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Synthesis, Calcium Antagonistic Activity and Structure-Activity Relationships of Cycloalkylesters of 1,4-Dihydropyridines

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A series of 1,4-dihydropyridine derivatives that incorporates cycloalkyl esters has been synthesized via a modified Hantzsch method. Specifically, the compounds were prepared by the reaction of a nitrobenzaldehyde with a cycloalkyl acetoacetate followed by further treatment with an ester of β-aminocrotonic acid. The compounds were evaluated for *in vitro* and *in vivo* calcium antagonistic activity and structure-activity relationships were established. In general, a cyclohexyl or cyclopentyl ester, along with a simple methyl ester in the 3- and 5-positions, was found to be optimal for maximum potency.

INTRODUCTION

The vital role of calcium ion in a large number of cellular processes such as stimulus secretion and excitation-contraction is of special importance [1-3]. The regulation of intracellular concentration of this ion makes possible the control of such Ca^{2+}-dependent processes. Entry of extracellular calcium ion can occur by different mechanisms, including the use of Ca^{2+} channels. These channels can be defined in terms of ion selectivity, electrophysiological properties and through the use of antagonists. The calcium channel antagonists usually operate through the inhibition of calcium movement via certain membrane channels [4,5].

Structurally different classes of organic compounds are known to be effective as calcium antagonists, among which 1,4-dihydropyridine derivatives have been recognized as being particularly potent [2,4,5]. These compounds usually exert a powerful negative ionotropic effect on heart muscle and a marked relaxation of smooth muscles [6,7]. Studies carried out to date suggest that 1,4-dihydropyridine calcium antagonists mainly operate at specific membrane sites rather than through nonspecific membrane interactions [8,9].

Structure-activity relationships derived for 1,4-dihydropyridine calcium antagonists reveal that the nature and position of the substitution on the aryl ring are determining factors in their activity [10,11]. Namely, activity is independent of the electronic character of the substituent on the phenyl ring (electron donating or withdrawing), however is highly dependent on the size of the substituent site. Excellent studies by Loev et al. [11] suggest that activity may be enhanced by the presence of bulky groups which cause the 4-substituent to prefer an orientation perpendicular to the plane of the dihydropyridine ring. Further, x-ray crystallographic studies of some 3,5-dicarbomethoxy-1,4-dihydropyridines indicate that substitution on the phenyl ring could influence the degree of puckering of the 1,4-dihydropyridine ring and also restrict the free rotation of the the phenyl ring about the C_4-C_7 bond due to the repulsion effect of the carbomethoxy groups at the 3- and 5- positions and, thereby, causing a more favorable conformation for drug-receptor interaction [12,13]. These results are in direct agreement with those reported by Loev et al.

Considering the above facts, it can be concluded that the phenyl ring commonly bisects the 1,4-dihydropyridine ring which possesses a boat-type conformation with the C_8 hydrogen residing approximately over the center of the ring. The other substituents on the phenyl ring are twisted out of planarity with the ring about their exocyclic bonds (Figure 1) [13].

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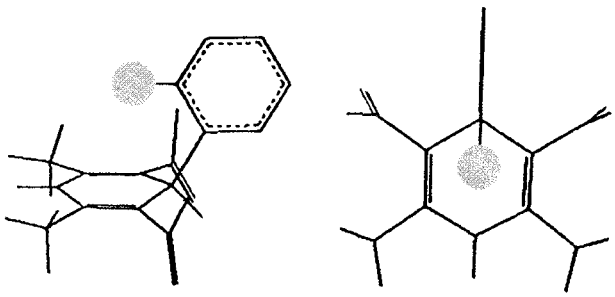


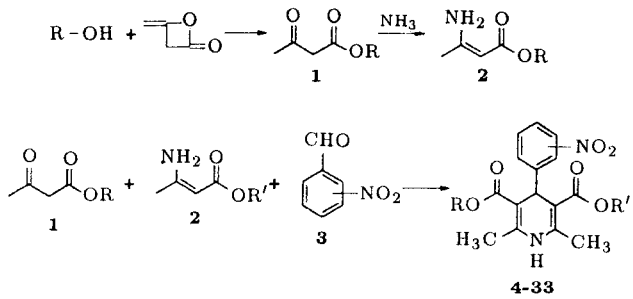
Figure 1. Side and top views of the phenyl ring.

In addition, the carbalkoxy groups at the C_3 and C_5 positions do not typically enjoy the same spatial arrangement. Namely, the carbalkoxy group at C_3 is synplanar to the C_3-C_4 bond, while that at C_5 is antiperiplanar to the C_5-C_4 bond. This arrangement makes the whole molecule more hindered and imparts activity to the compound.

With these factors in mind, it was decided to investigate the effect on activity of incorporating cycloalkyl esters in the C_3 and C_5 positions of the 4-nitrophenyl-1,4-dihydropyridine nucleus. It was theorized that the cycloalkyl moiety, due to its hindering nature, could contribute a superior fit to the receptor than moieties such as t-butyl which has been reported to result in a very low activity [11].

CHEMISTRY

The targetted cycloalkyl esters of 1,4-dihydropyridines (**4**) were synthesized utilizing a modified Hantzsch method (Scheme 1). Namely, condensation of nitrobenzaldehydes (**3**) with cycloalkylacetoacetic esters



Scheme 1. Synthesis of 1,4-dihydropyridines.

