Imidazol-1-yl and Pyridin-3-yl Derivatives of 4-Phenyl-1,4-dihydropyridines Combining Ca^{2+} Antagonism and Thromboxane A_2 Synthase Inhibition

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A series of derivatives of 4-phenyl-1,4-dihydropyridine bearing imidazol-1-yl or pyridin-3-yl moieties on the phenyl ring were synthesized with the aim of combining Ca^{2+} antagonism and thromboxane A_2 (TxA_2) synthase inhibition in the same molecule. Some of these compounds showed significant combined Ca^{2+} antagonism and TxA_2 synthase inhibition *in vitro*, while others showed only one single activity. Structural requirements for significant single or combined activities are discussed. Theoretical conformational analysis, by molecular mechanics and semiempirical AM1 calculations, was performed for 1,4-dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid, diethyl ester (FCE 24265) and two close congeners. FCE 24265, which inhibited TxB_2 production in rat whole blood with $IC_{50} = 1.7 \times 10^{-7}$ M and antagonized K^+ induced contraction in guinea pig aorta with $IC_{50} = 6.0 \times 10^{-8}$ M, was selected for further pharmacological evaluation. Our results show that this compound is less potent than nifedipine both *in vitro* and *in vivo* yet presents a favorable profile *in vivo*, lowering blood pressure without inducing reflex tachycardia. Moreover, its additional potent and selective TxA_2 synthase inhibitory activity makes this compound an interesting pharmacologic tool in pathologies where both enhanced TxA_2 synthesis and cellular Ca^{2+} overload are involved.

Introduction

Dihydropyridine (DHP) calcium antagonists (CA), i.e., nifedipine and structurally related drugs (Chart I), are a known subclass of a wider class of CA, which are among the most commonly used drugs for patients with cardiovascular diseases.

In particular, DHP-CA are extensively used for the treatment of hypertension,[1] subarachnoid hemorrhage,[2,3] myocardial infarction,[4-6] and stable[7,8] and unstable angina,[9,10] even though recently their therapeutic efficacy in myocardial infarction and angina has been questioned.[11] This class of compounds is also under clinical evaluation for the treatment of heart failure,[12] ischemic brain damage,[13] nephropathies,[14] and atherosclerosis.[15]

Thromboxane A_2 synthase inhibitors (TxSI) (Chart II) are a class of pharmacological agents under investigation for their potential role in counteracting proaggregative and vasoconstrictive effects of enhanced TxA_2 levels occurring in several vascular pathologies as myocardial infarction,[16] unstable angina,[17] and various glomerular diseases.[18,19] In particular, this class of compounds is under clinical evaluation in the treatment of asthma,[20] subarachnoid hemorrhage,[21] diabetic nephropathy,[22] and nephrotic syndrome.[23]

TxA_2 may also play a role in the development of atherosclerosis,[24] and a TxSI has proved to be beneficial as an antiatherosclerotic agent in an experimental model of atherosclerosis.[25]

Chart I. Representative Ca^{2+} Antagonists of Dihydropyridine Class

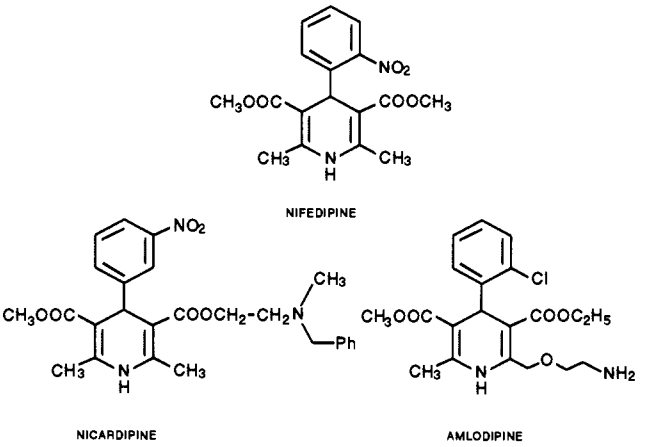
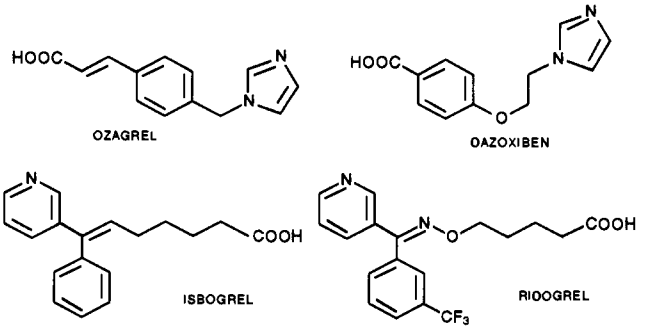


Chart II. Representative TXA_2 Synthase Inhibitors



Thus, in some cases, the established therapeutic applications of DHP-CA and the potential therapeutic targets of TxSI are coincident, and it may be speculated that in pathologies in which vasoconstriction is the result of multiple mechanisms and is associated with enhanced

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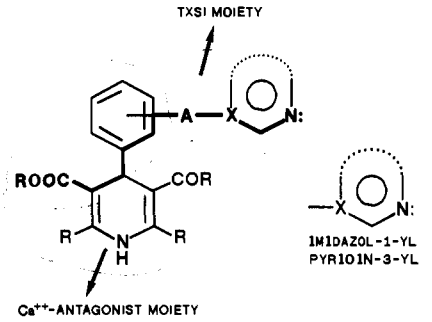
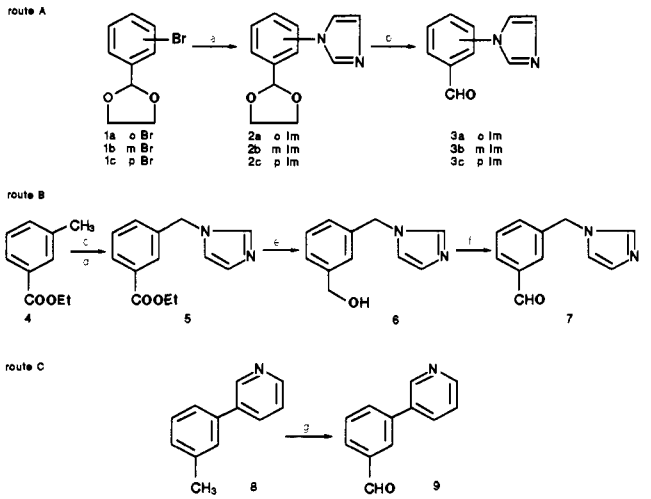


Figure 1.

Scheme I[a]



[a] Reagents: (a) imidazole–Na/Cu; (b) aq HCl; (c) NBS; (d) imidazole; (e) $LiAlH_4$; (f) DMSO–$(COCl)_2$/Et_3N; (g) CrO_3/Ac_2O.

platelet activation, such as myocardial infarction, unstable angina, subarachnoid hemorrhage, and progressive renal diseases, agents combining Ca^{2+} antagonism and TxA_2 synthase inhibition might show enhanced therapeutic efficacy.

As SAR requirements for DHP-CA and TxSI are known,[26,27] the aim of our synthetic work was to incorporate in the DHP frame what is considered the minimal structural requirement for potent and selective TxA_2 synthase inhibition: the presence of an unhindered "pyridine" nitrogen (sp_2 hybridized) of a pyridine, imidazole, or other suitable azole ring and a carboxy or carboxy-derived function (e.g., ester or amide) separated by a distance of about 9 Å[28,29] (Figure 1).

