of interest because in several systems significant selectivity of action occurs. Thus, the combination of some of these factors could contribute to explain some differences between radioligand binding and functional studies and will stimulate further investigation to establish the activity and/or selectivity toward other channels and receptors.

To further examine whether compounds **5Ac**, **5Al**, and **5Cq** bind directly to the $\alpha1_c$ subunit or allosterically modulate it, their effects on PN200-110, (+)-[5-methyl-^{3}H] specific binding were evaluated in intact HEK-293 cells stably expressing the cardiac $\alpha1_{c\text{-}a}$ or the vascular $\alpha1_{c\text{-}b}$ subunit.[26] No detectable specific binding of PN200-110, (+)-[5-methyl-^{3}H] was observed in non-transfected HEK-293 cells (data not shown). In transfected cells, the nonspecific binding of PN200-110, (+)-[5-methyl-^{3}H] was approximately 10–20% of the total binding.

Displacement of PN200-110, (+)-[5-methyl-^{3}H] specific binding by nifedipine, **5Ac**, **5Al**, and **5Cq** in transfected HEK-293 is shown in Figure 3. In agreement with literature data,[26] nifedipine exhibited a lower affinity for the recombinant $\alpha1_{c\text{-}a}$ (IC_{50} = 45 nM; Figure 3a) than $\alpha1_{c\text{-}b}$ (IC_{50} = 3.6 nM; Figure 3b) subunit. Similarly to nifedipine, compound **5Al** exhibited a lower affinity for the recombinant $\alpha1_{c\text{-}a}$ (IC_{50} = 265 nM; Figure 3a) than for the $\alpha1_{c\text{-}b}$ subunit (IC_{50} = 1.45 nM; Figure 3b). On the contrary, compound **5Ac**, which behaved as a weak competitor in displacement binding assays carried out on guinea pig cardiac membranes (Figure 2b), did not affect PN200-110, (+)-[5-methyl-^{3}H] specific binding in intact HEK-293 expressing either $\alpha1_{c\text{-}a}$ or $\alpha1_{c\text{-}b}$ subunit (Figure 3). Therefore, this behavior is suggestive of an allosteric rather than directly competitive binding of the compound **5Ac** to the $\alpha1_c$ cardiac subunit occurring in guinea pig ventricular membranes.

Compound **5Cq** behaved as a weak competitor for PN200-110, (+)-[5-methyl-^{3}H] specific binding both in ventricular membranes (Figure 2b) and in HEK-293 cells expressing the $\alpha1_{c\text{-}a}$ subunit (Figure 3a), whereas it did not displace PN200-110, (+)-[5-methyl-^{3}H] in HEK-293 cells expressing the $\alpha1_{c\text{-}b}$ subunit (Figure 3b). This latter compound showed a profile of cardiac versus vascular selectivity, which has been previously reported for other 1,4-dihidropiridine substitutes.[27]

C-Computational Studies. To get major insights on the molecular basis of the interactions between LTCC and the new 4-aryl-1,4-DHPs, docking simulations of the most active and structurally diverse compounds (**5Cb**, **5Cq**, and **5Ag**) were

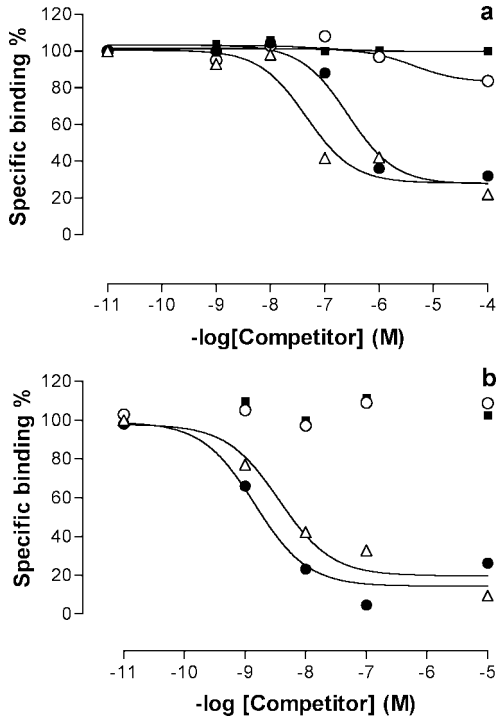


Figure 3. Effect of nifedipine (△), **5Al** (●), **5Ac** (■), and **5Cq** (○) on PN200-110, (+)-[5-methyl-^{3}H] specific binding in HEK-293 cells expressing the $\alpha 1_{c\text{-}a}$ (**a**) or $\alpha 1_{c\text{-}b}$ (**b**) subunit. Points are the mean from three independent experiments (S.E.M. smaller than the symbols).

