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Ligand Based Approach to L-Type Calcium Channel by Imidazo[2,1-*b*]thiazole-1,4-Dihydropyridines: from Heart Activity to Brain Affinity

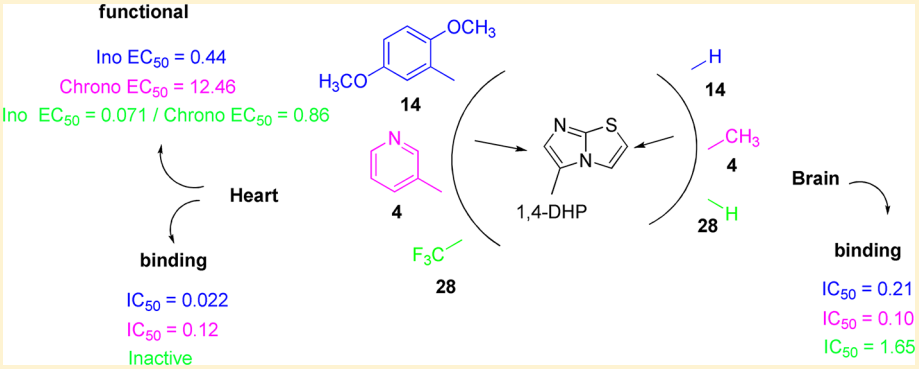
Alessandra Locatelli,[†] Sandro Cosconati,[‡] Matteo Micucci,[†] Alberto Leoni,*[,†] Marinelli Luciana,[§] Andrea Bedini,[†] Pierfranco Ioan,[†] Santi Mario Spampinato,[†] Ettore Novellino,[§] Alberto Chiarini,[†] and Roberta Budriesi*[,†]

[†]Dipartimento di Farmacia e Biotecnologie, Università degli Studi di Bologna, Via Belmeloro 6, 40126 Bologna, Italy

[‡]DiSTABiF, Seconda Università di Napoli, Via Vivaldi 43, 81100 Caserta, Italy

[§]Dipartimento di Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli "Federico II", Via Montesano 49, 80131 Napoli, Italy

Supporting Information



ABSTRACT: The synthesis, characterization, and functional in vitro assay in cardiac and smooth muscle (vascular and nonvascular) of a series of 4-imidazo[2,1-*b*]thiazole-1,4-dihydropyridines are reported. To define the calcium blocker nature of the imidazo[2,1-*b*]thiazole-1,4-DHPs and their selectivity on $Ca_v1.2$ and $Ca_v1.3$ isoforms, we performed binding studies on guinea pig atrial and ventricular membranes on intact cells expressing the cloned $Ca_v1.2a$ subunit and on rat brain cortex. To get major insights into the reasons for the affinity for $Ca_v1.2$ and/or $Ca_v1.3$, molecular modeling studies were also undertaken. Some physicochemical and pharmacokinetic properties of selected compounds were calculated and compared. All the biological data collected and reported herein allowed us to rationalize the structure−activity relationship of the 4-imidazo[2,1-*b*]thiazole-1,4-DHPs and to identify which of these enhanced the activity at the central level.

INTRODUCTION

Voltage-gated Ca^{2+} channels (VGCCs) play a critical role in many physiological functions, from muscle contraction to neurosecretion in a variety of tissues.[1] The 10 members of VGCCs family, with different role in cellular signal transduction, have been characterized in mammals.[2] The calcium channels are complex proteins of four or five distinct subunits. The α_1 subunit is the largest, and it incorporates the conduction pore and the majority of the known sites of channel regulation by drugs. This main site of action is located at α_1 subunit isoforms that belong to three different subfamilies (Ca_v1, Ca_v2, and Ca_v3). The accessory subunits (α_2-δ, β, and γ) fine-tune the channel function. L-Type Ca^{2+} channels (LTCCs) are formed by the Ca_v1 family, which comprises four isoforms: $Ca_v1.1$ (α_{1S} subunit), predominantly expressed in skeletal muscle, $Ca_v1.2$ (α_{1C} subunit), also known as cardiac isoform widely expressed in cardiovascular system where it regulates vascular tone and cardiac inotropy and in neurons, $Ca_v1.3$ (α_{1D} subunit), predominantly expressed in neurons and in cardiac pacemaker cells, and $Ca_v1.4$ (α_{1F} subunit), with physiological function as neurotransmitter release in retinal cells.[3] Several studies have demonstrated that specific drugs, activator or blocker, interact at discrete receptor sites in accordance with their chemical heterogeneity which has been extensively reviewed since the 1970s.[4,5] LTCC blockers are a group of leading drugs directed at cardiovascular diseases including hypertension, angina, peripheral vascular disease, and some arrhythmias.[6,7] In particular, LTCC isoforms $Ca_v1.2$ and $Ca_v1.3$ are predominantly expressed in neurons and, for this reason, are now considered viable drug targets to treat central nervous system (CNS) disease such as Parkinson or pain perception.[3,8,9] The

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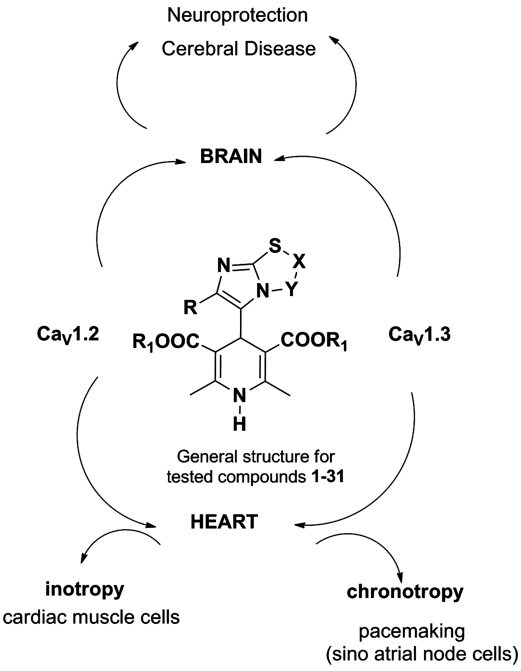
class of ligands with LTCCs higher affinity are 1,4-dihydropyridines, whose historical lead is Nifedipine. In cardiovascular systems, Nifedipine is selective for vascular smooth muscle, allowing its use and that of subsequent 1,4-DHP's generations in the treatment of hypertension.[10,11] It is well-known that high doses of 1,4-DHPs, such as Nifedipine, cause a variety of brain effects such as amelioration of age-related working memory deficits, anxiolytic and antidepressant-like actions, inhibition of fear memory extinction, anticonvulsant effects, and effects on drug-taking behavior.[12] Unfortunately, so far, it is not clear if these effects are mediated by LTCCs or by other blocking target because of the difficulty in finding selective LTCC modulators for the brain.[12] At the same time, it should not be underestimated the problem related to therapeutic use of the LTCC modulators for diseases of the CNS because of their poor selectivity. Indeed, Nifedipine binds both $Ca_v1.2$ and $Ca_v1.3$ isoforms with high affinity. Its low specificity together with its difficulty in overcoming the blood–brain barrier are key requirements for use in cardiovascular therapy but also greater limits.[12]

1,4-DHPs, known from different decades and spread across several generations, could still represent a valuable tool for the characterization of peripheral and central $Ca_v1.2$ and $Ca_v1.3$ isoforms LTCC. In the framework of our research, we recently[13] described the design, synthesis, and structure–activity relationship of a small 1,4-DHPs library bearing in position 4 a variously substituted imidazo[2,1-*b*]thiazole system. The substitutions on the heterocycle scaffold, along with the profile of activity shown on isolated cardiac tissues streamlined through computational studies and confirmed by binding studies on isolated cardiomyocytes and HEK-293 cells transfect with $\alpha1_{c\text{-}a}$ and $\alpha1_{c\text{-}b}$ subunit, made it possible to correlate the selectivity and potency both with specific substituents and both with their position on the heterocyclic system. Herein, to deepen the influence of the chemical modifications of imidazo[2,1-*b*]thiazole-1,4-DHPs on the peripheral and central activity and/or on the selectivity of calcium channels, we synthesized a new series of compounds **1–13** (Chart 1) using a ligand based approach and taking into account the previous data[13] of the inotropic or chronotropic effects caused by substituents in the imidazo[2,1-*b*]thiazole system. Moreover, we extended the investigation not only to the functional studies but also to the binding studies to verify the calcium blocker nature of the imidazo[2,1-*b*]thiazole-1,4-DHPs on $Ca_v1.2$ and $Ca_v1.3$.

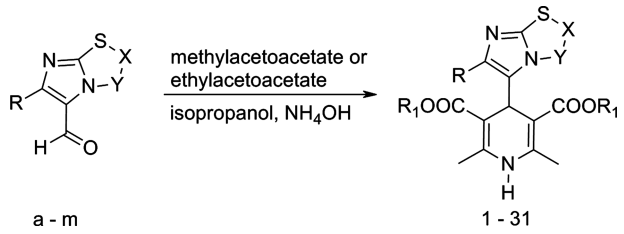
CHEMISTRY

As shown in Scheme 1, the synthesis of the new 4-imidazo[2,1-*b*]thiazole-1,4-dihydropyridines **1–13** (Table 1) was accomplished by means of the well-known Hantzsch reaction:[14] one-pot condensation of the appropriate *β*-ketoester, methylacetatoacetate, or ethylacetoacetate with the opportune aldehydes **a–m**[15–18] in a solution of aqueous ammonia and isopropyl alcohol. After standard purification the 1,4-DHPs were obtained with a range of 5–25% yield. All the structures of final products were confirmed with infrared and ^{1}H nuclear magnetic resonance spectra. The new compounds synthesized gave ^{1}H NMR spectra in agreement with the assigned structures and showed common features as regards the 1–4 DHP moiety: the methyl groups in position 2,6 appear as a singlet in the range 1.91 and 2.23 ppm, the methyl groups of the ester give a singlet in the range 3.21 and 3.44 ppm, the singlet corresponding to H-4 is evident in the range 4.99 and