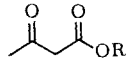
(**1**), followed by further reaction with appropriate esters of β-aminocrotonic acid (**2**), afforded compounds **4-33**. The cycloalkyl acetoacetic esters (**1**) were prepared in very good yields by the reaction of an appropriate cycloalkanol with ketene dimer. Esters of β-aminocrotonic acids (**3**) were synthesized by direct bubbling of liquid ammonia through the acetoacetic esters followed by distillation.

Although α-tropinyl acetoacetate (**1d**) and the related β-aminocrotonate (**2**) were synthesized in high yields, their reaction with nitrobenzaldehyde did not proceed, due to the instability of the esters. Typically, the reaction resulted in the recovery of nitrobenzaldehyde and polymerization of the β-ketoester. The physical characteristics of compounds $\mathbf{1}_{a\text{-}l}$, $\mathbf{2}_{a\text{-}d}$ and **4-33** are summarized in Tables 1,2 and 3.

PHARMACOLOGICAL ACTIVITY AND DISCUSSION

The 1,4-dihydropyridines synthesized were assayed for calcium antagonistic activity *in vivo* by the measurement of blood flow through the carotid and femural arteries in anesthetized dogs. The *in vitro* assay was carried out by measuring the relaxing effect of

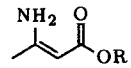
Table 1. Physical characteristics of acetoacetate esters ($1_{a\text{-}l}$).



No.	R	Yield(%)	b.p.(°C)	Formula[a]
1_a	cyclohexyl	82	104-106 (5 mm)	$C_{10}H_{16}O_3$
1_b	cyclopentyl	80	94-96 (8 mm)	$C_9H_{14}O_3$
1_c	exo-norbornyl	83	113-114 (5 mm)	$C_{11}H_{16}O_3$
1_d	α-tropinyl	94	130-136 (4 mm)	$C_{12}H_{19}NO_3$
1_e	3-methylcyclohexyl	73	107-110 (4 mm)	$C_{11}H_{18}O_3$
1_f	2-methylcyclohexyl	81	109-112 (4 mm)	$C_{11}H_{18}O_3$
1_g	endo-norbornyl	78	115-116 (4 mm)	$C_{11}H_{16}O_3$
1_h	trans-2-methylcyclopentyl	80	90-93 (4 mm)	$C_{10}H_{16}O_3$
1_i	3-methylcyclopentyl	85	110 (10 mm)	$C_{10}H_{16}O_3$
1_j	trans-3,3,5-trimethylcyclohexyl	82	115-120 (7 mm)	$C_{13}H_{22}O_3$
1_k	cis-3,3,5-trimethylcyclohexyl	90	118-121 (5 mm)	$C_{13}H_{22}O_3$
1_l	2,4-dimethylcyclopentyl	85	102 (5 mm)	$C_{11}H_{18}O_3$

[a] All analysis were within ±0.4% of the calculated values.

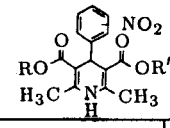
Table 2. Physical characteristics of β-aminocrotonate esters ($2_{a\text{-}d}$).



No.	R	Yield (%)	b.p. (°C)	Formula[a]
2_a	cyclohexyl	46	110-115 (4 mm)	$C_{10}H_{17}NO_2$
2_b	cyclopentyl	57	115-120 (5 mm)	$C_9H_{15}NO_2$
2_c	exo-norbornyl	60	135-140 (6 mm)	$C_{11}H_{17}NO_2$
2_d	α-tropinyl	55	123-126[b]	$C_{12}H_{20}N_2O_2$

[a] All analysis were within ±0.4% of the calculated values.
[b] Melting point.

Table 3. Physical characteristics of 1,4-dihydropyridines (**4-33**).



No.	R	R′	NO_2	Yield (%)	m.p. (°C)	Formula[a]
4	Me	cyclohexyl	ortho	44	129-131	$C_{22}H_{26}N_2O_6$
5	Me	cyclohexyl	meta	53	75-77	$C_{22}H_{26}N_2O_6$
6	Me	cyclopentyl	ortho	41	168-170	$C_{21}H_{24}N_2O_6$
7	Me	cyclopentyl	meta	81	85-87	$C_{21}H_{24}N_2O_6$
8	cyclohexyl	cyclohexyl	ortho	31	97-99	$C_{27}H_{34}N_2O_6$
9	cyclohexyl	cyclohexyl	meta	59	139-142	$C_{27}H_{34}N_2O_6$
10	cyclopentyl	cyclopentyl	ortho	29	167-169	$C_{25}H_{30}N_2O_6$
11	cyclopentyl	cyclopentyl	meta	90	169-171	$C_{25}H_{30}N_2O_6$
12	Me	exo-norbornyl	ortho	30	95-97	$C_{23}H_{26}N_2O_6$
13	Me	exo-norbornyl	meta	83	83-85	$C_{23}H_{26}N_2O_6$
14	exo-norbornyl	exo-norbornyl	ortho	40	187-189	$C_{29}H_{34}N_2O_6$
15	exo-norbornyl	exo-norbornyl	meta	75	95-97	$C_{29}H_{34}N_2O_6$
16	cyclopentyl	cyclohexyl	ortho	49	65-70	$C_{26}H_{32}N_2O_6$
17	cyclopentyl	cyclohexyl	meta	53	146-148	$C_{26}H_{32}N_2O_6$
18	exo-norbornyl	cyclopentyl	ortho	48	75-76	$C_{27}H_{32}N_2O_6$
19	exo-norbornyl	cyclopentyl	meta	56	161-163	$C_{27}H_{32}N_2O_6$
20	exo-norbornyl	cyclohexyl	ortho	53	85-86	$C_{28}H_{34}N_2O_6$
21	Me	3-methylcyclohexyl	ortho	49	73-75	$C_{23}H_{28}N_2O_6$
22	Me	3-methylcyclohexyl	meta	66	73-75	$C_{23}H_{28}N_2O_6$
23	Me	2-methylcyclohexyl	ortho	54	83-85	$C_{23}H_{28}N_2O_6$
24	Me	2-methylcyclohexyl	meta	68	75-77	$C_{23}H_{28}N_2O_6$
25	Me	endo-norbornyl	ortho	54	89-92	$C_{23}H_{26}N_2O_6$
26	Me	endo-norbornyl	meta	74	75-78	$C_{23}H_{26}N_2O_6$
27	Me	trans-2-methylcyclopentyl	ortho	29	68-70	$C_{22}H_{26}N_2O_6$
28	Me	trans-2-methylcyclopentyl	meta	58	66-68	$C_{22}H_{26}N_2O_6$
29	Me	(±) 3-methylcyclopentyl	ortho	48	67-69	$C_{22}H_{26}N_2O_6$
30	Me	(±) 3-methylcyclopentyl	meta	46	59-62	$C_{22}H_{26}N_2O_6$
31	Me	trans-3,3,5-trimethylcyclohexyl	meta	40	105-110	$C_{25}H_{32}N_2O_6$
32	Me	cis-3,3,5-trimethylcyclohexyl	meta	50	135-140	$C_{25}H_{32}N_2O_6$
33	Me	2,4-dimethylcyclopentyl	meta	58	65-68	$C_{25}H_{32}N_2O_6$