carried out using the published three-dimensional structure of LTCC.[28] Docking experiments were conducted employing the new version of AutoDock (AutoDock4) software.[29] The latest version of this software implements a new force field that, using an improved thermodynamic model of the binding process, allows inclusion of intramolecular terms in the estimated free energy. Moreover, the new force field includes a full desolvation model that contains terms for all atom types, including the favorable energetics of desolvating carbon atoms as well as the unfavorable energetics of desolvating polar and charged atoms. This force field also incorporates an improved model of directionality in hydrogen bonds, now predicting the proper alignment of groups with multiple hydrogen bonds. This program was employed to perform 100 independent docking runs for the selected compounds, which usually converged to a small number of different clusters ("clusters" of results differing by less than 1.5 Å rmsd). Even if the predicted free energy of binding associated with each solution should be used as a criterion for the choice of the "best" posing, herein the preference for one solution has been also governed by its consistency with experimental data (mutagenesis experiments and SARs). In the following section, a brief description of the calculated binding modes of the selected 1,4-DHPs into LTCC is given together with the rationalization of the new SARs data. Hereafter, to distinguish between the two sides of the 1,4-DHP ring, as suggested by Goldmann et al.,[30] the preferred conformation of 1,4-DHP moiety will be regarded as a flattered boat with C4 as the bow, the axial aryl ring as the bowsprit, and the N1 atom as the stern. Accordingly, the two sides of the 1,4-DHP ring will be referred as the port side (left) and the starboard side (right).[30] Docking of **5Cb** placed dihydropyridine ring in the cleft formed by IIIS6, IIIS5, and IVS6 segments in consonance with experimental evidences (photoaffinity labeling, construction of chimeric channels, and mutagenesis experiments) suggesting that they contain all the residues critical for DHP