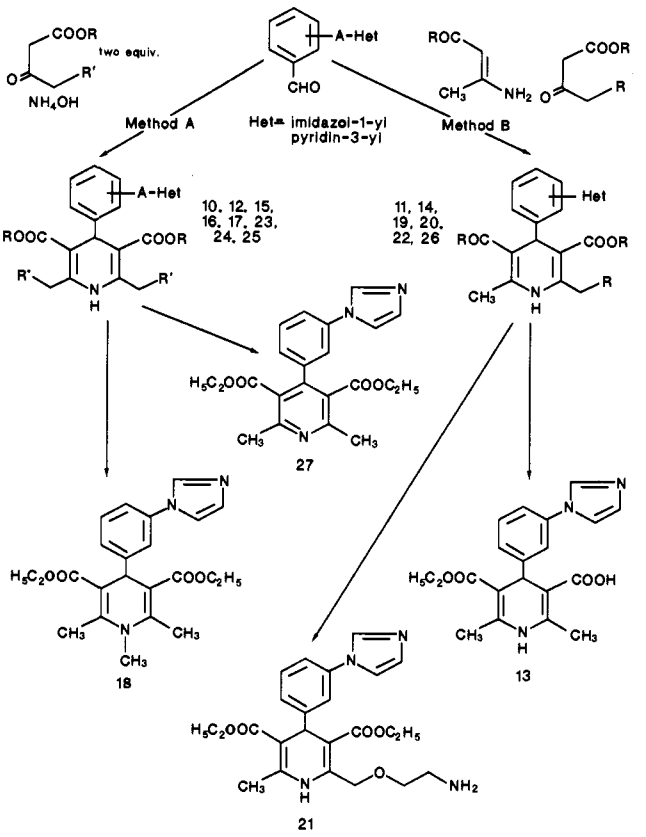
For reaching our target we followed the approach of replacing substituents on the phenyl ring of the 4-phenyl-1,4-dihydropyridine with an imidazol-1-yl or a pyridin-3-yl moiety. This paper describes the synthesis and biological evaluation of a series of imidazol-1-yl derivatives of 4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate esters and of some pyridin-3-yl congeners and related compounds, some of which have significant combined Ca^{2+} antagonism and TxA_2 synthase inhibitory activity.

Chemistry

Scheme I reports the synthesis of benzaldehydes which were key intermediates for subsequent synthesis, reported in Scheme II, of tested compounds listed in Table I.

Benzaldehydes of formula **3** were obtained in good overall yields by the reaction of appropriately substituted bromodioxolanes **1** (commercially available or prepared by known procedures) with the sodium salt of imidazole

Scheme II



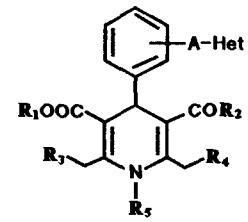
prepared *in situ*, followed by the subsequent acid hydrolysis of imidazolyl dioxolanes **2**. In particular, intermediates **2** were obtained in yields ranging from 65% (**2c**) to 87% (**2b**) with reaction times ranging from 4 to 6 h. Performing the reaction of bromo derivatives **1** with imidazole instead of its sodium salt resulted in lower yields and recovery of unreacted starting material with even larger reaction times. The synthesis of **3b** and **3c** has already been reported involving direct reaction of halogenobenzaldehydes and imidazole in various conditions,[31,32] but with low yields in the case of **3c** (about 30%) and with better yields in the case of **3b** (about 50%) and with a very long reaction time (3 days).[33] When we tried a direct reaction of bromobenzaldehydes with imidazole we obtained complex reaction mixtures.

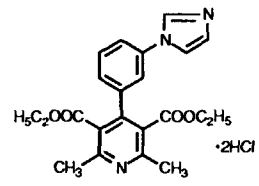
Benzaldehyde **7** was obtained following the sequence outlined in route B (Scheme I) from ethyl 3-methylbenzoate through subsequent bromination with NBS, reaction of ethyl 3-(bromomethyl)benzoate with imidazole in DMF, reduction of the ester function with $LiAlH_4$, and final Swern Oxidation of the carbinol obtained. Finally, benzaldehyde **9** (route C, Scheme I) was obtained by oxidation of the intermediate **8**, obtained in turn by a known procedure[34] from 3-bromotoluene and 3-bromopyridine, using chromic anhydride in acetic anhydride.

Scheme II reports the synthesis of the tested compounds starting from benzaldehydes **3**, **7**, and **9**, following two classic procedures of the Hantsch dihydropyridine synthesis.[35]

Procedure A, i.e., the reaction of the appropriate benzaldehyde with 2 equiv of the appropriate keto ester and concentrated ammonium hydroxide in EtOH, was followed for the synthesis of symmetric compounds. This procedure, in the case of compound **12**, was performed on a 60-g scale with a yield of 84%.

Table I. Imidazol-1-yl and Pyridin-3-yl Derivatives of 4-Phenyl-1,4-dihydropyridines. Physical Properties, Methods, Yields and *in Vitro* TXA_2 Synthase Inhibition and Ca^{2+} Antagonism



compd	Het[a]	A	phenyl substit	R_1	R_2	R_3	R_4	R_5	meth.	yield[b] (%)	mp[c] (°C)	formula[d]	IC_{50} (μM) TXB_2[e]	IC_{50} (μM) Ca^{++} ant.[f]
10	Im		meta	Me	OMe	H	H	H	A	40	212–16	$C_{23}H_{21}N_3O_4$	0.27 ± 0.03[g]	0.85 ± 0.01
11	Im		meta	Me	OEt	H	H	H	B	60	197–200	$C_{23}H_{21}N_3O_4$	0.24 ± 0.02[g]	0.26 ± 0.04
12	Im		meta	Et	OEt	H	H	H	A	84	202–4	$C_{25}H_{25}N_3O_4$	0.17 ± 0.04[g]	0.06 ± 0.01
13	Im		meta	H	OEt	H	H	H	*h*	*h*	117–21 dec	$C_{20}H_{21}N_3O_4 \cdot H_2O$	>1.00 (42%)	1.96 ± 0.30
14	Im		meta	Et	$O(CH_2)_2N(Me)Bzl$[a]	H	H	H	B	11	oil	$C_{30}H_{34}N_4O_4$	0.22 ± 0.02[g]	0.05 ± 0.01
15	Im		ortho	Et	OEt	H	H	H	A	18	208–10	$C_{25}H_{25}N_3O_4$	>1.00 (34%)	0.46 ± 0.03
16	Im		para	Et	OEt	H	H	H	A	22	231–3	$C_{25}H_{25}N_3O_4$	>1.00 (40%)	23.00 ± 0.5
17	Im	CH_2	meta	Et	OEt	H	H	H	A	40	196–9	$C_{26}H_{27}N_3O_4$	0.73 ± 0.1	1.67 ± 0.1
18	Im		meta	Et	OEt	H	H	Me	*i*	*i*	oil	$C_{26}H_{27}N_3O_4$	0.64 ± 0.2	8.84 ± 0.2
19	Im		meta	Me	OEt	H	OMe	H	B	31	153–4	$C_{25}H_{25}N_3O_5$	0.40 ± 0.01[g]	1.95 ± 0.2
20	Im		meta	Me	OEt	H	$O(CH_2)_2NHPht$[a]	H	B	49	168–72	$C_{31}H_{30}N_4O_7$	0.70 ± 0.06	3.03 ± 0.7
21	Im		meta	Me	OEt	H	$O(CH_2)_2NH_2$	H	*j*	*j*	138–40	$C_{29}H_{28}N_4O_5 \cdot C_4H_4O_4$	0.30 ± 0.02[g]	2.37 ± 0.5
22	Im		meta	Me	Me	H	H	H	B	42	226	$C_{25}H_{21}N_3O_3$	0.26 ± 0.02[g]	>10.00
23	Im		meta	Et	OEt	Me	Me	H	A	40	171–2	$C_{26}H_{29}N_3O_4$	>1.00 (38%)	0.97 ± 0.06
24	Py		meta	Et	OEt	H	H	H	A	57	183–6	$C_{26}H_{29}N_3O_4$	0.28 ± 0.03[g]	0.10 ± 0.01
25	Py		meta	iBu[a]	OiBu[a]	H	H	H	A	29	97–8	$C_{30}H_{37}N_3O_4$	>1.00 (4%)	1.50 ± 0.2
26	Py		meta	Et	$O(CH_2)_2N(Me)Bzl$[a]	H	H	H	B	15	oil	$C_{32}H_{35}N_3O_4$	>1.00 (15%)	0.06 ± 0.01
27									*k*	*k*	178–80	$C_{23}H_{23}N_3O_4 2HCl \cdot H_2O$	>1.00 (15%)	>10.00
nifedipine													>10.00	0.004 ± 0.001
dazoxiben													1.20 ± 0.05	