[a] All analysis were within ±0.4% of the calculated values.

the test compounds on isolated rat aorta contracted with 40 mM K^+, which their results are summarized in Tables 4 and 5. Nicardipine, a strong calcium antagonist, was used as a reference in both assays.

A consideration of reports on the higher activity of 1,4-dihydropyridines with nonidentical ester functions at the C_3 and C_5 positions, in comparison with their symmetrically substituted counterparts and on the effect of the ring puckering on pharmacological activity [12] promoted the decision to carry out the synthesis of bulky esters of Nifedipine analogs, in order to control the twisting of the phenyl ring about C_4 of the

Table 4. *In vivo* effect of 1,4-dihydropyridines (**4-33**) on blood flow.

No.	Dose (μg i.a.)	Increase in Blood Flow (%)					
		CCAF[a]	ED_{50}	Relative Potency[b]	FAF[c]	ED_{50}	Relative Potency[b]
4	0.3	9.1	0.55	1.0	33.3	0.40	1.3
	1.0	30.9			105.0		
5	0.3	6.4	0.80	0.69	22.7	0.69	0.77
	1.0	23.2			62.5		
6	0.3	10.7	0.51	1.1	31.0	0.74	0.72
	1.0	32.1			56.3		
7	0.3	9.0	0.81	0.68	35.2	0.55	0.96
	1.0	22.3			64.3		
8	0.3	0	ND[d]		0	ND	
	1.0	3.4			2.9		
9	0.3	1.7	ND		0	ND	
	1.0	3.4			2.9		
10	0.3	0	ND		2.9	ND	
	1.0	5.4			14.7		
11	0.3	0	ND		0	ND	
	1.0	5.4			5.7		
12	0.3	0	ND		0	ND	
	1.0	6.2			8.8		
13	0.3	3.4	ND		2.9	ND	
	1.0	5.8			15.6		
14	0.3	0	ND		0	ND	
	1.0	0			0		
15	0.3	0	ND		0	ND	
	1.0	0			0		
16	0.3	0	ND		0	ND	
	1.0	0			14.9		
17	0.3	0	ND		0	ND	
	1.0	1.1			23.6		
18	0.3	0	ND		0	ND	
	1.0	0			0		
19	0.3	0	ND		0	ND	
	1.0	0			6.4		
20	0.3	0	ND		0	ND	
	1.0	0			0		
21	0.3	6.0	0.85	0.65	11.8	0.63	0.84
	1.0	22.2			73.4		
22	0.3	6.4	1.39	0.40	13.0	0.75	0.71
	1.0	17.1			61.4		
23	0.3	4.8	0.78	0.71	18.1	0.99	0.54
	1.0	24.0			50.4		
24	0.3	0	1.14	0.48	10.7	1.85	0.29
	1.0	18.0			36.7		
25	0.3	0	ND		0	ND	
	1.0	2.9			4.8		
26	0.3	0	ND		0	ND	
	1.0	6.4			15.3		
27	0.3	0	ND		0	ND	
	1.0	2.8			8.9		

Table 4. *In vivo* effect of 1,4-dihydropyridines (**4-33**) on blood flow. (cont'd)

No.	Dose (μg i.a.)	Increase in Blood Flow (%)					
		CCAF[a]	ED_{50}	Relative Potency[b]	FAF[c]	ED_{50}	Relative Potency[b]
28	0.3	4.8	0.60	0.92	33.3	0.50	1.1
	1.0	31.4			73.1		
29	0.3	0	ND		0	ND	
	1.0	3.0			7.9		
30	0.3	0	1.45	0.38	11.6		
	1.0	15.3			15.6		
31	0.3	0	ND		0	ND	
	1.0	4.2			0		
32	0.3	0	ND		0	ND	
	1.0	0			8.7		
33	0.3	2.4	ND		1.0	3.09	0.17
	1.0	5.5			26.3		
Nicardipine	0.3	13.1	0.55	1.0	34.4	0.53	1.0
	1.0	27.0			67.6		