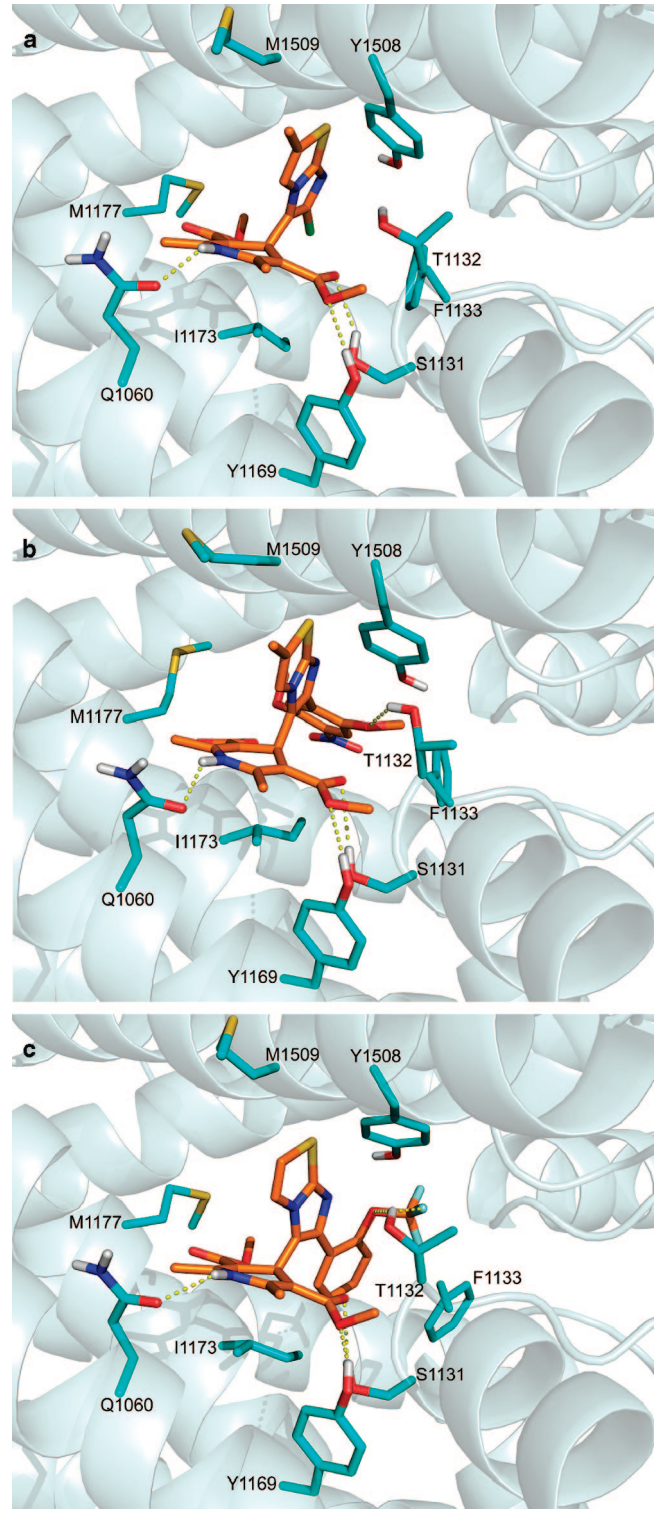


Figure 4. Docked structures of **5Cb** (a), **5Cq** (b), and **5Ag** (c) in the three-dimensional structure of L-type Ca^{2+} channel. DHPs are displayed as orange sticks, and key interacting residues are shown in cyan. Hydrogen bonds as represented with dashed yellow lines. The figure was created by using PYMOL software.[32]

binding.[31] Moreover, the plane of the ligand 1,4-DHP ring is parallel to the pore axis, the ligand NH group faces the IIIS5 segment, the starboard side of the heterocyclic ring points upward, and the plane of the imidazo[2,1-*b*]thiazole system is perpendicular to the pore axis. Such a binding pose allows the ligand to establish numerous interactions with the protein (Figure 4a).[32]

In particular, the N1 hydrogen atom of the 1,4-DHP heterocyclic ring H-bonds with the carbonyl oxygen of Q1060 side chain in IIIS5. This is in consonance with SAR studies reported for other 1,4-DHPs compounds which indicate that the N1 hydrogen atom has a key role in the binding of 1,4-DHPs to LTCC.[30] Moreover, mutagenesis data clearly demonstrated that Q1070 contributes to the binding of 1,4-DHPs.[31] As depicted in Figure 4a,[32] both the carbonyl and the ester oxygens on the starboard side of the dihydropyridine ring form two H-bonds with the S1131 and Y1169 side chains. The involvement of Y1169 in a H-bond with the carbonyl oxygen of the starboard side esters of the most common 1,4-DHPs is in agreement with mutagenesis data. In fact, when mutating Y1169 to A (*S*)-isradipine resulted to be 25-fold less active on the resulting mutant.[33] Moreover, in the Y1169F mutant (*S*)-isradipine demonstrated to be 12.4-fold less active if compared with the wild-type channel.[33] This demonstrates the involvement of Y1169 hydroxyl group in a H-bond with the ligand in agreement with the proposed binding pose. The involvement of S1131 in the binding of 1,4-DHPs was demonstrated by Yamaguchi et al., who reported that when mutating S1131 to A the IC_{50} value of (*S*)-nitrendipine was 39.4 times higher than that of rbCII (rat brain Ca^{2+} channel α_{1C} subunit type II).[34] Differently from other known 1,4-DHPs, **5Cb** features the presence of an imidazo[2,1-*b*]thiazole system instead of a phenyl ring. This system perfectly fits into the channel 1,4-DHP binding cavity and establishes with it favorable interactions. Most precisely, the aromatic system is well oriented to form a T-shaped interaction with Y1508, which is predicted to be reinforced by the electron-withdrawing effect of the chlorine atom in R_2 (see Table 1). The involvement of Y1508 in the binding of Ca^{2+}-antagonists 1,4-DHPs was experimentally proven by mutagenesis studies. In fact, replacement of this residue to A has large effects on 1,4-DHP activities, with the KD for 1,4-DHP binding in Y1508A mutant increased by 6.1-fold.[35] In addition, the methyl group in R takes favorable hydrophobic contacts with M1509. Indeed, the importance of an electron-withdrawing group in R_2 and a lipophilic one in R is further demonstrated by biological data. In fact, the structurally related compounds **5Ac** displayed comparable inotropic activities (Table 2), whereas the different chemical properties of the above-mentioned groups in **5Ba**, **5Bb**, and **5Db** results in a slightly lower activity. Docking calculations conducted on **5Cq** resulted in a binding pose similar to that found for **5Cb** (Figure 4b).[32] Although, differently to **5Cb**, in **5Cq** the imidazo[2,1-*b*]thiazole system is substituted in R_2 with a phenyl ring that establishes additional π-stacking interactions with Y1508 side chain. Moreover, the above cited interaction is further reinforced by the presence of the electron-withdrawing nitro group in *para*-position on the phenyl ring in R_2. In **5Cq** this phenyl ring is also substituted in *ortho*-position by a methoxy group, which with its ether oxygen H-bonds with T1132 side chain. On the contrary, the other *ortho*-methoxy group on the port side of the molecule engages hydrophobic interactions with M1177 side chain. Also, in this case, the above-cited residue has been confirmed to be critical for 1,4-DHP binding.[33] The presence of supplementary interactions with LTCC might explain why **5Cq** resulted to have a higher inotropic potency if compared with **5Cb**. Docking results on **5Cq** also allow to infer the reasons for the different inotropic potencies recorded for its structural congeners. In fact, good negative inotropic potencies, ranging from 0.026 to 0.36 μM, were found for structurally related compounds **5Ap**, **5Aq**, **5Bq**, **5Cp**, **5Cq**, and **5Gp**, which feature the same kind of substitutions on the phenyl ring in R_2 but in different positions, still