[a] Key: Im = imidazol-1-yl; Py = pyridin-3-yl; Bzl = benzyl; Pht = phthalyl; iBu = isobutyl. [b] No effort was made to optimize yields except for 12. [c] Compounds were usually purified by column chromatographic separation. [d] All compounds were analyzed for C, H, N; results were within ±0.4% of theoretical values. [e] Micromolar concentration (mean ± SEM of eight replications) required to inhibit by 50% TXB_2 production in rat whole blood during clotting at 37 °C for 1 h; TXB_2 assayed by RIA. [f] Micromolar concentration (mean ± SEM of four relications) required to block K^+-induced contraction in guinea pig ileum strips. [g] $p < 0.05$ from reference standard dazoxiben. [h] Hydrolysis of 12, yield 50%. [i] Methylation of 12, yield 48%. [j] Deprotection of 20, yield 65%. [k] Oxidation of 12, yield 72%.

Procedure B, i.e., the reaction of the appropriate benzaldehyde with 1 equiv of keto ester and 1 equiv of the appropriate aminocrotonate in EtOH, was followed for the synthesis of asymmetric compounds.

Compound 18 was obtained from 12 treated with powdered KOH in DMSO, by reaction with CH_3I. Compound 27 was obtained by oxidation of 12 with $NaNO_3$ in water. The monoethyl ester 13 was obtained by alkaline hydrolysis of the corresponding cyanoethyl ethyl ester obtained in turn by procedure B.

The amlodipine-like compound 21 was obtained from corresponding phthalimido derivative 20 by removal of the protecting group with methylamine dissolved in diisopropyl ether.

Results and Discussion

The compounds reported in Table I were evaluated *in vitro* as for the inhibition of the production of TxB_2 (stable metabolite of TxA_2) in rat whole blood during clotting and for the inhibition of potassium-induced contraction in guinea-pig ileum strips, as described in the Experimental Section.

Several compounds show relevant TxA_2 synthase inhibitory activity, often significantly superior to that of the reference compound dazoxiben (e.g., 10–12, 14, 19–22, and 24). This activity appears to be closely linked to *meta* substitution of the imidazolyl moiety on the phenyl ring (12 vs 15 and 16), and because this feature is also crucial for Ca^{2+}-antagonism, the investigation of the role of other moieties was performed with fixed *meta* substitution of the phenyl ring.

Apparently, TxA_2 synthase inhibitory activity is independent from a given 3,5-diester structure in the imidazolyl series (10–12, 14), while this is not the case in the pyridinyl series where only the 3,5-diethyl ester analogue 24 retains the same activity as its imidazolyl congener 12 (24 vs 12; 24 vs 25 and 26).

One of the 3,5 ester functions may be replaced by a ketone (22 vs 10) but not by a free carboxylic group (13 vs 12). As far as 2,6-substitution is concerned, replacement of 2,6-dimethyl with 2,6-diethyl groups leads to loss of activity (23 vs 12); surprisingly, the replacement of a methyl by a methoxymethyl or by an (aminoethoxy)methyl group (19 and 21 vs 11) maintains the activity.

It must be noted, however, that 19 and 21 are racemates, so that their activity may arise from enantioselectivity of action.

When the imidazolyl moiety is linked to the phenyl ring by means of a methylene bridge, the activity tends to decrease (17 vs 12). Finally, the replacement of DHP itself by a pyridine ring gives an inactive compound (27 vs 12).

As far as Ca^{2+} antagonism is concerned, two points deserve comment. First, the SAR, in most cases, does not differ substantially from that reported for classic DHP-CA, even though the potency is lower than that found with the most potent drugs of this class as, for example, reference compound nifedipine. In fact, Ca^{2+} antagonism is dramatically reduced by (a) replacement of DHP by a pyridine ring (27 vs 12), (b) substitution of DHP nitrogen (N-1) by a methyl group (18 vs 12), (c) para substitution on the phenyl ring (16 vs 12), and (d) replacement of one ester function by a ketone or carboxy group (18 and 13 vs 12). All these variations are also detrimental in classic DHP-CA.[26] However, in our series, at variance with the usual effect of the substitution pattern on phenyl ring, *ortho* substitution with the imidazole group leads to a loss of activity in comparison with *meta* substitution (15 vs 12).

The most potent derivatives are 3,5-diethyl esters and ethyl, [(*N*-methyl-*N*-benzylamino)ethoxy]methyl esters (nicardipine pattern) (12, 14, 24, 26), while the compound with the (aminoethoxy)methyl group, typical of amlodipine, is devoid of significant activity (21).

A second important point is that in most cases Ca^{2+} antagonism and TxA_2 synthase inhibition coexist. There are few exceptions, such as ester ketone 22 and compounds with asymmetric 2,6-substitution pattern, 19 and 21, which maintain only TxA_2 synthase inhibition, and nicarpidine-like derivative of pyridinyl series 26, which, on the contrary, maintains only Ca^{2+} antagonism.

In conclusion, significant combined Ca^{2+} antagonism and TxA_2 synthase inhibition appear to be linked to the presence of a 1,4-dihydro-4-phenyl-2,6-dimethylpyridine-3,5-dicarboxylate ester frame in which the 4-phenyl ring is *meta*-substituted by an imidazol-1-yl or pyridin-3-yl moiety. Compounds 12, 14, and 24 show most relevant combined activity.

Conformational analysis was performed for compound 12, which combines achirality with one of the best dual activities of the series, and for two of its closest congeners, compounds 15 and 16, in which the variation of substitution pattern of imidazolyl moiety on the phenyl ring dramatically affects the activities.