[a] Common carotid arterial flow. [b] Relative potency as compared to Nicardipine.
[c] Femoral arterial flow. [d] Not determined.

dihydropyridine ring and make it more puckered. The results of *in vitro* and *in vivo* tests for the synthesized compounds, in comparison with Nicardipine, reveal reasonable structure-activity relationships among these compounds and demonstrate the promising effect of cycloalkyl bulk on the stereochemistry of the 1,4-dihydropyridine nucleus. Several compounds have shown considerable calcium antagonistic activity with prolonged activity. According to the *in vivo* and *in vitro* assay results outlined in Tables 4 and 5, compounds **4-7** (R_1=CH_3; R_2=cyclopentyl, cyclohexyl) show a high degree of activity and long duration of action.

Employing exo-norbornyl esters (**12**,**13**) or endo-norbornyl (**25**,**26**) resulted in a sharp decrease in activity *in vivo*, which might be due to a loss in flexibility of the 4-substituted phenyl-1,4-dihydropyridine nucleus and an unsuitable interaction with the appropriate receptor. *In vitro* activity of these compounds at low concentration is minimal, while at higher concentrations, compounds **25** and **26** show considerable activity.

Changing simple cycloalkyl esters to mono-, di-, or tri-substituted cycloalkyl esters resulted in activity variation. Specifically, activity varied depending on the position of the methyl group on the cyclohexyl or cyclopentyl moiety. The (±)3-methylcyclohexyl ester (**21**,**22**) showed considerable *in vitro* and *in vivo* activity and compound **22** was found to be more active than Nicardipine. In contrast, (±)2-methylcyclohexyl esters (**23**,**24**) were demonstrated to be less active than their 3-methyl counterparts. The difference in activity seems to be due to conformational requisites for drug-receptor interaction. The (±) 3-methylcyclohexyl group renders less hindrance than (±) 2-methylcyclohexyl, allowing the nitrophenyl group to have a limited rotation about the C_4-C_7 carbons, which results in a more suitable conformation. In the case of (±)2-methylcyclohexyl analogs, electron repulsion effects between the nitro group on the phenyl ring and the methylcyclohexyl moiety might affect the free rotation of the phenyl ring, resulting in a suitable conformation for good interaction with the receptor. This theory could be supported by comparing the activity of compounds **21** and **22**. Electron repulsion between o-nitro group and the 3-methylcyclohexyl moiety in compound **21** is much greater than that between m-nitro and 3-methylcyclohexyl in compound **22**. Thus, the phenyl ring could rotate more freely about the C_4-C_7 bond in compound **22** than in **21** and the whole molecule could probably obtain a more suitable conformation while interacting with its receptor. To further prove the effect of the cycloalkyl group on rotation of the phenyl ring, more bulky cycloalkyl esters of 1,4-dihydropyridines, such as 3,3,5-trimethylcyclohexyl (**31**,**32**), were synthesized and found to exhibit no calcium antagonist activity. This result confirms that a limited rotation of the phenyl ring about C_4 is a requirement for potent calcium antagonistic activity. On the other hand, complete inhibition of the phenyl ring rotation, which might be due to electron repulsion between the nitro group and bulky cycloalkyl moiety, will more likely result in unsuitable conformations, causing a sharp decrease in activity.

Overall, the ortho or meta nitro group seems to be one of the critical factors in the rotation of the phenyl

Table 5. *In vitro* relaxation effect of 1,4-dihydropyridines (**4-33**) on isolated rat aorta.

No.	Relaxation (%)		ED_{50} ($\times 10^{-9}$ M)	Relative Potency[b]
	3×10^{-10} M[a]	3×10^{-9} M[a]		
4	3.3	49.2	3.12	0.23
5	3.7	64.6	1.73	0.42
6	17.8	81.5	0.96	0.76
7	9.1	82.5	1.08	0.68
8	0	0	ND[c]	
9	0	0	ND	
10	0	8.7	ND	
11	0	7.5	ND	
12	0	28.5	ND	
13	0	37	ND	
14	0	0.5	ND	
15	7.7	15.4	ND	
16	0	25.0	ND	
17	10.0	63.0	1.70	0.43
18	11.1	16.7	ND	
19	3.4	13.8	ND	
20	8.0	14.0	ND	
21	0	72.5	1.47	0.5
22	3.6	116.7	0.77	0.95
23	5.8	28.9	24.58	0.03
24	0	77.8	1.32	0.55
25	8.7	97.9	0.87	0.84
26	12.0	91.7	0.90	0.81
27	2.6	50	3.00	0.24
28	13.8	82.8	1.00	0.73
29	8.5	25.5	ND	
30	16.1	61.3	1.69	0.43
31	0	0	ND	
32	0	0	ND	
33	0	19.4	ND	
Nicardipine	17.7	101.1	0.73	1.0

[a] Test concentration. [b] Relative potency as compared to Nicardipine. [c] Not determined.