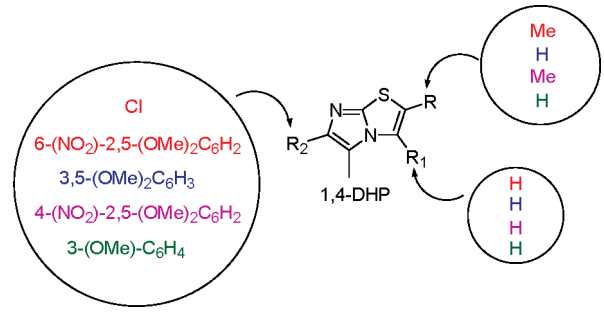


Figure 5. Synopsis of the SAR findings: the red groups for the best inotropic potency, the blue groups for the best chronotropic potency, the magenta groups for the best inotropic selectivity, and the green groups for the best chronotropic selectivity.

allowing the interaction with the above cited residues. It remains unknown the anomalous of **5Bp** (weak negative inotropic agent) and the low negative inotropic potency of **5Ar**. On the other hand, the absence of the electron-withdrawing nitro group on the phenyl ring could explain why compounds such as **5Ai**, **5Aj**, **5Am**, **5An**, **5Bk**, **5Ck**, and **5Fk** are endowed of lower inotropic potency (range from 0.90 to 2.64 μM). Docking on **5Ag** converged toward a well-defined solution in which the DHP ring fits in the cleft formed by IIIS6, IIIS5, and IVS6 segments similarly to what found for the above-described compounds. Nevertheless, if compared with **5Cq**, the presence of a single *ortho*-substitution in **5Ag** allows this ring to be nearly parallel to the imidazo[2,1-*b*]thiazole moiety (Figure 4c). This different orientation allows the R_2 aromatic portion to establish a T-shaped interaction with Y1508 and an off-centered parallel displaced $\pi-\pi$ interaction with F1133, which are strengthened by the trifluoromethoxy group that provides an electron-withdrawing effect on the aromatic ring. Moreover, the same group also H-bonds through its fluorine and oxygen atoms with T1132 side chain. The proposed binding mode is in accordance with the SARs presented herein. In fact, when the trifluoromethoxy group is moved from the *ortho*- to the *meta*-position (**5Ah**) lower inotropic potency was found. This might be rationalized by the absence of the H-bond interactions with T1132 side chain. Indeed, when the trifluorometoxy is substituted by a trifluoromethyl group (**5Ae**), the same interactions were detected (data not shown), which explains why similar potencies were recorded in functional studies. In the same way, if the H-bond accepting group is moved from the *ortho*- to the *meta*-position (**5Af**), lower inotropic activities were recorded due to the loss of a polar interaction with T1132.