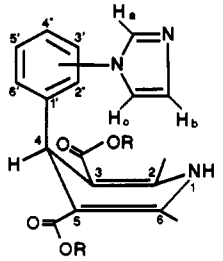
Regioselectivity of action linked to the substitution pattern on the phenyl ring is not surprising since this may interfere both with the conformation of dihydropyridine ring, known to be relevant for Ca^{2+} antagonism, and with the distance between imidazole N(3) nitrogen and 3,5-diester functions, hypothesized by us to be relevant for TxA_2 synthase inhibition. It is now largely accepted that the active conformation of DHP-CA involves a flat boat shape for the DHP ring with a pseudoaxial orientation of the 4-phenyl ring. A correlation between the degree of ring flattening and pharmacological activity was proposed,[36] and the relative position consequently adopted by the 4-aryl ring was demonstrated to be of paramount importance.[26]

A similar behavior was observed by us in the model molecules 12, 15, and 16, on which theoretical calculations were run. The geometric optimization of the structures of 12, 15, and 16, in which the aryl group was pseudoequatorially oriented, always gave rise to the corresponding pseudoaxial conformer. As for the role of antiperiplanarity (substituent toward DHP ring) or synperiplanarity (substituent away from DHP ring) for *ortho* and *meta* substitution on the phenyl ring, our results show that there is not a bias for one rotamer between syn and antiperiplanar conformers (see the heat of formation in Table II).

Free rotation around the C_1'–C_4 bond is confirmed by nuclear Overhauser effect (NOE) experiments in DMSO solution. In fact (structure in Table II), irradiation of C_4-H of 12 (*meta*) causes a NOE enhancement on C_2'-H (9%) and C_6'-H (5.9%), and irradiation of C_4-H of 15 (*ortho*) causes a NOE enhancement on C_6'-H (5.1%), H_A (4.6%), and H_C (7.1%), thus confirming for both regioisomers 12 and 15 the presence of both rotamers in solution.

These data are in agreement with reported theoretical calculations[37] but do not contradict the possible role of the synperiplanar semiaxial conformer as a bioactive

Table II. Theoretical Heat of Formation, Summation, and Selected Torsion Angles, Calculated Distance between Imidazole N(3) and DHP–COOR for Compounds 12, 15, and 16



compd	conformer[a]	heat of formn[b] (kcal/mol)	$\sum(\theta)$[c] (deg)	torsion 3–4–1′–2′ (deg)	N(3)–COOR[d] distance range (Å)
12	sp	−66.6	74.2	118.0	7.2–8.0
	ap	−66.4	79.2	233.9	7.7–8.4
15	sp	−61.4	81.0	130.7	5.1
	ap	−61.4	58.6	237.4	5.3–5.6
16		−61.7	81.6	142.6	8.6–9.0

[a] sp = synperiplanar, ap = antiperiplanar. [b] Determined by MOPAC software package using AM1 method. [c] Summation of torsion angles of the 1,4-dihydropyridine ring. [d] Distance range between nitrogen N(3) of imidazole and carbonyl of 3/5 diester function on DHP ring.

structure suggested by several authors.[38,39] The flatness of the 1,4-dihydropyridine rings and the orientation of the phenyl groups of 12, 15, and 16 are given in Table II as summations, $\sum|\theta|$, of torsional angles of the 1,4-dihydropyridine ring and as value of torsion angle C_3–C_4–C_1'–C_2', respectively. Lower values of $\sum|\theta|$ and torsion angle C_3–C_4–C_1'–C_2' mean an increase in the flatness of the 1,4-dihydropyridine which correspond to a better Ca^{2+} antagonism. It is interesting to observe that nifedipine, our Ca^{2+} antagonist of reference, shows in the crystal structure conformation the $\sum|\theta|$ value of 72.1° and the torsion angle of 101.0°.[36] These values are similar to those shown by the *sp* conformer of 12 and far from those shown by 15 and 16, and this, as a hypothesis, might explain better Ca^{2+} antagonist activity of 12.

Table II also reports the distances between N(3) imidazole nitrogen and the carbon of carbonyl ester functions, which prove, in the case of inactive *ortho* isomer 15, to be largely inferior to the lower limit of the range of 8.5–10 Å, calculated by different authors, for N(3)—C=O distances in most potent TxSI. In the case of *meta* isomer 12, upper limits of calculated distances, 8 Å for synperiplanar and 8.4 Å for antiperiplanar axial conformations, are near the lower limit of the optimal range for TxSI.

In the case of *para* isomer 16 both calculated limits, 8.6–9 Å, fall within the optimal range for TxSI. The lack of TxA_2-synthase inhibitory activity for 16, in spite of proper N(3)—C=O distance, may be explained by markedly different spatial arrangement for compounds 12 and 16 obtained superimposing imidazolylphenyl moieties of two isomers (Figure 2). It may be inferred that the presence of DHP ring allows the recognition of C=O ester function, by the enzyme active site, in the case of the *meta* but not in the case of the *para* isomer.

Compound 12, which was one of the most active compounds of the series and showed low acute toxicity (LD_{50} >800 mg/kg, single oral dose in the mouse), was selected for further evaluation. Its selectivity toward TxA_2 synthase was proved by the enhancement of PGE_2 levels in rat whole blood during clotting, which paralleled the

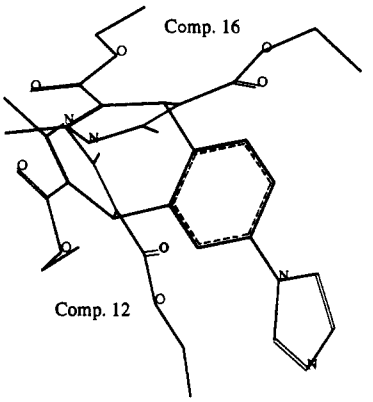


Figure 2. Superimposition of imidazolylphenyl moieties of compounds 12 and 16. Conformations, generated using the SYBYL molecular modeling program, in which the distances between imidazole N(3) nitrogen and ester carbonyl group are 8 Å.

Table III. Ca^{2+} Antagonistic Effect of 12 and Nifedipine *in Vitro*[a]

	guinea pig ileum IC_{50} (nM)	rabbit renal artery IC_{50} (nM)	guinea pig right ventricle IC_{50} (nM)	guinea pig atria IC_{25} (nM)
12	61.5 (23.4–158)	111.0 (59.6–206)	2150 (1750–2540)	94.2 (64.8–137)
nifedipine	4.7 (4.1–5.6)	6.9 (5.1–9.5)	57.8 (49.3–66.0)	31.5 (17.3–57.2)
potency ratio nifedipine/12	12 (11.8–12.2)	15.7 (15.6–15.8)	37.1 (37.0–37.2)	2.9 (2.8–3.0)

[a] Data are means of four replications. Limits for $p = 0.05$ are reported in parentheses. IC_{50} and IC_{25} were evaluated by regression analysis.

inhibition of TxA_2 production (EC_{200}, i.e., concentration doubling PGE_2 levels, was 0.14 μM). Ca^{2+} antagonism *in vitro* for compound 12 and reference standard nifedipine are summarized in Table III.