ring about C_4. In general, most of the o-nitrophenyl derivatives show lower activity, whereas m-nitrophenyl derivatives, which might have less electron repulsion with respect to that of cycloalkyl ester function, show superior potency. For example, while compound **27** which possesses (-) 2-methylcyclopentyl ester at the 3-position and o-nitrophenyl at the 4-position, has shown almost no *in vivo* and only mild *in vitro* activity, compound **28**, with the same ester group but with a m-nitrophenyl moiety, exhibited activity comparable with Nicardipine and a duration of action 4 times that of the reference, both *in vitro* and *in vivo*. Compound **29**, with a (±) 3-methylcyclohexyl ester and o-nitrophenyl, showed no *in vivo* activity, while its m-nitrophenyl analog (**30**) was significantly less active than the reference, although exhibiting some activity. The *in vitro* activity of these two compounds, although rather low, were in good agreement with the *in vivo* results. Expectedly, compound **33** with (±) 2,4-dimethylcyclopentyl ester and m-nitrophenyl showed very low activity, both *in vivo* and *in vitro*.

Further experiments concerning the requirement of a limited rotation of the phenyl ring about C_4 for calcium antagonistic activity were also carried out. Namely, compounds **8-11**, **14** and **15**, with identical 3- and 5-cyclohexyl, cyclopentyl and exo-norbornyl and compounds **16-20**, with nonidentical 3- and 5-cycloalkyl esters, were synthesized. Except compound **17** which showed very weak *in vivo* and *in vitro* activity, none of these derivatives exhibited calcium antagonist activity. In these cases, although inhibition of the phenyl ring rotation at C_4, due to extreme hindrance caused by two bulky groups, could be considered the main factor in the lack of calcium antagonist activity,

the possibility that an excessive bulkiness of the whole molecule results in an unfavorable fit in the putative receptor, should not be neglected. The results obtained from *in vivo* and *in vitro* tests of compound **17** and the other nonidentical esters also confirm the importance in pharmacological activity of nonsymmetry. Such nonsymmetry renders chirality to 1,4-dihydropyridine nucleus, resulting in selectivity of the receptor towards optically active 1,4-dihydropyridines.

CONCLUSIONS

On the basis of the above discussion, it is concluded that a bulky ester group at C_3 of the 1,4-dihydropyridine nucleus imparts activity to the resulting molecule. Therefore, in most cases, the products potency and duration of action improve remarkably. Although unsymmetrical esters are expected to be more active than their symmetrical analogs, the presence of two bulky esters at the C_3 and C_5 positions results in a loss of activity. This phenomenon could be related to the rigidity of the molecule, due to the inhibition of twisting of the substituted phenyl at C_4 by the bulky ester substituents. It is suggested that a semi-flexibility should remain in the 1,4-dihydropyridine nucleus, in order to maintain significant calcium antagonistic activity. The size of the cycloalkyl moiety is another critical factor in determining activity. Small cycloalkyls, such as cyclopentyl or cyclohexyl (**4-7**), seem to be more suitable than other bulkier ones, presumably because of a better fit with the putative receptor.

EXPERIMENTAL

In Vivo Arterial Blood Flow Studies

Mongrel dogs of either sex weighing 7-14 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). After intubation, artificial respiration was performed with a Harvard respirator using room air. A catheter connected to a pressure transducer was positioned in the left femoral artery to measure arterial blood pressure. The left common carotid artery and the right femoral artery were exposed and ligated. Both ends of a rubber catheter were inserted into the left common carotid artery proximally and distally at the ligature making an extra-corporeal circuit. Another extra-corporeal circuit was similarly constructed at the ligature of the right femoral artery. Blood flow through these circuits was measured using the probes of an electromagnetic flow meter interposed within the circuits. Test compounds were dissolved in 10% ethanol to give a concentration of 10 μg/ml. Nicardipine, the reference drug, was dissolved in 0.9% saline. Solutions of test compounds and Nicardipine were injected into the circuits at a volume of 30 or 100 μl by means of a microsyringe.

In Vitro Relaxation Studies on Isolated Rat Aorta

The thoracic aorta of male Wister rats, weighing 300-350 g, were removed, cleared of adhering fat and connective tissue and cut into 5 mm wide transverse rings. These rings were mounted on stainless steel hooks in organ baths containing Krebs-Henseleit solution at 37°C and gassed with 95% O_2 and 5% CO_2. The Krebs-Henseleit solution had the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; Na HCO_3, 25; and glucose, 10. Tissues were loaded with 0.5 g of resting tension, which was measured isometrically with force-displacement transducers. The aortic rings were allowed to equilibrate for 1 hour before experiments were initiated and were contracted using 40 mM K^+ prior to addition of the test compounds and Nicardipine, in concentrations of 3×10^{-10} and 3×10^{-9}. Relaxations are expressed as a percentage of complete relaxation of the 40 mM K^+-induced tone. Compounds were dissolved as described above.

Chemistry

All melting and boiling points are uncorrected. Infrared spectra were measured with a JASCO IRA-1 spectrometer and proton nuclear magnetic resonance spectra were recorded with a JEOL JNM-PMX60 spectrometer. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet.

Cyclohexyl Acetoacetate (1_a)

A three-neck flask fitted with a thermometer, dropping funnel and condenser was charged with cyclohexanol (10.7g, 0.107 mol) and the temperature was raised to 80-85°C. The heat source was removed and anhydrous sodium acetate (0.04 g, 0.04 mmol) was added while stirring. Diketene (9.6 g, 0.114 mol) was added dropwise over 1.5 hours, resulting in a moderate increase in temperature. The mixture was stirred for an additional hour and then the brown solution was distilled in vacuo. The fraction boiling at 104-106°C (5 mm Hg) was collected to give $\mathbf{1}_a$ in 82% yield.