Chart 1



Scheme 1



5.96, and finally, the NH gives rise to a broad singlet in the range 8.01 and 9.13 which exchange by treatment with D_2O. The signals corresponding to the imidazothiazole substituted moietys were assigned on the basis of previous papers.[13,19]

PHARMACOLOGY

Functional Assays. The pharmacological profile of all compounds was derived on guinea pig isolated left and right atria to evaluate their inotropic and/or chronotropic effects, respectively, and on K^+-depolarized (80 mM) guinea pig vascular (aortic strips) and nonvascular [ileum longitudinal smooth muscle (GPILSM)] to assess the calcium antagonist activity. Compounds were checked at increasing doses to evaluate: (i) the percent decrease of developed tension on isolated left atrium driven at 1 Hz and on spontaneously beating right atrium (negative inotropic activity), (ii) the percent decrease in atrial rate on spontaneously beating right atrium (negative chronotropic activity), and (iii) the percent inhibition of calcium-induced contraction on K^+-depolarized aortic strips and GPILSM (vascular and nonvascular relaxant activity, respectively). Details have been reported in Supporting Information. Data were analyzed using Student's *t*-test and are presented as mean ± SEM.[20] Because the analyzed compounds 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} and IC_{50} was evaluated from log concentration–response

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Table 1. 1,4-Dihydropyridines

compd	starting aldhedyes	x−y	R	R_1
1	**a**[b]	HC=CH	2-Pyr	CH_3
2	**b**[c]	H_3CC=CH	2-Pyr	CH_3
3	**c**[b]	HC=CH	3-Pyr	CH_3
4	**d**[c]	H_3CC=CH	3-Pyr	CH_3
5	**e**[c]	HC=CH	4-Pyr	CH_3
6	**f**[c]	H_3CC=CH	4-Pyr	CH_3
7	**g**[d]	HC=CH	2-thio	CH_3
8	**h**[d]	H_3CC=CH	2-thio	CH_3
9	**i**[d]	HC=CH	3-thio	CH_3
10	**j**[d]	H_3CC=CH	3-thio	CH_3
11	**k**[d]	HC=CH	2,4-Cl_2-3-thio	CH_3
12	**l**[d]	H_3CC=CH	2,4-Cl_2-3-thio	CH_3
13	**m**[e]	HC=CH	2,5-$(OCH_3)_2$-C_6H_3	C_2H_5
14[a]		HC=CH	2,5-$(OCH_3)_2$-C_6H_3	CH_3
15[a]		H_3CC=CH	2,5-$(OCH_3)_2$-C_6H_3	CH_3
16[a]		ClC=CH	2,5-$(OCH_3)_2$-C_6H_3	CH_3
17[a]		HC=CH	2,4-$(OCH_3)_2$-C_6H_3	CH_3
18[a]		HC=CH	3,4-$(OCH_3)_2$-C_6H_3	CH_3
19[a]		HC=CH	3,5-$(OCH_3)_2$-C_6H_3	CH_3
20[a]		HC=CH	3-(OCH_3)-C_6H_4	CH_3
21[a]		HC=CH	6-NO_2-2,5-$(OCH_3)_2$-C_6H_2	CH_3
22[a]		H_3CC=CH	6-NO_2-2,5-$(OCH_3)_2$-C_6H_2	CH_3
23[a]		$H_3C(CH_2)_2C$=CH	6-NO_2-2,5-$(OCH_3)_2$-C_6H_2	CH_3
24[a]		HC=CH	4-NO_2-2,5-$(OCH_3)_2$-C_6H_2	CH_3
25[a]		H_3CC=CH	4-NO_2-2,5-$(OCH_3)_2$-C_6H_2	CH_3
26[a]		H_3CC=CCH_3	C_6H_5	CH_3
27[a]		HC=CH	2-(CF_3)-C_6H_4	CH_3
28[a]		HC=CH	(CF_3)	CH_3
29[a]		H_3CC=CH	Cl	CH_3
30[a]		HC=CCH_3	Cl	CH_3
31[a]		H_3CC=CH	CH_3	CH_3

[a]Taken from ref 13. [b]Taken from ref 15. [c]Taken from ref 16. [d]Taken from ref 17. [e]Taken from ref 18.

curves (Probit analysis using Litchfield and Wilcoxon[20] or GraphPad Prism software[21,22]) in the appropriate pharmacological preparations.

Binding Studies. Compounds were screened for their affinity for calcium channels from guinea pig heart ventricles and atria and rat brain cortex. Binding site was determined by using a competitive radiometric receptor binding assay. PN200−110, (+)-[5-methyl-^{3}H] was used to label 1,4-DHPs binding site. Binding affinity were expressed as K_i and IC_{50} values (mean ± SEM).[23] 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 the rabbit $Ca_v1.2a$ ($\alpha_{1c\text{-}a}$) encoding plasmid. $Ca_v1.2a$ plasmid was 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 ± SEM).[23]

RESULTS

A. Functional Study on Cardiac System. All the newly synthesized (**1**−**13**, Chart 1) 1,4-DHPs bearing in C-4 a differently substituted imidazo[2,1-*b*]thiazole system were tested for their cardiovascular profile on guinea pig left atrium driven at 1 Hz and on a spontaneously beating right atrium to evaluate their inotropic and chronotropic effects, respectively, as previously done for the analogues that have already been published (**14**−**31**).[13] Data of cardiac activity for all compounds (**1**−**31**) are collected in Table 2 using Nifedipine as the reference drug. All compounds show interesting cardiovascular activity profile, except **5** and **31**, which are devoid of activity. As shown in Table 2, most of the tested compounds (**1**, **11**, **13**, **15**−**18**, **20**, **24**−**26**, and **28**) exhibited both negative inotropic and chronotropic properties with different selectivity. **26** is the most potent of the series [EC_{50} = 0.59 μM (c.l. 0.43−0.81); EC_{50} = 0.66 μM (c.l. 0.51−0.85) negative inotropic and chronotropic potency, respectively]. **1** and **11** have negative inotropic potency 45 and 47 times greater than the negative chronotropic potency, respectively. Compounds **12**, **14**, **21**−**23**, **27**, and **29**−**30** possess selective negative inotropic effect also on the spontaneously beating right atrium, even though in this case, the potency is lower. **12**, **22**−**23**, and **27** are significantly more potent than Nifedipine as negative inotropic compounds. Among the compounds studied, **2**−**4**, **6**−**10**, and **19** have only negative chronotropic properties and they are less potent than Nifedipine.

B. Functional Studies on Guinea Pig Smooth Muscles. All new 1,4-DHPs derivatives (**1**−**13**) were tested on K^+-depolarized (80 mM) guinea pig smooth muscles: aortic strips and ileum longitudinal smooth muscle to assess their vascular- or nonvascular-relaxant activity, respectively. The data of the new compounds (**1**−**13**) and of the previously published compounds (**14**−**31**)[13] selected for the particular cardiovascular profile are collected in Table 3 using Nifedipine as the reference drug. All compounds (**1**−**31**) had no effect on vascular smooth muscle. On the contrary, all compounds (**1**−**31**) inhibited K^+ induced contraction on guinea pig nonvascular smooth muscle. Only the compound **9** showed an activity comparable to that of Nifedipine [EC_{50} = 0.0023 μM (c.l. 0.0018−0.0030); EC_{50} = 0.0015 μM (c.l. 0.0011−0.0022); respectively]. **13**, **19**, and **20** resulted from 30−50 times less potent than Nifedipine.

C. Binding Studies on Guinea Pig Atria, Ventricles, and on Intact Cells Expressing the Cloned $Ca_v1.2a$ Subunit. For compounds **1**−**31**, competition binding assays on guinea pig atrial and ventricular membranes have been carried out; Nifedipine was employed as a reference compound. All compounds were first dissolved in DMSO in order to obtain solutions at 10^{-2} M concentration. These stock solutions were diluted with buffer. All compounds at the concentrations used were soluble in DMSO and in buffer. Addition of the drug vehicle had no appreciable effect in binding experiments. Binding assays have been also carried out on intact HEK-293 cells stably expressing the cardiac $Ca_v1.2a$ subunit of the LTCC.[24] PN200−110, (+)-[5-methyl-^{3}H] (0.5 nM) was incubated with increasing concentrations (0.001−100 μM) of all the tested compounds; IC_{50} and K_i values of all the tested compounds are reported in Table 4 together with those of Nifedipine. As expected, the reference compound Nifedipine significantly bind to LTCC in both atrial and ventricular membranes as well as in intact HEK-293 cells expressing the

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Table 2. Cardiac Activity of Compounds 1-31