Compound 12 antagonized in a dose-dependent way (30–300 μM) K^+-induced contractions in rabbit renal artery preparations with an IC_{50} of 111 nM. Its potency was about 15 times lower than that of nifedipine in this isolated vessel, as previously found in the guinea pig ileum screening test. A negative inotropic effect was observed in electrically stimulated guinea pig right ventricles with both compounds, but at relatively higher concentrations with compound 12, as it was about 35 times less active than nifedipine. On the other hand, 12 was only three times less active than nifedipine in reducing spontaneous heart rate (IC_{25} = 94.2 vs 31 nM).

These data show that compound 12, although less potent than nifedipine in inhibiting smooth muscle contraction, is endowed with a different pharmacologic profile, having a relatively less marked negative inotropic effect, yet a relatively more pronounced negative chronotropic activity. The lower potency *in vitro* of compound 12 translated into lower potency in reducing mean blood pressure when given intravenously to spontaneously breathing anesthetized dogs, as compared to nifedipine (potency ratio 1:12). However, unlike nifedipine, it did not cause a reflex increase of heart rate, in line with its relatively more pronounced negative chronotropic effect observed *in vitro* (Figure 3).

Conclusions

Active compounds reported here prove the possibility of combining significant Ca^{2+} antagonism and TxA_2

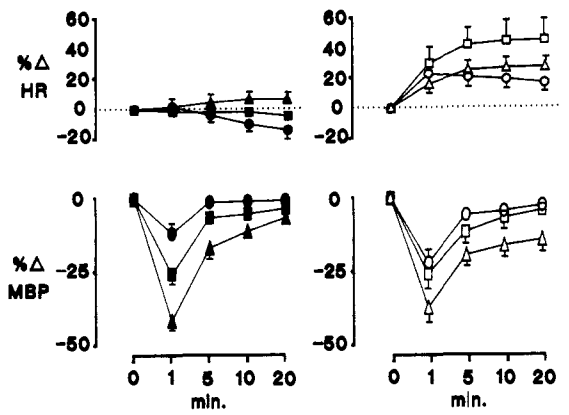


Figure 3. Time course of the effect on heart rate (HR) and mean blood pressure (MBP) of FCE 24285 [180 (●); 540 (■); 1620 (▲) μg/kg iv] and nifedipine [15 (○); 45 (□); 135 (△) μg/kg iv] in anesthetized dogs. Basal values of HR and MBP were, respectively, 142.2 ± 14.2 b/min and 114.2 ± 2.8 mmHg. Data are means ± SEM of six animals.

synthase inhibition in the same molecule by placing an imidazolyl or pyridyl moiety on the phenyl ring *meta* position of the 4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate frame typical of DHP-Ca^{2+} antagonists. At the end of our program of synthesis, this combined activity appeared in the literature for a compound in which a basic imidazolyl moiety was on the side-chain in position 2 of the DHP ring, a feature recalling amlodipine structure.[40]

Compound 12 (FCE 24265), in addition to significant TxA_2 synthase inhibition *in vitro* and low acute toxicity, presents a favorable profile of activity as a Ca^{2+} antagonist both *in vitro* and *in vivo* in comparison with nifedipine, lowering blood pressure without inducing reflex tachicardia. As already discussed, it may be speculated that agents of this kind may be useful in a variety of pathological conditions. In particular, the fact that ozagrel, the sole TxSI on the market, and some DHP-CA are among the few therapeutic tools available for treatment of subarachnoid hemorrhage may suggest this pathology as a possible elective field of application.

Experimental Section

Chemistry. Melting points were determined in open glass capillaries with a Buchi melting point apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 instrument, and C, H, and N results were within ±0.4% of theoretical values. ^{1}H NMR spectra for all the compounds were recorded on Varian VXR-200, Varian VXR 400S, and Bruker WP-80 SY instruments using the solvent as the internal standard, and chemical shifts are expressed in parts per million (δ). Column chromatographic separations were performed by the flash technique on 40/60 μm silica gel (Merck no. 9385). Of the compounds used as reference standards in pharmacological tests, dazoxiben was prepared according to the literature[41] and nifedipine was purchased from Research Biochemicals Inc.

2-[3-(1*H*-Imidazol-1-yl)phenyl]-1,3-dioxolane (2b). To a suspension of sodium hydride (55% mineral oil dispersion) (0.44 g, 0.01 mol) in 2 mL of DMF was added imidazole (0.68 g, 0.01 mol) with stirring at room temperature until hydrogen evolution ceased. Then, 2-(3-bromophenyl)-1,3-dioxolane (2.29 g, 0.01 mol) and copper powder (0.063 g, 0.001 mol) were added to the reaction mixture which was stirred at 150 °C for 4 h. The mixture was cooled to room temperature, diluted with $CHCl_3$ and water, stirred for 1 h, and filtered. The organic layer was separated, washed with water, dried over $CaCl_2$, and evaporated to dryness under vacuum. The residue was purified over flash silica gel column (eluant: CH_2Cl_2/MeOH = 93/7), yielding 1.81 g (84%) of the title compound, as an oil; the crude product can also be used without further purification.

^{1}H-NMR ($CDCl_3$) δ ppm: 3.84–4.22 (4H, m, $-OCH_2CH_2O-$), 5.85 (1H, s, $-OCHO-$), 7.11–7.61 (6H, m, phenyl ring + imidazole H^4, H^5), 7.86 (1H, br s, imidazole H^2).

By the same procedure, 2-[4-(1*H*-imidazol-1-yl)phenyl]-1,3-dioxolane (2c) (mp 125–6 °C (65%)) and 2-[2-(1*H*-imidazol-1-yl)phenyl]-1,3-dioxolane (2a) (mp 95–8 °C (77%)) were obtained.

3-(1*H*-Imidazol-1-yl)benzaldehyde (3b). 2-[3-(1*H*-Imidazol-1-yl)phenyl]-1,3-dioxolane (2b, 1.81 g, 0.0084 mol) was stirred at room temperature for 2 h in 18 mL of 1 N HCl. The reaction mixture was neutralized with aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with aqueous NaCl, dried over Na_2SO_4, and evaporated to dryness under vacuum, yielding 1.39 g (96%) of the pure product, as a pale yellow solid. Mp: 74.5–77.5 °C (lit.[33] mp 76–7 °C).

^{1}H-NMR ($CDCl_3$) δ ppm: 7.22 and 7.34 (2H, 2 br s, imidazole H^4, H^5), 7.50–8.06 (5H, m, phenyl ring + imidazole H^2), 10.06 (1H, s, $-CHO$).

By the same procedure, 4-(1*H*-imidazol-1-yl)benzaldehyde (3c) (mp 143–5 °C (90%) (lit.[32] mp 146–7 °C)) and 2-(1*H*-imidazol-1-yl)benzaldehyde (3a) (mp 46–8 °C (93%)) were obtained.

Ethyl 3-[(1*H*-Imidazol-1-yl)methyl]benzoate (5). Ethyl 3-methylbenzoate, (9.46 g, 0.058 mol) and *N*-bromosuccinimide (11.26 g, 0.063 mol) were stirred under reflux for 3 h in 100 mL of CCl_4. The reaction mixture was cooled to room temperature, the succinimide filtered off, and the organic solvent evaporated under vacuum. The crude product was directly allowed to react with imidazole (14 g, 0.206 mol) in 15 mL of DMF at room temperature for 5 h. The reaction mixture was poured into water and extracted with $CHCl_3$ and the organic layer treated with 10% HCl.