Cyclopentylacetoacetate ($\mathbf{1}_b$) was prepared in an identical manner.

Exo-Norbornyl Acetoacetate (1_c)

Exo-norborneol (11.9 g, 0.107 mol) was dissolved in 30 ml benzene and anhydrous sodium acetate (0.04 g, 0.04 mmol) was added. The resulting mixture was heated to reflux and diketene (9.6 g, 0.114 mol) was added dropwise over 1.5 hours. The mixture was then heated to reflux for 1 additional hour, then the benzene evaporated. The residue was distilled in vacuo with 1_c

Table 6. Proton NMR spectral characteristics of acetoacetate esters ($\mathbf{1}_{a\text{-}l}$).

No.	Solvent	Keto:Enol	^{1}HNMR δ
$\mathbf{1}_a$	$CDCl_3$	3:1	1.13-1.83 (m, 10H), 1.93 (s, 0.75H), 2.26 (s, 2.25H), 3.4 (s, 1.5H), 4.66-4.86 (m, 1H), 4.93 (s, 0.25H), 12.23 (br, 0.25H)
$\mathbf{1}_b$	$CDCl_3$	4:1	1.30-1.90 (m, 8H), 1.93 (s, 0.6H), 2.16 (s, 2.4H), 3.40 (s, 1.6H), 5.20 (m, 1H), 5.23 (s, 0.2H), 12.10 (br, 0.2H)
$\mathbf{1}_c$	CCl_4	2:1	0.96-1.76 (m, 10H), 1.90 (s, 1H), 2.16 (s, 2H), 3.26 (s, 1.4H), 4.46-4.73 (m, 1H), 4.86 (s, 0.3H), 12.10 (br, 0.3H)
$\mathbf{1}_d$	CCl_4	1:0	1.40-2.06 (m, 8H), 2.16 (s, 6H), 2.96 (s, 2H), 3.30 (s, 2H), 4.80-5.10 (m, 1H)
$\mathbf{1}_e$	CCl_4	2:1	0.90-1.86 (m, 12H), 1.90 (s, 1H), 2.16 (s, 2H), 3.26 (s, 1.3H), 4.50-4.86 (m, 1H), 4.83 (s, 0.35H), 12.13 (br, 0.35)
$\mathbf{1}_f$	CCl_4	2:1	0.90 (d, 3H), 1.20-1.90 (m, 9H), 1.93 (s, 1H), 2.23 (s, 2H), 3.36 (s, 1.3H), 4.20-4.70 (m, 1H), 4.96(s, 0.35H), 12.20(br, 0.35H)
$\mathbf{1}_g$	CCl_4	2:1	0.80-1.80 (m, 10H), 0.90 (s, 1H), 2.20 (s, 2H), 3.30 (s, 1.3H), 4.70-5.00 (m, 1H), 4.86 (s, 0.35H), 12.10 (br, 0.35H)
$\mathbf{1}_h$	CCl_4	2:1	0.96 (d, J=6Hz, 3H), 1.16-1.86 (m, 7H), 1.90 (s, 1H), 2.16 (s, 2H), 3.26 (s, 1.3H), 4.60-4.83 (m, 1H), 4.86 (s, 0.35H), 12.10 (br, 0.35H)
$\mathbf{1}_i$	CCl_4	2:1	1.00-1.20 (m, 3H), 1.26-1.56 (m, 1H), 1.66-2.16 (m, 6H), 1.93 (s, 1H), 2.20 (s, 2H), 3.26 (s, 1.3H), 4.86 (s, 0.35H), 5.03-5.36 (m, 1H), 12.13 (br, 0.35H)
$\mathbf{1}_j$	CCl_4	2:1	0.86-1.10 (m, 9H), 1.16-1.80 (m, 7H), 1.93 (s, 1H), 2.20 (s, 2H), 3.26 (s, 1.3H), 4.86 (s, 0.35H), 5.00-5.23 (m, 1H), 12.16 (br, 0.35H)
$\mathbf{1}_k$	CCl_4	2:1	0.76-1.16 (m, 9H), 1.23-1.86 (m, 7H), 1.93 (s, 1H), 2.20 (s, 2H), 3.66 (s, 1.3H), 4.60-5.13 (m, 1H), 4.83 (s, 0.35H), 12.16 (br, 0.35H)
$\mathbf{1}_l$	CCl_4	2:1	0.96 (d, J=2Hz, 3H), 1.08 (d, J=2Hz, 3H), 1.23-2.10 (m, 6H), 1.93 (s, 1H), 2.20 (s, 2H), 3.30 (s, 1.3H), 4.50-4.83 (m, 1H), 4.86 (s, 0.35H), 12.13 (br, 0.35H)

boiling at 113-114°C (5 mm Hg), producing a yield of 83%.

Acetoacetate esters $\mathbf{1}_{d\text{-}l}$ were prepared in a similar manner as described for $\mathbf{1}_c$. The physical characteristics of $\mathbf{1}_{a\text{-}l}$ are summarized in Table 1 and the spectral characteristics can be found in Table 6.

Cyclopentyl-β-Aminocrotonate (2_b)

Anhydrous ammonia gas was bubbled through a solution of cyclopentyl acetoacetate ($\mathbf{1}_b$) (17.0 g, 0.1 mol) in 8 ml dioxane for a period of 7 hours. The mixture was kept in a refrigerator overnight and then ether was added. Afterwards the mixture was filtered. The filtrate was dried over sodium sulfate and the solvent evaporated. The residue was distilled in vacuo with $\mathbf{2}_b$ boiling at 115-120°C (5 mm Hg), producing a yield of 57%.