Conclusions

In this paper we report the *in vitro* biological characterization of a new set of 1,4-DHPs. In summary, it is notable the lack of vasorelaxant activity of new derivatives of 1,4-DHPs in which the phenyl ring on the C-4 position was replaced with imidazo[2,1-*b*]thiazole. As a matter of fact, in a previous work we underlined this for some 1,4-DHPs bearing different heterocyclic systems.[4] A number of structure–activity relationship trends have been identified and rationalized through molecular modeling studies. We found that the group in position 6 (R_2) is determining to influence the cardiovascular activity (Figure 5).

Nevertheless, the weak affinity to calcium channel measured by binding assay on the new 4-aryl-DHPs showed a peculiar cardiovascular activity profile. Morel et al. comparing some well-known 1,4-DHPs in cells expressing the cardiac $Ca_v1.2a$ and smooth muscle $Ca_v1.2b$ subunits demonstrated that these subunits are different in sensitivity to 1,4-DHPs.[26] There are

several explanations for selective action of 1,4-DHPs toward a tissue: the existence of various types and heterogeneous distribution of voltage-operated Ca^{2+} channels,[36] discovery of splicing variants of α_1 subunits,[37] actions other than calcium channel blocking,[38] frequency and voltage-dependency of the action,[39] and physicochemical properties of drugs[40] are some descriptors for this effect.

We used HEK-293 cells transfected either with cDNA encoding for the $\alpha 1_{c\text{-}a}$ or $\alpha 1_{c\text{-}b}$ subunit of the LTCC to ascertain that some of these novel compounds, such as **5Ac**, may allosterically modulate binding affinity of PN200-110, (+)-[5-methyl-^{3}H] to the LTCC.

Compounds that selectively act on cardiac tissue with a negative inotropic and/or chronotropic effect and devoid of effects on vascular tissues might be used in the treatment of many cardiovascular dysfunctions, such as cardiac hypertrophy and ischemia. Although attempts have been made to improve the prognosis of ischemia-evoked cardiac failure with second-generation calcium antagonists, this could be an interesting approach.[41] In fact, the most common agents used to treat the above cited pathologies are the β_1 adrenoreceptor antagonists, which most of the time are not devoid of side effects due to the lack of β_1/β_2-adrenoreceptor selectivity. In conclusion, the encouraging and interesting pharmacological profile of the new imidazo[2,1-*b*]thiazole 1,4-DHPs certainly deserves further studies that are currently in progress to identify new tissue-selective calcium antagonists useful to treat common cardiovascular pathologies such as cardiac hypertrophy and ischemia.

Experimental Section

A. Chemistry. The melting points are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values (see Supporting Information, Table S3). TLC was performed on Bakerflex plates (Silica gel IB2-F); the eluent was a mixture of petroleum ether 60–80 °C/acetone in various proportions. Kieselgel 60 (Merck) was used for flash chromatography. The IR spectra were recorded in nujol on a Nicolet Avatar 320 E.S.P.; ν_{max} is expressed in cm^{-1}. The ^{1}H NMR spectra were recorded on a Varian Gemini (300 MHz); the chemical shift (referenced to solvent signal) is expressed in δ (ppm) and J in Hz (see Supporting Information, Table S2). All solvents and reagents, unless otherwise stated, were supplied by Aldrich Chemical Company Ltd. and were used as supplied.

For a new starting compound, please see Table S1 in Supporting Information.

General Procedure for the Synthesis of the Dihydropyridines 5Ac−5Gr. Methylacetoacetate (2 mM) and 30% NH_4OH (4 mM) were added to a stirred solution of the appropriate aldehyde **4** (1 mM) dissolved in isopropyl alcohol (50 mL). The reaction mixture was refluxed for 1–4 days (according to a TLC test acetone/petroleum ether 55–85 °C, 1:9 v/v, 2:8 v/v) and added of methylacetoacetate (4 mM) and 30% NH_4OH (2 mM) every 12 h. After cooling, the mixture was evaporated to dryness under reduced pressure. All the derivatives were purified by flash chromatography. See Table 1 for eluent and yield.

B. Functional Studies. For details, please see Supporting Information, section S11.

C. Receptor Binding Studies. For details, please see Supporting Information, section S14.

D. Computational Methods. For details, please see Supporting Information, section S15.

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Supporting Information Available: Chemistry, analytical data, functional assays, receptor binding studies, and computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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