The aqueous acidic phase was neutralized with 10% NaOH, extracted with ethyl acetate, and dried over Na_2SO_4. The organic solvent was evaporated under vacuum, yielding 6 g (45%) of title compound as an oil. The crude product was pure enough to be used in the next reaction step.

^{1}H-NMR ($CDCl_3$) δ ppm: 1.35 (3H, t, $-CO_2CH_2CH_3$), 4.34 (2H, q, $-CO_2CH_2CH_3$), 5.13 (2H, s, $-CH_2N-$), 6.87–7.40 (4H, m, phenyl ring + imidazole H^4, H^5), 7.53 (1H, m, imidazole H^2), 7.76–8.07 (2H, m, H^2, H^6 phenyl ring).

3-[(1*H*-Imidazol-1-yl)methyl]benzyl Alcohol (6). Ethyl 3-[(1*H*-imidazol-1-yl)methyl]benzoate (5, 0.8 g, 0.0035 mol) and lithium aluminum hydride (0.265 g, 0.007 mol) were stirred in 10 mL of dry THF for 1 h at room temperature. The reaction mixture was poured into water, neutralized with 1 N HCl, diluted with ethyl acetate, and filtered. After separation of the organic layer, the aqueous phase was extracted twice with ethyl acetate and the organic extracts were collected, washed with aqueous NaCl, dried over Na_2SO_4, and evaporated under vacuum yielding 0.53 g (80%) of title compound as an oil, which was used for the next step without further purification.

^{1}H-NMR ($CDCl_3$) δ ppm: 4.58 (2H, s, $-CH_2OH$), 4.95 (2H, s, $-CH_2N=$), 5.43 (1H, br s, $-CH_2OH$), 6.70–7.43 (7H, m, phenyl ring + imidazole ring).

3-[(1*H*-Imidazol-1-yl)methyl]benzaldehyde (7). Oxalyl chloride (0.28 mL, 0.0031 mol) in 5 mL of CH_2Cl_2 and DMSO (0.44 mL, 0.00629 mol) in 1.5 mL of methylene chloride were stirred under nitrogen at −37 °C.

3-[(1*H*-Imidazol-1-yl)methyl]benzyl alcohol (6, 0.53 g, 0.0028 mol) in 2.5 mL of CH_2Cl_2 was added at −50 to −60 °C to the previous solution and allowed to react for 30 min. Triethylamine (1.96 mL, 0.0140 mol) was added, and the reaction mixture was allowed to warm to room temperature. Water and CH_2Cl_2 were added, the two layers were separated, and the organic phase was washed with aqueous NaCl, dried over $CaCl_2$, and evaporated under vacuum. The residue was purified by flash silica gel column (eluant: CH_2Cl_2/MeOH = 96/4), yielding 0.21 g (40%) of the title compound as an oil.

^{1}H-NMR ($CDCl_3$) δ ppm: 4.80 (2H, s, $-CH_2N=$), 6.95, 7.16 (2H, 2br s, imidazole H^4, H^5), 7.35–8.03 (5H, m, phenyl ring + imidazole H^2), 10.00 (1H, s, $-CHO$).

3-(3-Pyridyl)benzaldehyde (9). To a mixture of 3-(3-methylphenyl)pyridine (4 g, 0.0236 mol), prepared in turn following the literature,[34] glacial acetic acid (3.75 mL), and acetic anhydride (37 mL) was added 96% H_2SO_4 (5.7 mL) and then, after cooling at 5 °C, further added portionwise CrO_3 (6.53 g, 0.0653 mol).

After 30 min the reaction mixture was poured into ice–water, neutralized with 2 N NaOH, and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated. The oily residue was dissolved in 50% ethanol (10 mL), 96% H_2SO_4 (0.5 mL) was added, and the solution was refluxed for 30 min. After cooling, the resulting mixture was neutralized with 2 N NaOH and extracted with ethyl acetate. The organic layer, dried over Na_2SO_4, was evaporated, affording 1.2 g (28%) of title compound as an oil which was used as such for further reactions.

^{1}H-NMR ($CDCl_3$) δ ppm: 7.16–8.03 (8H, m, phenyl ring + pyridine H^4, H^5), 8.40–8.86 (2H, m, pyridine H^2, H^6), 10.00 (1H, s, –CHO).

Hantsch Synthesis: Procedure A. 1,4-Dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinecarboxylic Acid, Diethyl Ester (12). A mixture of 3-(1*H*-imidazol-1-yl)benzaldehyde (**3b**, 3.8 g, 0.022 mol), ethyl acetoacetate (6.89 g, 0.053 mol), and 30% ammonium hydroxide (3.6 mL) in absolute ethanol (40 mL) was stirred under reflux for 6 h. The reaction mixture was poured into ice–water, and the aqueous solution was extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over $CaCl_2$, and evaporated to dryness under vacuum. The residue was purified by silica gel column (eluant: CH_2Cl_2/MeOH = 95/5) giving a solid, which after crystallization from ethyl acetate afforded 7.31 g (84%) of title compound, mp 204–6 °C.

^{1}H-NMR ($CDCl_3$) δ ppm: 1.23 (6H, t, 2 $COOCH_2CH_3$), 2.38 (6H, s, =CCH_3), 4.12 (4H, q, $COOCH_2CH_3$), 5.08 (1H, s, dihydropyridine H^4), 6.27 (1H, br s, NH), 7.1–7.4 (6H, m, phenyl ring + imidazole H^4, H^5), 7.82 (1H, br s, imidazole H^2). Anal. $C_{22}H_{25}N_3O_4$ (C, H, N).

Hantsch Synthesis: Procedure B. (±)-1,4-Dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic Acid, Ethyl Methyl Ester (11). A mixture of 3-(1*H*-imidazol-1-yl)benzaldehyde (**3b**, 0.27 g, 1.57 mol), ethyl acetoacetate (0.204 g, 1.57 mmol), and methyl 3-aminocrotonate (0.18 g, 1.56 mmol) in absolute ethanol (10 mL) was refluxed for 6 h. The mixture was concentrated and poured into ice–water (20 mL), and the aqueous solution was extracted with CH_2Cl_2. The organic layers were collected, dried over $CaCl_2$, and evaporated under vacuum. The crude product was purified by flash silica gel column (eluant: ethyl acetate/*n*-hexane = 1/4), yielding 0.36 g (60%) of the title compound, mp 197–200 °C.

^{1}H-NMR (DMSO-d_6) δ ppm: 1.10 (3H, t, $COOCH_2CH_3$), 2.28 (6H, s, 2=CCH_3), 3.57 (3H, s, $COOCH_3$), 4.01 (2H, 1, $COOCH_2CH_3$), 4.93 (1H, s, dihydropyridine H^4), 7.05–7.6 (6H, m, phenyl ring + imidazole H^4, H^5), 8.09 (1H, dd, imidazole H^2), 8.88 (1H, s, NH). Anal. $C_{21}H_{23}N_4O_4$ (C, H, N).