	left atrium			right atrium					
	negative inotropy			negative inotropy			negative chronotropy		
compd	activity[c] (M ± SEM)	EC_{50}[d] (μM)	95% conf lim ($\times 10^{-6}$)	activity[e] (M ± SEM)	EC_{50}[d] (μM)	95% conf lim ($\times 10^{-6}$)	activity[f] (M ± SEM)	EC_{50}[d] (μM)	95% conf lim ($\times 10^{-6}$)
1	58 ± 2.7[g]	0.54	0.36−0.79				71 ± 1.3	24.39	18.50−29.21
2	42 ± 3.2[h]						72 ± 0.9	39.91	32.71−45.72
3	25 ± 1.6[g]						98 ± 0.7	28.44	21.38−36.96
4	18 ± 0.7[h]						86 ± 1.6[i]	12.46	9.67−16.10
5	46 ± 1.7[h]						25 ± 1.2[g]		
6	44 ± 2.6[h]						93 ± 2.1[i]	7.49	3.52−9.91
7	46 ± 1.4						85 ± 1.6[j]	1.30	1.04−1.62
8	40 ± 1.3[h]						70 ± 3.4	40.50	31.62−48.87
9	47 ± 2.2						87 ± 3.3[j]	1.06	0.80−1.41
10	12 ± 0.7						85 ± 1.8	2.30	1.74−3.04
11	74 ± 2.1	0.046	0.032−0.064				77 ± 1.4[j]	2.16	1.83−2.55
12	60 ± 1.9[g]	0.31	0.093−0.81	82 ± 1.6	0.73	0.35−1.12	22 ± 1.1		
13	93 ± 1.2	1.16	0.85−1.68				88 ± 2.1[g]	2.63	2.10−3.28
14[a]	92 ± 3.4[i]	0.44	0.29−0.65	85 ± 2.7	0.56	0.38−0.82	31 ± 1.6[g]		
15[a,b]	78 ± 0.9[i]	1.43	1.02−1.94				58 ± 3.4[i]	6.62	4.37−10.02
16[a,b]	55 ± 2.3	0.90	0.56−1.44				84 ± 2.7	0.98	0.73−1.33
17[a]	71 ± 2.8[i]	1.24	0.93−1.48				55 ± 2.5[i]	8.60	7.47−9.90
18[a,b]	61 ± 2.7	2.64	2.03−3.01				80 ± 1.9[i]	3.01	2.41−3.76
19[a]	26 ± 1.3						90 ± 0.5[j]	1.36	1.08−1.78
20[a,b]	90 ± 3.7	1.90	1.65−2.27				94 ± 2.0[j]	1.36	0.97−1.70
21[a,b]	71 ± 0.9	0.36	0.25−0.51	87 ± 2.1	1.11	0.74−1.63	12 ± 0.9		
22[a]	71 ± 0.1[j]	0.093	0.063−0.14	80 ± 1.1	0.31	0.18−0.48	47 ± 3.2		
23[a]	55 ± 1.6[h]	0.039	0.030−0.051	75 ± 3.4[j]	0.39	0.25−0.62	25 ± 1.3[i]		
24[a]	54 ± 3.4[j]	0.054	0.036−0.079				67 ± 2.3[g]	6.08	5.19−7.12
25[a]	75 ± 3.6[j]	0.026	0.018−0.036				87 ± 5.3[g]	3.59	2.71−4.76
26[a]	76 ± 1.4	0.59	0.43−0.81				94 ± 2.4[j]	0.66	0.51−0.85
27[a]	56 ± 2.4[h]	0.093	0.068−0.12	90 ± 2.5[j]	0.16	0.12−0.22	25 ± 1.8[g]		
28[a,b]	90 ± 3.8[i]	0.071	0.021−0.14				54 ± 4.2[k]	0.86	0.74−1.01
29[a,b]	63 ± 2.7[k]	0.056	0.041−0.076	95 ± 1.7	1.70	1.26−2.04	22 ± 0.7[i]		
30[a,b]	79 ± 3.1[i]	0.10	0.08−0.15	67 ± 1.8[h]	1.89	1.32−2.09	39 ± 1.5[i]		
31[a,b]	43 ± 3.3[l]						33 ± 2.6[i]		
Nif	97 ± 2.0[g]	0.26	0.19−0.36				85 ± 4.2[k]	0.039	0.031−0.051

[a]Taken from ref 13. [b]Taken from ref 19. [c]Decrease in developed tension on 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. The 10^{-4} M concentration gave the maximum effect for most compounds. [d]Calculated from log concentration−response curves (Probit analysis by Litchfield and Wilcoxon[20] with n = 6−7). When the maximum effect was <50%, the EC_{50} ino., EC_{50} chrono, values were not calculated. [e]Decrease in developed tension on guinea pig spontaneously beating isolated right atrium at 10^{-5} M, expressed as percent changes from the control (n = 7−8). The 10^{-5} M concentration gave the maximum effect for most compounds. [f]Decrease in atrial rate on guinea pig spontaneously beating isolated right atrium at 10^{-4} M, expressed as percent changes from the control (n = 7−8). The 10^{-4} M concentration gave the maximum effect for most compounds. Pretreatment heart rate ranged from 165 to 190 beats/min. [g]At the 10^{-5} M. [h]At the 10^{-6} M. [i]At the 5×10^{-5} M. [j]At the 5×10^{-6} M. [k]At the 10^{-7} M. [l]At the 5×10^{-7} M.

$Ca_v1.2a$ subunit, showing similar affinity and potency in all the three above-mentioned experimental settings. In regard to the tested compounds, **4** and **14** significantly displace PN200−110, (+)-[5-methyl-^{3}H] binding to LTCC in atrial membranes but not in ventricular membranes or in intact HEK-293 stably expressing the cardiac α subunit. Thus **4** and **14** showed a good selectivity toward atrial LTCC and an affinity within the nanomolar range; however, neither **4** nor **14** could displace more than 60% of PN200−110, (+)-[5-methyl-^{3}H] binding to atrial LTCC. Compounds **27** and **29** selectively inhibited the PN200−110, (+)-[5-methyl-^{3}H] binding to atrial LTCC, with a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] greater than 91%. As with **4** and **14**, compounds **27** and **29** have similar affinity values. Compound **7**, on the contrary, could not significantly alter PN200−110, (+)-[5-methyl-^{3}H] binding to LTCC expressed on atrial membranes, but it was effective in displacing radioligand binding to both ventricular LTCC and to the cloned alpha subunit, with similar affinity and potency in both the experimental settings. Nevertheless, **7** could not displace more than 50% of the PN200−110, (+)-[5-methyl-^{3}H] binding to both ventricular LTCC and the cloned α subunit. Compound **20** was able to significantly reduce PN200−110, (+)-[5-methyl-^{3}H] binding to LTCC in both atrial and ventricular membranes but not in intact HEK-293 expressing the cloned α subunit. These findings suggest that **20** may bind to LTCC subunits other than $Ca_v1.2a$. Compound **20** showed affinity values in the nanomolar range and a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] of about 92%. Compounds **9**, **13**, **18**, **19**, and **23** significantly displaced PN200−110, (+)-[5-methyl-^{3}H] binding to LTCC in both atrial and ventricular membranes as well as in HEK-293 cells stably expressing $Ca_v1.2$ α subunit, with affinity values and

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Table 3. Relaxant Activity of Compounds 1−31 on K^+-Depolarized Guinea Pig Vascular and Nonvascular Smooth Muscle

	aorta	ileum		
compd	activity[c] (M ± SEM)	activity[c] (M ± SEM)	IC_{50}[d] (μM)	95% conf lim ($\times 10^{-6}$)
1	28 ± 1.3[e]	87 ± 2.4	2.57	1.97−3.35
2	13 ± 1.2	78 ± 2.1	2.81	2.20−3.57
3	36 ± 2.4	91 ± 0.4	3.55	2.05−6.16
4	19 ± 0.7[e]	83 ± 2.5[e]	15.96	10.93−20.10
5	14 ± 0.5	90 ± 2.1[i]	0.96	0.74−1.06
6	4 ± 0.1	97 ± 0.5[j]	9.69	7.61−12.35
7	23 ± 1.4[f]	91 ± 0.3[k]	0.11	0.086−0.13
8	9 ± 0.8	81 ± 2.3[i]	1.37	1.08−1.72
9	11 ± 0.1	85 ± 1.7[l]	0.0023	0.0018−0.0030
10	6 ± 0.2	74 ± 2.6	3.52	2.68−4.61
11	22 ± 1.3[e]	95 ± 2.6[k]	0.18	0.10−0.31
12	31 ± 3.1	87 ± 1.1	3.23	2.39−4.36
13	29 ± 1.1	85 ± 2.3[k]	0.046	0.029−0.071
14[a]	24 ± 1.4	51 ± 0.6	12.35	8.56−17.80
15[b]	15 ± 0.9[e]	74 ± 1.4	4.89	3.74−6.40
16[a,b]	11 ± 1.0	91 ± 2.3[e]	1.14	0.73−1.85
17[a]	42 ± 3.1	90 ± 1.7	1.21	0.91−1.61
18[a,b]	15 ± 0.9[e]	90 ± 2.3[g]	0.45	0.10−0.91
19[a]	40 ± 2.4[g]	89 ± 3.5[k]	0.055	0.043−0.070
20[a,b]	48 ± 1.7[f]	51 ± 2.4[l]	0.083	0.066−0.103
21[a,b]	17 ± 0.9	86 ± 5.2[j]	11.04	8.18−14.90
22[a]	27 ± 1.9	67 ± 3.2[i]	1.84	1.49−2.26
23[a]	5 ± 0.3	89 ± 2.4[j]	12.81	10.00−16.41
24[a]	35 ± 1.4	97 ± 1.4	2.56	1.90−3.11
25[a]	32 ± 2.5	94 ± 3.5[g]	0.24	0.19−0.29
26[a]	12 ± 1.1	74 ± 3.8	2.96	2.24−3.92
27[a]	24 ± 1.4	92 ± 1.5	1.66	1.36−2.05
28[a,b]	34 ± 2.1[e]	70 ± 2.3[m]	0.21	0.14−0.31
29[a,b]	19 ± 1.3	93 ± 0.5	2.06	1.55−2.73
30[a,b]	47 ± 3.1	97 ± 0.6[i]	0.51	0.39−0.62
31[a,b]	38 ± 1.5	70 ± 1.3	8.83	5.53−10.41
Nif	82 ± 1.3[g,h]	70 ± 0.36[n]	0.0015	0.0011−0.0022

[a]Taken from ref 13. [b]Taken from ref 19. [c]Percent inhibition of calcium-induced contraction on K^+-depolarized (80 mM) guinea pig aortic strips (at 10^{-4} M) and longitudinal smooth muscle (at 10^{-5} M). The 10^{-4} M and the 10^{-5} M concentration gave the maximum effect for most compounds respectively. [d]Calculated from log concentration−response curves (Probit analysis by Litchfield and Wilcoxon[20] with n = 6−7). When the maximum effect was <50%, the IC_{50} values were not calculated. [e]At the 5×10^{-5} M. [f]At the 10^{-5} M. [g]At the 10^{-6} M. [h]IC_{50} = 0.009 μM (c.l. 0.003−0.020). [i]At the 5×10^{-6} M. [j]At the 10^{-4} M. [k]At the 5×10^{-7} M. [l]At the 10^{-7} M. [m]At the 3×10^{-7} M. [n]At the 5×10^{-9} M.

potency profiles similar to Nifedipine. The remaining compounds **1−3**, **5−6**, **8**, **10−12**, **15−17**, **21−22**, **24−26**, **28**, and **30−31** did not significantly affect the PN200−110, (+)-[5-methyl-^{3}H] binding to calcium channels in atrial and ventricular membranes as well as in HEK-293 intact cells stably expressing the cloned $Ca_v1.2a$ subunit, therefore showing no affinity greater than 50% to cardiac LTCC up to 100 μM concentration.