The other β-aminocrotonates ($\mathbf{2}_{a,c,d}$) were similarly prepared. However, in the case of $\mathbf{2}_d$, distillation was not necessary as the product crystallized out of ether in a pure form. The physical characteristics of $\mathbf{2}_{a\text{-}d}$ are summarized in Table 2 and the spectral characteristics can be found in Table 7.

3-Cyclohexyloxycarbonyl-2, 6-Dimethyl-5-Methoxycarbonyl-4-(2-Nitrophenyl)-1, 4-Dihydropyridine (4)

A mixture of cyclohexyl acetoacetate (1_a) (1.84 g, 0.01 mol), methyl-β-aminocrotonate (1.73g, 0.015mol) and o-nitrobenzaldehyde (1.51 g, 0.01 mol) in 10 ml isopropanol, was heated to reflux for 24 hours. The solvent was evaporated and the residue eluted through an alumina column, using chloroform as eluent. The resulting nearly pure oil was recrystallized from methanol-water to give pure, crystalline **4** in 44% yield.

Compounds **5-33** were similarly prepared and purified via alumina or silica chromatography, as well as via recrystallization techniques. The physical characteristics of **4-33** are summarized in Table 3 and the spectral characteristics can be found in Table 8.

Table 7. Proton NMR spectral characteristics of β-aminocrotonate esters ($\mathbf{2}_{a\text{-}d}$).

No.	Solvent	^{1}H NMR δ
$\mathbf{2}_a$	$CDCl_3$	1.13-1.86 (m, 10H), 1.90 (s, 3H), 4.50 (s, 1H), 4.56-5.00 (m, 1H), 5.30-7.13 (br, 2H)
$\mathbf{2}_b$	CCl_4	1.40-1.76 (m, 8H), 1.83 (s, 3H), 4.33 (s, 1H), 4.90-5.20 (m, 1H), 4.90-7.00 (br, 2H)
$\mathbf{2}_c$		
$\mathbf{2}_d$	$CDCl_3$	1.60-2.20 (m, 8H), 1.86 (s, 3H), 2.26 (s, 3H), 3.10 (s, 2H), 4.53 (s, 1H), 4.83-5.10 (m, 1H), 6.90 (br, 2H)

Table 8. Proton NMR spectral characteristics of 1,4-dihydropyridines (**4-33**).