(±)-1,4-Dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic Acid, Ethyl Ester (13). A mixture of (±)-1,4-dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)-phenyl]-3,5-pyridinedicarboxylic acid-2 cyanoethyl ethyl ester (0.4 g, 0.95 mmol), 1 mL of 2 N NaOH, and 2 mL of ethanol was stirred for 1 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate. The aqueous phase was acidified to pH = 6 with 1 N HCl. The precipitate was collected, washed with water, and dried under vacuum, yielding 0.183 g (50%) of the title compound (monohydrate), mp 117–121 °C dec.

^{1}H-NMR (DMSO) δ ppm: 1.10 (3H, t, $COOCH_2CH_3$), 2.22 (6H, s, 2=CCH_3), 4.02 (2H, q, $COOCH_2CH_3$), 4.92 (1H, s, dihydropyridine H^4), 7.0–7.60 (6H, m, phenyl ring + imidazole H^4, H^5), 8.05 (1H, dd, imidazole H^2), 8.75 (1H, s, NH). Anal. $C_{20}H_{23}N_3O_5$ (C, H, N).

(±)-1,4-Dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid 2-cyanoethyl ethyl ester used as starting material was prepared in turn following method B in 60% yield. Anal. $C_{23}H_{24}N_4O_4$ (C, H, N).

1,4-Dihydro-1,2,6-trimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic Acid, Diethyl Ester (18). To finely powdered KOH (0.144 g, 0.0026 mol) in 15 mL of DMSO was added 1,4-dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid, diethyl ester (**12**, 0.26 g, 0.000 66 mol), and the reaction mixture was stirred for 2 h at room temperature under nitrogen atmosphere. Then methyl iodide (0.187 g, 0.001 31 mol) was added to the mixture. After 2 h of stirring, the reaction mixture was poured into water and extracted with ethyl acetate; the organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by flash silica gel column (eluant: $CHCl_3$/MeOH 1% and 2%), yielding 0.13 g (48%) of pure oil.

^{1}H-NMR ($CDCl_3$) δ ppm: 1.27 (6H, t, 2 $COOCH_2CH_3$), 2.49 (6H, s, 2=CCH_3), 3.21 (3H, s, =NCH_3), 4.19 (4H, q, 2 $COOCH_2CH_3$), 5.12 (1H, s, dihydropyridine H^4), 7.0–7.3 (6H, m, phenyl ring + imidazole H^4, H^5), 7.78 (1H, bs, imidazole H^2). Anal. $C_{23}H_{27}N_3O_4$ (C, H, N).

(±)-1,4-Dihydro-2-[(2-aminoethoxy)methyl]-4-[3-(1*H*-imidazol-1-yl)phenyl]-6-methyl-3,5-pyridinedicarboxylic Acid, 3-Ethyl 5-Methyl Ester Maleate (21). A mixture of (±)-1,4-dihydro-2-[(2-phthalimidoethoxy)methyl]-6-methyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid, 3-ethyl 5-methyl ester (**20**, 0.8 g, 0.0014 mol), methylamine 20% solution in diisopropyl ether (9 mL), and absolute ethanol (10 mL) was stirred for 2 days at room temperature. The reaction mixture was evaporated to dryness, the residue taken up with ethyl acetate and diethyl ether, and the precipitated solid filtered off. The organic solution was evaporated under vacuum, and the residue was purified by flash silica gel column (eluant: $CHCl_3$/MeOH/ammonium hydroxide = 90/10/0.2), yielding 0.4 g (65%) of an oily product, which was dissolved in absolute ethanol (6 mL), and maleic acid (0.106 g) dissolved in ethanol (3 mL) was added. After evaporation of the solvent the residue was taken up in diethyl ether, affording the pure title compound, mp 138–40 °C.

^{1}H-NMR (DMSO) δ ppm: 1.13 (3H, t, $COOCH_2CH_3$), 2.35 (3H, s, =CCH_3), 3.05 (2H, m, $CH_2NH_3^+$), 3.56 (3H, s, $COOCH_3$), 3.64 (2H, m, $OCH_2CH_2NH_3^+$), 4.05 (2H, m, $COOCH_2CH_3$), 4.59, 4.74 (2H, 2d, =CCH_2O), 4.96 (1H, s, dihydropyridine H^4), 6.03 (2H, s, HOOCCH=CHCOOH), 7.1–8.02 (7H, m, phenyl ring + imidazole ring), 7.80 (3H, br s, NH_3^+), 8.47 (1H, s, NH). Anal. $C_{27}H_{32}N_4O_9$ (C, H, N).

2,6-Dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic Acid, Diethyl Ester Dihydrochloride (27). 1,4-Dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid, diethyl ester (**12**, 2.16 g, 0.0055 mol) was dissolved in glacial acetic acid (20 mL), and the solution added of $NaNO_3$ (1.88 g, 0.022 mol) was refluxed for 20 min under stirring. After cooling, ice–water (250 mL) was added, and the solution was extracted three times with ethyl acetate (250 mL × 3). The organic layer, washed with water, was dried over Na_2SO_4 and evaporated. The residue was dissolved in ethyl acetate (60 mL), and dry gaseous HCl was bubbled in the solution for 10 min. The precipitate was collected, washed with ethyl ether, and dried, affording 1.91 g (72%) of title compound (monohydrate), mp 178–80 °C.

^{1}H-NMR (DMSO) δ ppm: 0.83 (6H, t, $COOCH_2CH_3$), 2.53 (6H, s, CH_3), 3.99 (4H, q, $COOCH_2$–), 7.35 (1H, d), 7.72 (2H, m), 7.96 (2H, m), 8.31 (1H, s), 9.80 (1H, s, imidazole H^2).

Additional NMR Experiments. For the evaluation of NOE effects, proton NMR spectra at 400 MH_z were obtained for compounds **12** (1,4-dihydro-2,6-dimethyl-4-[3-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid, diethyl ester) and **15** (1,4-dihydro-2,6-dimethyl-4-[2-(1*H*-imidazol-1-yl)phenyl]-3,5-pyridinedicarboxylic acid, diethyl ester).

Stationary NOE experiments were performed with a Varian VXR-400S instrument at 27 °C, using DMSO as solvent because in $CDCl_3$ significant signals were not well resolved.

The numbering of protons in following spectra makes reference to the structure in Table II.

Compound **12**. ^{1}H-NMR (DMSO) δ ppm: 1.10 (6H, t, $COOCH_2CH_3$), 2.26 (6H, s, CH_3), 3.9–4.05 (4H, m, $COOCH_2$), 4.91 (1H, s, dihydropyridine C_4-H), 7.8 (1H, s, imidazole H_b), 7.14 (1H, m, phenyl C_6'-H), 7.26 (1H, s, phenyl C_2'-H), 7.36 (2H, m, phenyl C_5'-H and C_4'-H), 7.55 (1H, s, imidazole H_c), 8.07 (1H, s, imidazole H_a), 8.84 (1H, bs, NH).

Compound **15**. ^{1}H-NMR (DMSO) δ ppm: 1.03 (6H, t, $COOCH_2CH_3$), 2.16 (6H, s, CH_3), 3.75–4.0 (4H, m, $COOCH_2$), 5.09 (1H, s, dihydropyridine-C_4-H), 7.05 (2H, m, imidazole H_b and phenyl C_3'-H), 7.24 (1H, ddd, phenyl C_4'-H), 7.35 (2H, m, phenyl C_6'-H and C_5'-H), 7.53 (1H, s, imidazole H_c), 7.83 (1H, s, imidazole H_a), 8.70 (1H, s, NH).