D. Binding Studies on Rat Cortex. Compounds **1−31** have been also assayed in competition binding experiments carried out on rat cortex membranes; Nifedipine was employed in the same experiments as a reference compound. All compounds were first dissolved in DMSO in order to obtain solutions at 10^{-2} M concentration. These stock solutions were diluted with buffer. All compounds at the concentrations used were soluble in DMSO and in buffer. Addition of the drug vehicle had no appreciable effect in binding experiments. PN200−110, (+)-[5-methyl-^{3}H] was incubated with increasing concentrations (0.001−100 μM) of all the tested compounds; affinity of **1−31** for rat cortex LTCC (as IC_{50} and K_i) and their maximal [^{3}H]PN200−100 displacement are listed in Table 5. As expected, Nifedipine could significantly compete with PN200−110, (+)-[5-methyl-^{3}H] for the 1,4-dihydropiridine binding site on rat cortex LTCC, displaying affinity values within the nanomolar range and a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] greater than 90%. Potency was in a nanomolar range (EC_{50} = 0.0061 ± 0.0008). Compound **4** displayed the best affinity toward rat cortex LTCC (K_i = 0.059 μM) and displaced the 70% of PN200−110, (+)-[5-methyl-^{3}H] binding. On the contrary, compounds **27** and **29−30** had the worst affinity to rat cortex LTCC, with K_i values of 13.9, 340, and 34.9 μM, respectively, as their maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] was lower than 60%. **13−14**, **18−20**, and **28** significantly bound to the rat cortex LTCC, showing an affinity ranging from 0.1 to 0.5 μM and a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] between 56 and 98%. The remaining 20 compounds (**1−3**, **5−8**, **10−12**, **15−17**, **21−26**, and **31**) did not affect PN200−110, (+)-[5-methyl-^{3}H] binding to calcium channels in rat cortex membranes, therefore showing no affinity greater than 50% to cerebral LTCC up to 100 μM concentration.

E. Computational Studies. In a previous study, a theoretical model of the LTCC $Ca_v1.2$ isoform inner pore was constructed by some of us and used to suggest a viable binding mode for several 1,4-DHPs endowed with affinity for this isoform.[13,25] In the present inspection, for some of the new 1,4-DHPs the binding to the $Ca_v1.3$ isoform of LTCC was also demonstrated. Therefore, in the attempt of getting major insights into the reasons for the affinity for both LTCC isoforms ($Ca_v1.2$ and $Ca_v1.3$) of these new compounds, we also constructed a model of the LTCC $Ca_v1.3$ starting from the $Ca_v1.2$ one. The sequence alignment (see Supporting Information) between the two isoforms demonstrated that the two channels displays a high sequence homology (≈66% identity).[2] Moreover, the residues lining the putative 1,4-DHPs binding site in the two isoforms are identical, with the conservative I1173($Ca_v1.2$)/V1139($Ca_v1.3$) mutation being the only exception. Construction of the two LTTCs allowed for the docking of the 1,4-DHPs that displayed diverse binding properties for the two channels. In particular, for this inspection, we considered compounds **4** and **28**, which can bind only the $Ca_v1.3$ and, with strong selectivity, the $Ca_v1.2$ isoforms, respectively, and **29**, which can bind both isoforms. These simulations were conducted with the AutoDock4.2 (AD4) software that was previously used to propose reasonable binding hypothesis for the other 1,4-DHPs.[13,25] As happened for our previous inspections, also in this case the choice of the "best" posing was also made by considering the consistency with experimental data (mutagenesis experiments and SARs). Analysis of docking results revealed that all the three 1,4-DHPs are able to share the same binding pose (Figure 1) within the LTCC regardless the considered isoform ($Ca_v1.2$ or $Ca_v1.3$), which was somehow expected given the high sequence identity of the two binding sites ($Ca_v1.2$ and $Ca_v1.3$). Moreover, the same interaction pattern has also been proposed by us for different 1,4-DHPs in our previous papers, demonstrating its

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Table 4. Cardiac Membrane (Atrium and Ventricle) and $Ca_v1.2$ Cloned α_1-Subunits Affinities of Compounds

	heart						cloned α_1-subunits		
	atrium			ventricle			$Ca_v1.2a$		
compd[a]	% of max disp[b] (M ± SEM)	IC_{50} ± SEM (μM)[c]	K_i ± SEM (μM)[c]	% of max disp[d] (M ± SEM)	IC_{50} ± SEM (μM)[c]	K_i ± SEM (μM)[c]	% of max disp[e] (M ± SEM)	IC_{50} ± SEM (μM)[c]	K_i ± SEM (μM)[c]
4	52 ± 4	0.022 ± 0.001	0.0048 ± 0.0004	*f*			*f*		
7	*f*			*f*			50 ± 5	2.15 ± 0.12	0.71 ± 0.07
9	60 ± 4	0.24 ± 0.003	0.052 ± 0.002	70 ± 3	2.05 ± 0.12	0.74 ± 0.05	65 ± 5	0.49 ± 0.04	0.15 ± 0.03
13	76 ± 6	0.078 ± 0.009	0.017 ± 0.008	91 ± 8	1.43 ± 0.05	0.51 ± 0.05	94 ± 7	0.86 ± 0.07	0.30 ± 0.05
14	65 ± 3	0.12 ± 0.005	0.044 ± 0.002	*f*			*f*		
18	50 ± 5	0.39 ± 0.06	0.14 ± 0.04	93 ± 4	0.046 ± 0.009	0.016 ± 0.005	55 ± 5	0.062 ± 0.013	0.023 ± 0.009
19	54 ± 3	0.25 ± 0.01	0.09 ± 0.005	60 ± 5	0.84 ± 0.09	0.31 ± 0.05	50 ± 3	0.61 ± 0.11	0.19 ± 0.07
20	92 ± 4	0.29 ± 0.05	0.090 ± 0.02	96 ± 5	0.45 ± 0.01	0.16 ± 0.05	*f*		
23	53 ± 7	0.065 ± 0.009	0.023 ± 0.008	50 ± 4	3.32 ± 0.19	1.16 ± 0.18	55 ± 5	0.0044 ± 0.0003	0.0016 ± 0.0002
27	91 ± 6	0.14 ± 0.007	0.049 ± 0.005	*f*			*f*		
29	95 ± 5	0.12 ± 0.011	0.042 ± 0.008	*f*			*f*		
Nif	72 ± 3	0.0051 ± 0.0008	0.0015 ± 0.0005	80 ± 5	0.0057 ± 0.0007	0.0020 ± 0.0004	75 ± 5	0.086 ± 0.006	0.032 ± 0.005

[a]Compounds **1**–**3**, **5**, **6**, **8**, **10**–**12**, **15**–**17**, **21**, **22**, **24**–**26**, **28**, **30**, and **31** have not been put in the table because they have an affinity greater than 50% at 100 μM. [b]Percent of maximal [^{3}H]PN200–110 displacement on guinea pig atrial membranes at 100 μM. [c]The concentration of the tested compounds that inhibited [^{3}H]PN200–110 binding on cells homogenate by 50% (IC_{50}) was determined by −log probit analysis with six concentration of the displacers, each performed in triplicate. The IC_{50} values were used to calculate apparent inhibition constant (K_i) by Prusoff method. Values are the mean ± SEM of 3–5 separate experiments performed in triplicate. [d]Percent of maximal [^{3}H]PN200–110 displacement on guinea pig ventricular membranes at 100 μM. [e]Percent of maximal [^{3}H]PN200–110 displacement on HEK-293 cells expressing the $\alpha 1_{c\text{-}a}$ at 100 μM. [f]No affinity greater than 50% up to 100 μM.

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Table 5. Rat Cortex Binding Affinity of Compounds 1−31

compd[a]	% of max disp[b] (M ± SEM)	IC_{50} ± SEM (μM)[c]	K_i ± SEM (μM)[c]
4	70 ± 3	0.10 ± 0.005	0.059 ± 0.003
9	53 ± 2	1.03 ± 0.006	0.74 ± 0.03
13	98 ± 4	0.24 ± 0.03	0.17 ± 0.02
14	56 ± 5	0.21 ± 0.01	0.16 ± 0.03
18	77 ± 6	0.35 ± 0.02	0.11 ± 0.02
19	79 ± 5	1.21 ± 0.08	0.41 ± 0.09
20	90 ± 4	0.99 ± 0.05	0.33 ± 0.04
27	52 ± 3	38.60 ± 1.5	13.90 ± 1.3
28	75 ± 5	1.65 ± 0.051	0.52 ± 0.020
29	60 ± 4	1023 ± 24	340 ± 21
30	52 ± 2	106 ± 9	34.7 ± 2.3
Nif	92 ± 6	0.0061 ± 0.008	0.0019 ± 0.005

[a]Compounds **1−3**, **5−8**, **10−12**, **15−17**, **21−26**, and **31** have not been put in the table because they have an affinity greater than 50% at 100 μM. [b]Percent of maximal [^{3}H]PN200−110 displacement on rat cortex homogenate at 100 μM [c]The concentration of the tested compounds that inhibited [^{3}H]PN200−110 binding on rat cortex homogenate by 50% (IC_{50}) was determined by −log probit analysis with six concentration of the displacers, each performed in triplicate. The IC_{50} values were used to calculate apparent inhibition constant (K_i) by Prusoff method. Values are the mean ± SEM of 3−5 separate experiments performed in triplicate.

correlation with both mutagenesis data and the wide amount of SARs data available for the 1,4-DHPs.[13,25]

Thus, it is clear that from docking alone that the different binding selectivity profile displayed by **4**, **28**, and **29** cannot be explained. Obviously, other differences between $Ca_v1.2$ and $Ca_v1.3$, not residing in the 1,4-DHPs binding site, should exist and contribute to the selective recognition of the above-mentioned compounds. Indeed, it is well-known that ligand entrance/exit may strongly affect the binding processes, thus also mutations in these paths should be considered.[27−29] Unfortunately, in the cases of LTCC ligands, no accurate information is available about the ligand entrance path. Nevertheless, it can be postulated that the different chemical composition as well the extension of the entrance/exit path could have an influence on the $Ca_v1.2/Ca_v1.3$ selectivity. According to this theory, **4**, with its bulky 3-pyridine substituent, should only be able to reach the $Ca_v1.3$ binding site while the small and highly electron-withdrawing CF_3 group in **28** should allow this compound to easily reach interact with the $Ca_v1.2$ 1,4-DHPs binding site. However, this is a pure theoretical hypothesis that has to be confirmed by future experimental data.