No.	^{1}H NMR ($CDCl_3$) δ
4	0.90-2.00 (m, 10H), 2.26 (s, 3H), 2.33 (s, 3H), 3.56 (s, 3H), 4.43-4.93 (br, 1H), 5.83 (s, 1H), 6.10 (br, 1H), 7.06-7.80 (m, 4H)
5	0.90-2.00 (m, 10H), 2.40 (s, 6H), 3.80 (s, 3H), 4.60-5.00 (br, 1H), 5.20 (s, 1H), 6.66 (br, 1H), 7.20-8.00 (m, 3H), 8.13 (s, 1H)
6	1.20-2.00 (m, 8H), 2.30 (s, 3H), 2.40 (s, 3H), 3.60 (s, 3H), 5.00-5.40 (br, 1H), 5.90 (br, 2H), 7.10-7.90 (m, 4H)
7	1.10-1.90 (m, 8H), 2.35 (s, 6H), 3.65 (s, 3H), 5.00-5.30 (br, 1H), 5.10 (s, 1H), 6.83 (br, 1H), 7.50-8.00 (m, 3H), 8.10 (s, 1H)
8	0.60-2.00 (m, 20H), 2.30 (s, 6H), 4.50-4.90 (br, 2H), 5.96 (s, 1H), 6.06 (br, 1H), 7.20-8.20 (m, 4H)
9	1.00-2.00 (m, 20H), 2.33 (s, 6H), 4.56-5.00 (br, 2H), 5.13 (s, 1H), 6.23 (br, 1H), 7.20-8.03 (m, 3H), 8.23 (s, 1H)
10	1.13-1.93 (m, 16H), 2.26 (s, 6H), 4.93-5.33 (m, 2H), 5.86 (s, 1H), 5.93 (br, 1H), 7.13-7.86 (m, 4H)
11	1.30-1.96 (m, 16H), 2.33 (s, 6H), 4.40-4.66 (br, 1H), 4.96 (s, 1H), 5.06-5.23 (m, 1H), 5.88 (br, 1H), 7.20-8.00 (m, 3H), 8.10 (s, 1H)
12	0.80-1.83 (m, 10H), 2.26 (s, 3H), 2.36 (s, 3H), 3.56 (s, 3H), 4.40-4.66 (m, 1H), 5.80 (s, 1H), 5.93 (br, 1H), 7.20-7.80 (m, 4H)
13	0.80-1.80 (m, 10H), 2.36 (s, 6H), 3.66 (s, 3H), 4.50-4.70 (m, 1H), 5.10 (br, 1H), 6.40 (br, 1H), 7.30-8.03 (m, 3H), 8.20 (s, 1H)
14	0.76-1.76 (m, 20H), 2.26 (s, 6H), 4.36-4.70 (m, 2H), 5.80 (s, 1H), 5.93 (s, 1H), 7.30-7.83 (m, 4H)
15	0.90-1.80 (m, 20H), 2.36 (s, 6H), 4.46-4.73 (m, 2H), 5.06 (s, 1H), 6.06 (s, 1H), 7.30-8.00 (m, 3H), 8.10 (s, 1H)
16	0.80-1.90 (m, 18H), 2.23 (s, 6H), 4.40-4.73 (m, 1H), 4.93-5.20 (m, 1H), 5.83 (s, 1H), 6.40 (br, 1H), 7.23-8.13 (m, 4H)
17	1.20-2.00 (m, 18H), 2.36 (s, 6H), 4.60-4.90 (m, 1H), 5.10 (s, 1H), 5.03-5.30 (m, 1H), 6.20 (br, 1H), 7.30-8.03 (m, 3H), 8.16 (s, 1H)
18	0.80-1.93 (m, 18H), 2.26 (s, 6H), 4.36-4.40 (m, 1H), 4.90-5.23 (m, 1H), 5.83 (s, 1H), 6.23 (br, 1H), 7.10-7.90 (m, 4H)
19	0.96-1.90 (m, 18H), 2.33 (s, 6H), 4.46-4.70 (m, 1H), 5.03 (s, 1H), 5.00-5.26 (m, 1H), 6.10 (br, 1H), 7.26-8.00 (m, 3H), 8.10 (s, 1H)
20	0.70-1.83 (m, 20H), 2.26 (s, 6H), 4.33-4.90 (m, 2H), 5.90 (br, 1H), 6.40 (br, 1H), 7.20-8.10 (m, 4H)
21	0.60-1.80 (m, 12H), 2.26 (s, 3H), 2.30 (s, 3H), 3.56 (s, 3H), 4.40-5.10 (m, 1H), 5.83 (s, 1H), 6.06 (br, 1H), 7.20-7.90 (m, 4H)
22	0.70-1.90 (m, 12H), 2.33 (s, 6H), 3.63 (s, 3H), 4.30-4.80 (m, 1H), 5.06 (s, 1H), 6.06 (br, 1H), 7.23-7.93 (m, 3H), 8.06 (s, 1H)
23	0.80-1.90 (m, 12H), 2.26 (s, 3H), 2.33 (s, 3H), 3.60 (s, 3H), 4.80-5.16 (m, 1H), 5.83 (br, 1H), 6.00 (s, 1H), 7.40-7.86 (m, 4H)
24	0.80-1.90 (m, 12H), 2.33 (s, 6H), 3.63 (s, 3H), 4.80-5.20 (m, 2H), 6.10 (br, 1H), 7.20-7.90 (m, 3H), 8.06 (s, 1H)
25	0.80-1.76 (m, 10H), 2.16 (s, 3H), 2.33 (s, 3H), 3.63 (s, 3H), 4.66-5.13 (m, 1H), 5.83 (s, 1H), 6.30 (br, 1H), 7.20-7.83 (m, 4H)
26	0.80-1.80 (m, 10H), 2.36 (s, 6H), 3.63 (s, 3H), 4.66-5.00 (m, 1H), 5.10 (s, 1H), 6.26 (br, 1H), 7.26-7.93 (m, 3H), 8.06 (s, 1H)
27	0.60-1.13 (m, 3H), 1.40-2.10 (m, 7H), 2.26 (s, 3H), 2.36 (s, 3H), 3.60, (s, 3H), 4.50-5.83 (m, 1H), 5.86 (s, 1H), 6.30 (br, 1H), 7.30-7.86 (m, 4H)
28	0.70-1.10 (m, 3H), 1.23-2.10 (m, 7H), 2.33 (s, 6H), 3.63 (s, 3H), 4.50-4.83 (m, 1H), 5.06 (s, 1H), 6.43 (br, 1H), 7.20-8.00 (m, 3H), 8.06 (s, 1H)
29	0.80-1.10 (m, 3H), 1.16-1.40 (m, 1H), 1.43-2.06 (m, 6H), 2.23 (s, 3H), 2.33 (s, 3H), 3.56 (s, 3H), 4.86-5.26 (m, 1H), 5.80 (s, 1H), 6.66 (br, 1H), 7.20-7.80 (m, 4H)
30	0.80-1.13 (m, 3H), 1.20-1.36 (m, 1H), 1.43-2.10 (m, 6H), 2.30 (s, 6H), 3.63 (s, 3H), 5.06 (br, 2H), 6.10 (br, 1H), 7.23-7.90 (m, 3H), 8.10 (s, 1H)
31	0.86-1.06 (m, 9H), 1.16-2.03 (m, 7H), 2.30 (s, 3H), 2.36 (s, 3H), 3.63 (s, 3H), 5.00-5.23 (m, 1H), 5.16 (s, 1H), 6.40 (br, 1H), 7.16-7.96 (m, 3H), 8.06 (s, 1H)
32	0.70-1.13 (m, 9H), 1.20-2.00 (m, 7H), 2.33 (s, 6H), 3.60 (s, 3H), 4.53-5.00 (m, 1H), 5.03 (s, 1H), 6.16 (br, 1H), 7.20-8.00 (m, 3H), 8.10 (s, 1H)
33	0.70-1.13 (m, 6H), 1.20-2.13 (m, 6H), 2.33 (s, 6H), 3.63 (s, 3H), 4.50-4.80 (m, 1H), 5.06 (s, 1H), 6.10 (br, 1H), 7.26-7.96 (m, 3H), 8.10 (s, 1H)

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