Molecular Modeling. The molecular modeling of compounds **12**, **15**, and **16** was carried out using the program SYBYL 5.3 (TRIPOS Ass. Inc., St. Louis, MO), with MNDO methods of

semiempirical molecular orbital software MOPAC,[42] running on Silicon Graphic Workstation 4D.

We built the structures of examined compounds starting from the 2,6-dimethyl-4-phenyl-3,5-bis(ethoxycarbonyl)-1,4-dihydropyridine, whose atomic coordinates were extracted from the Cambridge Structural Database.[43] To this template the imidazole group was added as present in the Fragment Database of SYBYL in the *meta* (12), *ortho* (15), and *para* (16) positions. All the compounds were with the phenyl group in the pseudoaxial conformations and the imidazole in synperiplanar form for 12 and 15. At the end of every building step an energy minimization was performed by molecular mechanics with the Tripos Force Field, without charge interaction calculations and keeping the 1,4-dihydropyridine ring fixed, because of lack of suitable parameters for treating this structural moiety when widely substituted.

After calculation of the charges with MOPAC geometry, energy minimization with molecular mechanics was rerun using the charge contributions also, followed by a complete conformational analysis, scanning every free rotational bond using an angle increment of 10° for the bond joining the imidazole to the phenyl and the phenyl to the 1,4-dihydropyridine, 180° for the ester groups, and 30° for the ester alkyl chains excluding the methyl in the terminal position. After the first two torsional angles for 12 and 15 were analyzed, two different minima were obtained for the syn- and the anti-periplanar forms. The same procedure was followed starting from the pseudoequatorial conformations of 12, 15, and 16. All these structures were optimized geometrically by MOPAC with the AM1 method using the option PRECISE and then measuring on final structures the torsional angles used for correlation with Ca^{2+} antagonism.

During conformational analysis we recorded attainable distances between the nitrogen N(3) of imidazole and the carbonyl of the 3/5-diester functions.

Pharmacology. Ca^{2+} Antagonistic Activity in Isolated Tissues. Guinea Pig Ileum. The terminal ileum of male guinea pigs (0.5–0.8 kg) was immediately removed, washed, and mounted in a 20-mL organ bath containing Tyrode's solution (composition mM: NaCl 136.8, KCl 2.68, $CaCl_2$, 1.8, NaH_2PO_4 0.41, $NaHCO_3$ 11.9, $MgCl_2$ 1.03, glucose 5.55) gassed with 95% O_2 and 5% CO_2 and thermoregulated at 37 °C. The tissue was loaded with 1 g of tension, and contractions were recorded by a Basile DYA isometric transducer on a Watanabe Mark V recorder. Contractions to KCl (60 mM) were obtained at 15-min intervals in the absence and presence of increasing concentrations of screening compounds. The antagonistic potency was expressed as the IC_{50} value (the concentration of antagonist which inhibits KCl response by 50%) by means of least-squares regression analysis.[44]

Rabbit Renal Artery. Male New Zealand rabbit (2.5–3 kg) renal artery strips were suspended in a 20-mL organ bath containing Krebs-Henseleit solution (composition mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.17, $CaCl_2$ 2.52, KH_2PO_4 1.17, $NaHCO_3$ 25, glucose 5.5) oxygenated with a mixture of 95% O_2 and 5% CO_2 and thermoregulated at 37 °C. Tissues were stretched with 1 g of tension and contractions recorded with Basile isometric transducers connected to a Watanabe Mark V recorder. KCl (60 mM) induced contractions were repeated at 30 min intervals, in the absence and presence of increasing concentrations of the compound under test or nifedipine, a reference dihydropyridine Ca^{2+} antagonist.

Ventricular Strips and Beating Guinea Pig Atria. Male guinea pig (0.5–0.8 kg) right ventricular strips (10 × 2 mm) and atria were mounted in 20 mL of Tyrode (29 °C) and Locke (composition mM: NaCl 154, KCl 5.64, $CaCl_2$ 2.16, $NaHCO_3$ 5.95, glucose 5.55; at 37 °C) solutions, respectively, oxygenated with a mixture of 95% O_2 and 5% CO_2. Tissue contractions (preloaded with 1 g) were recorded by Basile isometric transducers on a Basile Gemini recorder. Right ventricular strips were electrically stimulated (3 ms, 2 Hz, v_{max}), for 60 min and then depolarized with a 22 mM KCl solution. The contractility was restored with histamine (10 μM) and a frequency of stimulation reduced at 0.2 Hz. Spontaneously beating atria were rested for 60 min. Testing compounds were then added cumulatively with a 30 min contact time for each concentration. The antagonistic potency of compounds was calculated from a computerized regression analysis and expressed in terms of IC_{50} (ventricular strips) or IC_{25} (atria).

TxA_2 Synthase Inhibitory Activity in Rat Whole Blood. TxA_2 synthase inhibitory activity was evaluated by measuring the production of the TxA_2 chemically stable metabolite, TxB_2, in rat whole blood during clotting. Known concentrations of the compound under test, or control solvent, were incubated with 0.5 mL of rat whole blood for 1 h at 37 °C. After centrifugation the serum was collected and stored at −20 °C until assayed for TxB_2 levels.

Data are presented as IC_{50} derived from a dose–response curve calculated from at least three concentrations (n = 8 each concentration). Statistical significance of the difference between reference standard (dazoxiben) and compounds under test was evaluated by unpaired t-test.[47]

TxB_2 radioimmunoassay was performed as previously described.[45] Briefly, 12 000 dpm of ^3H-TxB_2 (114 Ci·mmol^{-1}) and an aliquot of specific rabbit antiserum (final dilution 1:125 000) sufficient to bind 40–50% of the tritiated compound were incubated for 16–24 h at 4 °C in a final volume of 1.5 mL for each assay tube. Separation of antibody bound from free-labeled antigen was achieved by rapid addition of 0.1 mL of a charcoal suspension (100 mg·mL^{-1}) and subsequent centrifugation at 4 °C. The supernatant solution containing antibody-bound TxB_2 was decanted directly into 10 mL of Instagel (Packard Instrument Co. Inc., Downers Grove, IL). Radioactivity of samples was counted in a liquid scintillation counter (Model LS 1800, Beckman Instruments, Irvine, CA) for 2 min. Results were expressed as ng·mL. The smallest concentration of TxB_2 that could be measured with 95% confidence was 2 pg·mL^{-1}.

The enzyme selectivity of interesting compounds was tested by measuring whole blood production of PGE_2 (a product of a different isomerase), the decrease of which could indicates cyclooxygenase inhibition.

Hemodynamic Studies in Anesthetized Dogs. Six mongrel dogs (average bw 25.5 kg) were used. Anesthesia was induced by sodium pentobarbital (25–30 mg/kg iv). Spontaneous respiration was always maintained throughout the experiment. Mean blood pressure was measured by a high-fidelity Mikro-Tip pressure transducer (MPC 500, 5F, Millar Instr., Houston, TX). Heart rate was measured with a cardiotachometer triggered by the arterial pulse.

All the signals were recorded on an HP computerized system through an HP multichannel A/D converter and successively analyzed. Both FCE 24265 and nifedipine were injected intravenously in each dog at 180, 540, 1620 and 15, 45, 135 μg/kg, respectively, administered at 45-min intervals following a latin square design.

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