F. Physicochemical and Pharmacokinetic Properties Prediction. Some physicochemical and pharmacokinetic properties of **4**, **28**, and **29** were calculated and compared as a coarse assessment of the drug-like character of our 1,4-DHPs. For such a purpose, we employed the Qikprop program (Schrödinger. LLC New York). The results are summarized in Table S1, Supporting Information. Quantitative structure−activity relationship (QSAR) studies on CNS active compounds and their derivatives, together with analyses based on CNS drugs, have suggested the physical and chemical properties that CNS compounds must possess. They are: molecular weight (MW) less than 450, ClogP less than 5, number of H-bond acceptor atoms less than 7, polar surface area (PSA) less than 90 Å, number of rotatable bonds (RB) less or equal to 10. Thus, for CNS penetration, the physical properties, usually, have a smaller range than general therapeutics (the latter ranges are reported in Table S1, Supporting Informations). On the basis of these premises, all the inspected 1,4-DHPs are predicted to have a good probability of entering the CNS, as all its estimated physicochemical parameters fall in the aforementioned ranges.

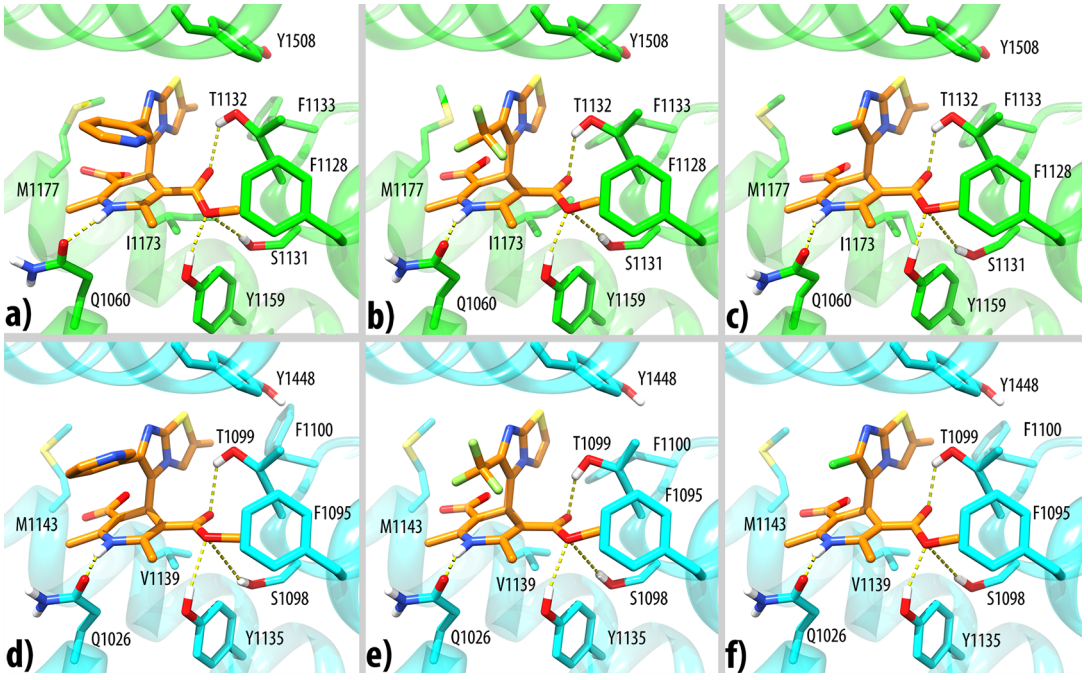

Figure 1. Docked structures of **4** (a,d), **28** (b,e), and **29** (c,f) in the three-dimensional structure of $Ca_v1.2$ (a−c) and $Ca_v1.3$ (d−f) LTCC. DHPs are displayed as orange sticks, and key interacting residues are shown in green ($Ca_v1.2$) and cyan ($Ca_v1.3$). Hydrogen bonds as represented with dashed yellow lines. The figure was created using the UCSF Chimera software.[26]

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DISCUSSION

Novel studies describe $Ca_v1.3$ as a determinant of heart rate but not of ventricular excitation−contraction coupling. The development of $Ca_v1.3$ selective blockers might be an attractive possibility for managing cardiac ischemia and other coronary artery diseases without the concomitant negative inotropy due to $Ca_v1.2$ channel inhibition.[3] Moreover, a $Ca_v1.3$ knockouts study revealed unexpected physiological and pathophysiological roles that should prompt development of $Ca_v1.3$-selective drugs with potential therapeutic effects of their pharmacological inhibition: antidepressant-like effects, neuroprotection of CNS dopaminergic neurons, and prevention neuronal loss in Parkinson's disease.[12] Taking into account these emerging biological results and some data published from us in a previous paper,[13] we designed and synthesized a small library of new 1,4-DHPs bearing a 4-imidazo[2,1-*b*]thiazole ring. Using a ligand-based approach, we tried to determine the SARs of imidazo-[2,1-*b*]thiazole ring in binding the two different LTCC isoforms: $Ca_v1.2$ and $Ca_v1.3$. The functional studies have allowed us to delineate the pharmacological profile of all compounds. We evaluated their inotropic and/or chronotropic effects on guinea pig isolated left atria driven at 1 Hz and on spontaneously beating right atria, respectively. By these preliminary functional studies, first of all we have hypothesized for some derivatives a $Ca_v1.2$ and/or $Ca_v1.3$ channel inhibition knowing that $Ca_v1.2$ is expressed in heart tissue and it is proposed to influence the physiological function of excitation-contraction coupling while $Ca_v1.3$ is expressed in sinoatrial node, atrioventricular node, heart atria and influences sinoatrial heart rate control, and atrioventricular conduction.[3] To support the hypothesis that emerged from functional studies, we performed binding studies on guinea pig atria and ventricular cells using [^{3}H]PN200−100. Only K_i and IC_{50} of compounds having an intrinsic activity greater than 50% are reported in Table 4. As in the CNS $Ca_v1.2$ accounts for approximately 80% of Ca_v1 channels, whereas $Ca_v1.3$ appears to be restricted to a much smaller subset of neurons,[3] compounds **1**−**31** have been assayed in competition binding experiments carried out on rat membranes cortex so as to investigate the affinity of **1**−**31** for rat cortex LTCC (as IC_{50} and K_i) and their maximal [^{3}H]PN200−100 displacement. Data are listed in Table 5. Comparing the results of binding on cardiomyocytes and on rat cortex, it has been possible to assess the presence of substituents of the skeleton of imidazo[2,1-*b*]thiazole able to discriminate between the central and peripheral LTCC isoforms. Finally, it is well-known that $Ca_v1.2a$ is also distributed in smooth muscle, including blood vessels, intestine, lung, and uterus and that $Ca_v1.3a$ is only expressed in vascular smooth muscle.[2,24] Therefore, all new 1,4-DHPs derivatives (**1**−**13**) were tested on K^+-depolarized (80 mM) guinea pig smooth muscles: aortic strips and ileum longitudinal smooth muscle to assess their vascular- and/or nonvascular-relaxant activity, respectively. The data of the new compounds (**1**−**13**) together with those of compounds previously published (**14**−**31**)[13] selected for the particular cardiovascular profile are collected in Table 3 using Nifedipine as the reference drug. All compounds (**1**−**31**) had no effect on vascular smooth muscle. On the contrary, all compounds (**1**−**31**) inhibited K^+-induced contraction on guinea pig nonvascular smooth muscle. The study of the LTCC isoforms selectivity was therefore concluded by binding assays carried out on intact HEK-293 cells stably expressing the cardiac $Ca_v1.2a$ subunit of the LTCC. IC_{50} and K_i values of all the tested compounds are reported in Table 4 together with those of Nifedipine. Considering the overall results of binding assays, we note that a considerable number of compounds show a weak affinity to calcium channel (intrinsic activity less than 50%): **1**−**3**, **5**, **6**, **8**, **10**−**12**, **15**−**17**, **21**, **22**, **24**−**26**, and **31**. For the remaining compounds **4**, **7**, **9**, **13**, **14**, **18**−**20**, **23**, and **27**−**30**, considering the percentage of maximal [^{3}H]PN200−110 displacement and the potency of displacement (IC_{50} and K_i), a difference of affinity toward 1,4-DHPs binding site of LTCC isoform emerges. It is interesting to note that these data are confirmed by functional studies on isolated organs, reflecting the different distribution of LTCC. In particular, **14** possess negative inotropic activity [EC_{50} = 0.44 μM (c.l. 0.29−0.65)] and showed a good selectivity toward atrial LTCC and an affinity within the nanomolar range; **14** displaces also more than 60% of PN200−110, (+)-[5-methyl-^{3}H] binding to atrial LTCC (65 ± 3). It is enough to modify from methyl to ethyl ester of the 1,4-dihydropyridine core to have a compound more potent but less selective: **13** significantly displaced PN200−110, (+)-[5-methyl-^{3}H] binding to LTCC in both atrial and ventricular membranes [IC_{50} = 0.078 ± 0.009 and IC_{50} = 1.432 ± 0.051, respectively] as well as in HEK-293 cells stably expressing $Ca_v1.2a$ subunit [IC_{50} = 0.86 ± 0.07], with affinity values and potency profiles similar to Nifedipine [IC_{50} = 0.0051 ± 0.0008; IC_{50} = 0.0057 ± 0.0007; IC_{50} = 0.086 ± 0.006; respectively]. **13** possesses both negative inotropic and chronotropic activities [EC_{50} = 1.16 μM (c.l. 0.85−1.68), EC_{50} = 2.63 μM (c.l. 2.10−3.28), respectively]. **14** and **13** significantly bind the rat cortex LTCC, showing an affinity potency in a μM range (IC_{50} = 0.21 ± 0.05 and IC_{50} = 0.24 ± 0.03, respectively) and a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] between 56 ± 5 and 98 ± 4%. The position of the two methoxy groups on the phenyl ring influences the functional and binding profile. **17** does not affect PN200−110, (+)-[5-methyl-^{3}H] binding to calcium channels in rat cortex membranes, therefore showing no affinity greater than 50% for cerebral LTCCs up to 100 μM concentration. **17** and **18** exhibited both negative inotropic and chronotropic properties, but in binding studies on guinea pig atria and ventricular membranes, no specific binding was observed. **19** only has negative chronotropic properties. [Decrease in atrial rate on guinea pig spontaneously beating isolated right atrium at 5×10^{-5} M is 90 ± 0.5, EC_{50} = 1.36 μM (c.l. 1.08−1.78)]. **18** and **19** significantly displace PN200−110, (+)-[5-methyl-^{3}H] binding to LTCC in both atrial [IC_{50} = 0.39 ± 0.06 and IC_{50} = 0.25 ± 0.01, respectively] and ventricular membranes [IC_{50} = 0.046 ± 0.009 and IC_{50} = 0.84 ± 0.09, respectively], with affinity values and potency profiles similar to Nifedipine. **18** and **19** bind the rat cortex LTCC. The elimination of a methoxy group in **19** to give **20** increases the potency but reduced selectivity. The introduction of a nitro group in the ortho or para position on the aromatic ring leads to uninteresting derivatives: comparing the biological activity of **14** with **21**−**25**, the potency and selectivity decreases. Only with the introduction of a short aliphatic chain in position two of the imidazo[2,1-*b*]thiazole nucleus (see the structure of **21**−**23**), an appreciable negative inotropic effect with a weak affinity to calcium channel (an intrinsic activity about 50%) in cardiac membranes can be recorded. The lack of the methoxy groups on the aromatic ring in position 6, **26**, or replacement with a bioisoster moiety as thienyl or pyridyl **1**−**10** leads to derivatives in which the negative chronotropic activity is potentiated with respect to the negative inotropic one, **3**−**4**, **6**, **9**, **10**, and **26**.

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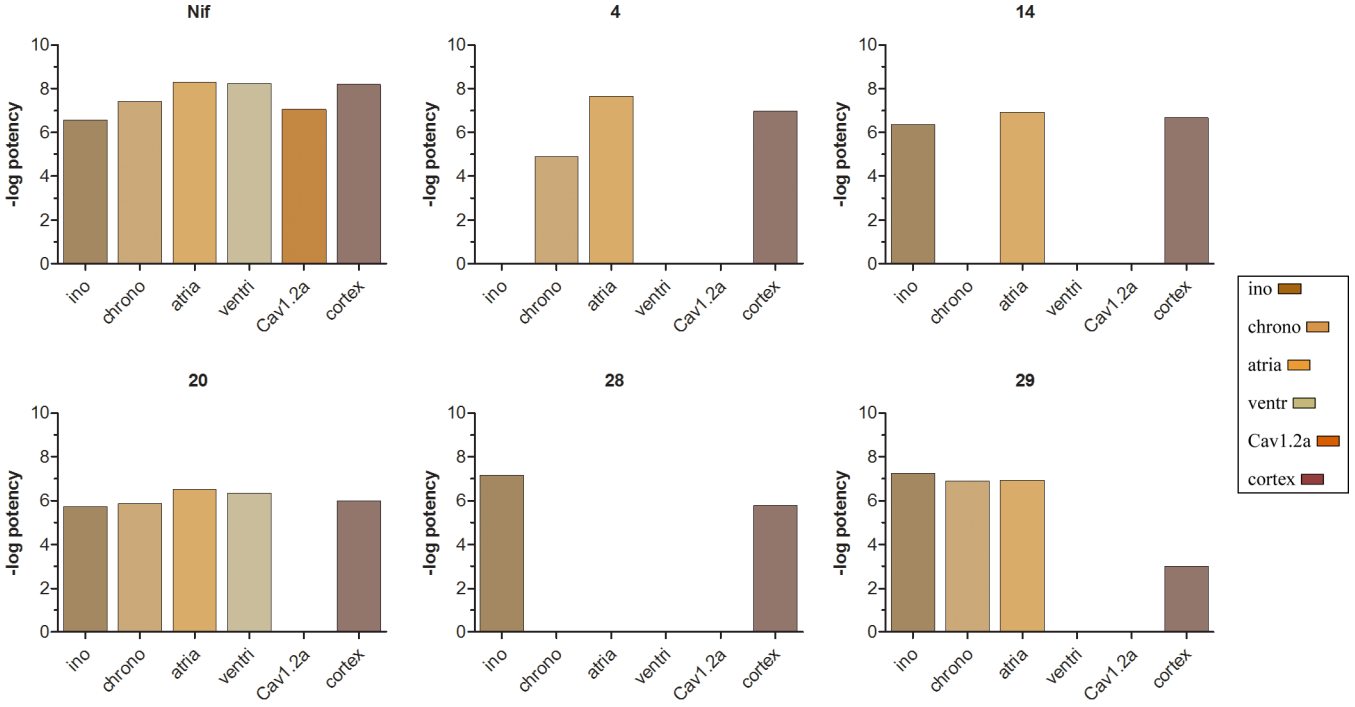

Figure 2. Potency of compounds with the best interesting profile and Nifedipine as reference in different biological preparations: isolated tissues (ino, decrease in developed tension on isolated guinea pig left atrium; chrono, decrease in atrial rate on guinea pig spontaneously beating isolated right atrium) and in binding assays (atria, displacement on guinea pig atrial membranes; ventr, displacement on guinea pig ventricular membranes; Cav1.2a, displacement on HEK-293 cells expressing the $\alpha 1_{c\text{-}a}$; cortex, displacement on rat cortex homogenate).

These latter compounds do not exhibit any appreciable affinity in binding assays, with the exception of **4** and **9**. **4** has minimal structural differences in comparison to other analogues with a pyridyl in position 6, **1**−**6**, but binds to the rat cortex LTCC, showing a IC_{50} = 0.105 ± 0.005 μM and a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] of 70 ± 3%. **9** is the only one of the series of derivatives with a thienyl group in 6, **7**−**8** and **10**−**12**, which has negative chronotropic potency [EC_{50} = 1.06 μM (c.l. 0.80−1.41)] and an appreciable binding in ventricle membranes [IC_{50} = 2.05 ± 0.12, maximal displacement equal to 70 ± 3%]. It is worth noting that, in binding assays, the more cortex selective compound of the series is **28** (IC_{50} = 1.65 ± 0.51 μM and a maximal displacement of PN200−110, (+)-[5-methyl-^{3}H] of 75 ± 5%). This 1,4-DHP exhibited high negative inotropic effect and potency on the left atrium [90 ± 3.8 at 5 × 10^{-5} M concentration with EC_{50} = 0.071 μM (c.l. 0.021−0.14)] and selectivity versus negative chronoscopic potency [EC_{50} = 0.86 μM (c.l. 0.74−1.01)]. Replacement of the trifluoromethyl moiety in **28** with chlorine, **29** and **30**, or a methyl, **31**, causes significant changes in activity. In particular, the methyl group abrogates the LTCC affinity while the chlorine potentiates negative inotropic potency on spontaneously beating right atria, decreases the developed tension on isolated guinea pig left atrium at 10^{-5} M = 95 ± 1.7, and induces an appreciable binding in atrial membranes. When the methyl in position 2 of the imidazo[2,1-*b*]thiazole ring is moved to position 3, **30**, binding and functional data show a weak calcium antagonist activity. Finally, the selectivity of **30** for the calcium channels of the cortex is lost when the trifluoromethyl group is not bound directly to the heterocycle nucleus but it is in the ortho position of a phenyl ring (**27**). This structural change causes a shift in the affinity for calcium channels of the atrium membranes as occurs for other derivatives with a phenyl in position 6 **14** and **20**.

CONCLUSION

Because $Ca_v1.2$ and $Ca_v1.3$ channels have diverse and essential functions (often in the same organ), they can be considered as potential therapeutic target in individual organs. Indeed, the development of an isoform-selective activator or blocker could be therapeutically valuable in a wide range of diseases. However, despite the fact that all the isoforms can bind 1,4-DHPs currently used in cardiovascular diseases such as Nifedipine, because the expression and function of each isoform is not restricted to one tissue or cell type, it is necessary to be aware of potential side effects.[3] At the peripheral level, in concert with previous studies[13] the present investigation suggests that 1,4-DHP scaffold bearing at C-4 a imidazo[2,1-*b*]thiazole ring is critical to provide compounds with cardiac selectivity over vascular one. On the basis of their activity profile in isolated cardiovascular tissues, the substituents at imidazo[2,1-*b*]thiazole ring can modulate the activity of compounds on different heart function influenced by cardiac L-type calcium channels isoforms $Ca_v1.2$ and $Ca_v1.3$. In particular the choice of an appropriate substituent in position 6 in the heterocyclic nucleus of the imidazo[2,1-*b*]thiazole allows obtaining of some 1,4-DHPs with a particular activity probably due to their binding with $Ca_v1.2$ or $Ca_v1.3$ with potential therapeutic effects. An appropriately substituted phenyl ring with one or two methoxy group as in **20** or in **14** enhances the negative inotropic activity. Bioisosteric replacement, as for the pyridine ring (**4**), leads to a derivative with higher negative chronotropic potency (Figure 2). All three of these compounds have different affinities of binding to the channels $Ca_v1.2$ and/or $Ca_v1.3$ of the various tissues. The affinities of heteroge-

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neously expressed $Ca_v1.2$ and $Ca_v1.3$ channels in heart and in brain is even more evident when the aromatic ring in position 6 is substituted with a chlorine or a trifluoromethyl. Despite the small diversity in substitution at imidazo[2,1-*b*]thiazole in 1,4-DHP ring, **29** has selectivity in cardiovascular parameters (negative chronotropic) and is able of displacing labeled PN200−110, (+)-[5-methyl-^{3}H] from brain tissue. It is interesting to note that compound **28** has both negative inotropic and negative chronotropic effects and does not displace the labeled from atrium and ventricle cells, but it is active at the central level (Figure 2). The results obtained with these compounds encourages us to further study the 1,4-DHP bearing imidazo[2,1-*b*]thiazole scaffold SARs to clarify the impact of altered channel regulation in brain functions.

EXPERIMENTAL SECTION

A. Chemistry. The melting points are uncorrected. Analyses (C, H, N) were within (0.4% of the theoretical values. 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. All solvents and reagents, unless otherwise stated, were supplied by Aldrich Chemical Co. Ltd. and were used as supplied.

*General Procedure for the Synthesis of the 1,4-Dihydropyridines **1**−**13**.* Methylacetoacetate or ethylaacetoacetate (2 mM) and 30% NH_4OH (4 mM) were added to a stirred solution of the appropriate aldehyde **a**−**m** (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 methylacetoacetate or ethylaacetoacetate (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 on silica gel (acetone/petroleum ether 40−60 °C from 1.9 to 4.6 v/v for **1**−**9**, **11**, and **13**; petroleum ether 40−60 °C/diethyl ether:9/1 v/v for **10** and **12**) to provide the pure-DHPs as pale-yellow syrup. The resulting oily residue was diluted with cold acetone to afford white solid collected by filtration for **2**, **4**, **5**, and **8**. For **1**, **3**, **6**, **7**, **9**, **11**, and **13**, resulting oils were taken with acetone/petroleum ether 40−60 °C 1/1 v/v and with diethyl ether for **10** and **12** to obtain solid products collect by filtration.

*Dimethyl 2,6-Dimethyl-4-(6-(pyridin-2-yl)imidazo[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**1**).* Yield 18%. IR: 1699, 1646, 1514, 1282, 1089. ^{1}H NMR: 2.16 (6H, s, CH_3), 3.22 (6H, s, $COOCH_3$), 5.96 (1H, s, dhp), 7.20 (1H, qd, py-4, J = 4.7, J = 1.1), 7.28 (1H, d, th, J = 4.4), 7.63(1H, d, th, J = 4.4), 7.75 (1H, td, py-5, J = 7.9, J = 1.1), 7.92 (1H, d, py-6, J = 7.9), 8.56 (1H, dd, py-3, J = 4.7, J = 1.1), 8.95 (1H, s, NH, ex D_2O). Melting point = 190 °C. MW = 424.4729. Anal. ($C_{21}H_{20}N_4O_4S$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(2-methyl-6-(pyridin-2-yl)imidazo[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**2**).* Yield 20%. IR: 1703, 1587, 1270, 1201, 1093. ^{1}H NMR: 2.15 (6H, s, CH_3), 2.45 (3H, s, CH_3), 3.23 (6H, s, $COOCH_3$), 5.74 (1H, s, dhp), 7.17 (1H, td, py-4, J = 5.1, J = 1.3), 7.41 (1H, s, th), 7.73 (1H, td, py-5, J = 7.8, J = 1.3), 7.89 (1H, d, py-6, J = 7.8), 8.54 (1H, dd, py-3, J = 5.1, J = 1.3), 8.92 (1H, s, NH, ex D_2O). Melting point = 245 °C. MW = 438. 4995. Anal. ($C_{22}H_{22}N_4O_4S$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(6-(pyridin-3-yl)imidazo[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**3**).* Yield 10%. IR: 1701, 1649, 1567, 1216, 1122. ^{1}H NMR: 2.19 (6H, s, CH_3), 3.22 (6H, s, $COOCH_3$), 5.64 (1H, s, dhp), 7.31 (1H, d, th, J = 4.6), 7.33 (1H, d, th, J = 4.6), 7.46 (1H, dd, py-5, J = 7.8, J = 5.1), 8.19 (1H, dt, py-6, J = 7.8, J = 1.7), 8.52 (1H, dd, py-4, J = 5.1, J = 1.7), 8.97 (1H, d, py-2, J = 1.7), 9.06 (1H, s, NH, ex D_2O). Melting point = 209 °C. MW = 424.4279. Anal. ($C_{21}H_{20}N_4O_4S$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(2-methyl-6-(pyridin-3-yl)imidazo[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**4**).* Yield 16%. IR: 1699, 1670, 1643, 1210, 1120. ^{1}H NMR: 2.18 (6H, s, CH_3), 2.42 (3H, s, CH_3), 3.23 (6H, s, $COOCH_3$), 5.59 (1H, s, dhp), 7.08 (1H, s, th), 7.43 (1H, dd, py-5, J = 7.9, J = 4.9), 8.15 (1H, d, py-6, J = 7.8), 8.49 (1H, d, py-4, J = 4.9), 8.94 (1H, s, py-2), 8.99 (1H, s, NH, ex D_2O). Melting point = 225 °C. MW = 438.4995. Anal. ($C_{22}H_{22}N_4O_4S$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(6-(pyridin-4-yl)imidazo[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**5**).* Yield 10%. IR: 1709, 1665, 1600, 1505, 1211. ^{1}H NMR: 2.23 (6H, s, CH_3), 3.21 (6H, s, $COOCH_3$), 5.79 (1H, s, dhp), 7.27 (1H, d, th, J = 4.6), 7.36 (1H, d, th, J = 4.6), 7.89 (2H, d, py-2,6, J = 6.2), 8.60 (2H, d, py-3,5, J = 6.2), 9.13 (1H, s, NH, ex D_2O). Melting point = 243−245 °C. MW= 424.4729. Anal. ($C_{21}H_{20}N_4O_4S$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(2-methyl-6-(pyridin-4-yl)imidazo[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**6**).* Yield 25%. IR 1703, 1672, 1602, 1207, 1115. ^{1}H NMR: 2.23 (6H, s, CH_3), 2.42 (3H, s, CH_3), 3.21 (6H, s, $COOCH_3$), 5.75 (1H, s, dhp), 7.01 (1H, s, th), 7.87 (2H, d, py-2,6, J = 6.2), 8.59 (2H, d, py-3,5, J = 6.2), 9.13 (1H, s, NH, ex D_2O). Melting point = 240 °C. MW= 437.4995. Anal. ($C_{22}H_{22}N_4O_4S$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(6-(thiophen-2-yl)imidazo[2,1-b]-thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**7**).* Yield 10%. IR 1701, 1634, 1269, 1200, 1093. ^{1}H NMR: 2.23 (6H, s, CH_3), 3.25 (6H, s, $COOCH_3$), 5.74 (1H, s, dhp), 7.12 (1H, dd, thio-4, J = 5.1, J = 3.6), 7.20 (1H, d, th, J = 4.4), 7.30 (1H, d, th, J = 4.4), 7.48 (1H, d, thio-5, J = 5.1), 7.55 (1H, d, thio-3, J = 3.6), 9.13 (1H, s, NH, ex D_2O). Melting point = 215−220 °C. MW = 429.5126. Anal. ($C_{20}H_{19}N_3O_4S_2$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(2-methyl-6-(thiophen-2-yl)imidazo-[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**8**).* Yield 10%. IR: 1701, 1620, 1306, 1215, 1102. ^{1}H NMR: 2.23 (6H, s, CH_3), 2.41 (3H, s, CH_3), 3.25 (6H, s, $COOCH_3$), 5.69 (1H, s, dhp), 6.97(1H, s, th), 7.10 (1H, dd, thio-4, J = 3.4, J = 2.4), 7.45 (1H, dd, thio-5, J = 3.4, J = 0.8), 7.51 (1H, d, thio-3, J = 2.4), 9.08 (1H, s, NH, ex D_2O). Melting point = 195 °C. MW= 443.5391. Anal. ($C_{21}H_{21}N_3O_4S_2$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(6-(thiophen-3-yl)imidazo[2,1-b]-thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**9**).* Yield 10%. IR: 1701, 1620, 1306, 1215, 1102. ^{1}H NMR: 2.23 (6H, s, CH_3), 3.23 (6H, s, $COOCH_3$), 5.72 (1H, s, dhp), 7.18 (1H, d, th, J = 4.4), 7.28 (1H, d, th, J = 4.4), 7.58 (2H, m, thio-4−5), 7.79 (1H, s, thio-2), 9.12 (1H, s, NH, ex D_2O). Melting point = 220−223 °C. MW = 429.5126. Anal. ($C_{20}H_{19}N_3O_4S_2$) C, H, N.

*Dimethyl 2,6-Dimethyl-4-(2-methyl-6-(thiophen-3-yl)imidazo-[2,1-b]thiazol-5-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**10**).* Yield 12%. IR: 1706, 1301, 1204, 1107, 1025. ^{1}H NMR: 2.22 (6H, s, CH_3), 2.40 (3H, s, CH_3), 3.24 (6H, s, $COOCH_3$), 5.67 (1H, s, dhp), 6.94 (1H, s, th), 7.56 (2H, m, thio-4−5), 7.74 (1H, m, thio-2), 9.03 (1H, s, NH, ex D_2O). Melting point = 210 °C. MW = 443.5391. Anal. ($C_{21}H_{21}N_3O_4S_2$) C, H, N.

*Dimethyl 4-(6-(2,5-Dichlorothiophen-3-yl)imidazo[2,1-b]thiazol-5-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**11**).* Yield 11%. IR: 1705, 1672, 1308, 1213, 1119. ^{1}H NMR: 2.12 (6H, s, CH_3), 3.34 (6H, s, $COOCH_3$), 5.17 (1H, s, dhp), 6.90 (1H, s, thio), 7.28 (1H, d, th, J = 4.4), 7.79 (1H, d, th, J = 4.4), 8.66 (1H, s, NH, ex D_2O). Melting point = 202 °C. MW = 498.4027. Anal. ($C_{20}H_{17}Cl_2N_3O_4S_2$) C, H, N.

*Dimethyl 4-(6-(2,5-Dichlorothiophen-3-yl)-2-methylimidazo[2,1-b]thiazol-5-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**12**).* Yield 10%. IR: 1705, 1670, 1209, 1112, 1020. ^{1}H NMR: 2.11 (6H, s, CH_3), 2.45 (3H, s, CH_3), 3.44 (6H, s, $COOCH_3$), 5.10 (1H, s, dhp), 6.86 (1H, s, thio), 7.55 (1H, s, th), 8.61 (1H, s, NH, ex D_2O). Melting point = 225 °C. MW = 512.4293. Anal. ($C_{21}H_{19}Cl_2N_3O_4S_2$) C, H, N.

*Diethyl 4-(6-(2,5-Dimethoxyphenyl)imidazo[2,1-b]thiazol-5-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**13**).* Yield 10%. IR: 1697, 1666, 1511, 1202, 1124. ^{1}H NMR: 0.94 (6H, t, $COOCH_2CH_3$, J = 7.1), 1.91 (6H, s, CH_3), 3.48 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.89 (4H, q, $COOCH_2CH_3$, J = 7.1), 5.05 (1H, s,

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dhp), 6.46 (1H, s, ar-6), 6.85 (1H, s, ar-3,4), 7.17 (1H, d, th, J = 4.6), 7.84 (1H, d, th, J = 4.6), 8.04 (1H, s, NH, ex D_2O). Mp = 235−240 °C. MW = 510.5820. Anal. ($C_{26}H_{28}N_3O_6S$) C, H, N.

B. Receptor Binding Studies. For details, see Supporting Information, section S2.

C. Functional Assays. For details, see Supporting Information, section S4.

D. Computational Methods. For details, see Supporting Information, section S5.

■ ASSOCIATED CONTENT

Supporting Information

Details for functional assays and for receptor binding studies and for computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For R.B.: phone, +39-051-2099737; fax, +39-051-2099721; E-mail, roberta.budriesi@unibo.it. For A.L.: phone, +39-051-2099714; fax, +39-051-2099721; E-mail, alberto.leoni@unibo.it.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CNS, central nervous system; LTCC, L-type calcium channel; 1,4-DHPs, 1,4-dihydropyridines; GPILSM, guinea pig ileum longitudinal smooth muscle; NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; SEM, standard error mean; QSAR, quantitative structure−activity relationship; PSA, polar surface area; RB, rotatable bonds

■ REFERENCES

(1) Triggle, D. J. L-Type calcium channels. *Curr. Pharm. Des.* **2006**, *12*, 443−457.

(2) Catterall, W. A.; Perez-Reyes, E.; Snutch, T. P.; Striessnig, J. International Union of Pharmacology. XLVIII. Nomenclature and structure−function relationships of voltage-gated calcium channels. *Pharmacol. Rev.* **2005**, *57*, 411−425.

(3) Zuccotti, A.; Clementi, S.; Reinbothe, T.; Torrente, A.; Vandael, D. H.; Pirone, A. Structural and functional differences between L-type calcium channels: crucial issues for future selective targeting. *Trends Pharmacol. Sci.* **2011**, *6*, 366−375.

(4) Fleckenstein, A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol.* **1977**, *17*, 149−166.

(5) Striessnig, J.; Grabner, M.; Mitterdorfer, J.; Hering, S.; Sinnegger, M. J.; Glossmann, H. Structural basis of drug binding to L Ca^{2+} channels. *Trends Pharmacol. Sci.* **1998**, *19*, 108−115.

(6) McDonough, S. I., Ed. *Calcium Channels Pharmacology*; Kluwer Academic/Plenum Publishers: New York, 2004.

(7) Triggle, D. J. Calcium channel antagonists: clinical uses—past, present and future. *Biochem. Pharmacol.* **2007**, *74*, 1−9.

(8) Hetzenauer, A.; Sinnegger-Brauns, M. J.; Striessnig, J.; Singewald, N. Brain activation pattern induced by stimulation of L-type Ca^{2+}-channels: contribution of Ca(V)1.3 and Ca(V)1.2 isoforms. *Neuroscience* **2006**, *139*, 1005−1015.

(9) Sinnegger-Brauns, M. J.; Huber, I. G.; Koschak, A.; Wild, C.; Obermair, G. J.; Einzinger, U.; Hoda, J. C.; Sartori, S. B.; Striessnig, J. Expression and 1,4-dihydropyridine-binding properties of brain L-type calcium channel isoforms. *Mol. Pharmacol.* **2009**, *75*, 407−414.

(10) Ioan, P.; Carosati, E.; Micucci, M.; Cruciani, G.; Broccatelli, F.; Zhorov, B. S.; Chiarini, A.; Budriesi, R. 1,4-Dihydropyridine Scaffold in Medicinal Chemistry, The Story So Far and Perspectives. Part 1: Action in Ion Channels and GPCRs. *Curr. Med. Chem.* **2011**, *18*, 4901−4922.

(11) Carosati, E.; Ioan, P.; Micucci, M.; Broccatelli, F.; Cruciani, G.; Zhorov, B. S.; Chiarini, A.; Budriesi, R. 1,4-Dihydropyridine Scaffold in Medicinal Chemistry, The Story So Far and Perspectives. Part 2: Action in Other Targets and Antitargets. *Curr. Med. Chem.* **2012**, *19*, 4306−4323.

(12) Striessnig, J.; Koschak, A.; Sinnegger-Brauns, M. J.; Hetzenauer, A.; Nguyen, N. K.; Busquet, P.; Pelster, G.; Singewald, N. Role of voltage-gated L-type Ca^{2+} channel isoforms for brain function. *Biochem. Soc. Trans.* **2006**, *34*, 903−909.

(13) Budriesi, R.; Ioan, P.; Leoni, A.; Locatelli, A.; Cosconati, S.; Ugenti, M. P.; Andreani, A.; Di Toro, R.; Bedini, A.; Spampinato, S.; Marinelli, L.; Novellino, E.; Chiarini, A. Imidazo[2,1-*b*]thiazole System: A Scaffold Endowing Dihydropyridines With Selective Cardiodepressant Activity. *J. Med. Chem.* **2008**, *51*, 1592−1600.

(14) Phillips, A. P. Hantzsch's pyridine synthesis. *J. Am. Chem. Soc.* **1949**, *71*, 4003−4007.

(15) Andreani, A.; Rambaldi, M.; Andreani, F.; Bossa, R.; Galatulas, I. 6-Pyridinylimidazo[2,1-*b*]thiazoles and Thiazolines as Potential Cardiotonic Agents. *Eur. J. Med. Chem.* **1985**, *20*, 93−94.

(16) Andreani, A.; Burnelli, S.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Varoli, L.; Farruggia, G.; Stefanelli, C.; Masotti, L.; Kunkel, M. W. Synthesis and Antitumor Activity of Guanylhydrazones from 6-(2,4-Dichloro-5-nitrophenyl)imidazo[2,1-*b*]thiazoles and 6-Pyridilimidazo[2,1-*b*]thiazoles. *J. Med. Chem.* **2006**, *49*, 7897−7901.

(17) Andreani, A.; Burnelli, S.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Varoli, L.; Calonghi, N.; Cappadone, C.; Farruggia, G.; Zini, M.; Stefanelli, C.; Masotti, L.; Radin, N. S.; Shoemaker, R. H. New Antitumor Imidazo[2,1-*b*]thiazole Guanylhydrazones and Analogues. *J. Med. Chem.* **2008**, *51*, 809−816.

(18) Andreani, A.; Rambaldi, M.; Locatelli, A.; Bossa, R.; Galatulas, I.; Ninci, M. Synthesis and cardiotonic activity of 2,5-dimethoxyphenylimidazo[2,1-*b*]thiazoles. *Eur. J. Med. Chem.* **1992**, *27*, 431−433.

(19) Budriesi, R.; Ioan, P.; Leoni, A.; Pedemonte, N.; Locatelli, A.; Micucci, M.; Chiarini, A.; Galietta, L. J. Cystic Fibrosis: A New Target for 4-Imidazo[2,1-*b*]thiazole-1,4-DHPs. *J. Med. Chem.* **2011**, *54*, 3885−3094.

(20) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, 2nd ed.; Springer-Verlag: New York, 1987.

(21) Motulsky, H., Christopoulos, A. *Fitting Models to Biological Data Using Linear and Nonlinear Regression*, 2003, www.graphpad.com. Accessed on 1/12/2011.

(22) Motulsky, H. J. *Prism 5 Statistics Guide*; GraphPad Software Inc.: San Diego, CA, 2007; www.graphpad.com. Accessed on 1/12/2011.

(23) *GraphPad*, version 3.0; GraphPad Software, Inc.: San Diego, CA.

(24) Tarabová, B.; Nováková, M.; Lacinová, L. Haloperidol moderately inhibits cardiovascular L-type calcium current. *Gen. Physiol. Biophys.* **2009**, *28* (3), 249−529.

(25) Cosconati, S.; Marinelli, L.; Lavecchia, A.; Novellino, E. Characterizing the 1,4-dihydropyridines binding interactions in the L-Type Ca^{2+} channel: model construction and docking calculations. *J. Med. Chem.* **2007**, *50*, 1504−1513.

(26) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera−a visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605−1612.

(27) Limongelli, V.; Marinelli, L.; Cosconati, S.; La Motta, C.; Sartini, S.; Mugnaini, L.; Da Settimo, F.; Novellino, E.; Parrinello, M. Sampling protein motion and solvent effect during ligand binding. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 1467−1472.
(28) Limongelli, V.; Bonomi, M.; Marinelli, L.; Gervasio, F. L.; Cavalli, A.; Novellino, E.; Parrinello, M. Molecular basis of cyclooxygenase enzymes (COXs) selective inhibition. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 5411−5416.
(29) Lo Monte, F.; Kramer, T.; Gu, J.; Anumala, U. R.; Marinelli, L.; La Pietra, V.; Novellino, E.; Franco, B.; Demedts, D.; Van Leuven, F.; Fuertes, A.; Dominguez, J. M.; Plotkin, B.; Eldar-Finkelman, H.; Schmidt, B. Identification of Glycogen Synthase Kinase-3 Inhibitors with a Selective Sting for Glycogen Synthase Kinase-3α. *J. Med. Chem.* **2012**, *55*, 4407−4424.