reaction time was in the range of 1.8–2 s. The peptides were dissolved in 0.01 N HCl, neutralized with 0.1 M NaOH, and injected intracerebroventricularly into conscious animals at a constant volume of 5 μL. The Student's t test was used for statistical comparisons.

Acknowledgment. We thank the Consejo Superior de Investigaciones Cientificas and the Comisión Asesora de Investigación Cientifica y Técnica for financial support.

Registry No. **3**, 111237-79-7; **4**, 111237-80-0; **5**, 111237-81-1; **6**, 111237-82-2; **7**, 111237-83-3; **8**, 111237-84-4; **9**, 111237-85-5; **10**, 111237-86-6; **11**, 111237-87-7; **12**, 111237-89-9; **13**, 111237-90-2; **14**, 111237-91-3; **15**, 111237-92-4; **15** (free base), 111238-00-7; **16**, 111237-93-5; **16** (free base), 111238-01-8; **17**, 111237-94-6; **17** (free base), 111238-02-9; **18**, 111237-95-7; **18** (free base), 111238-03-0; **19**, 111237-96-8; **20**, 111237-97-9; **20** (free base), 111320-54-8; **21**, 111320-51-5; **21** (free base), 111407-15-9; **22**, 111320-52-6; **22** (free base), 111407-16-0; **23**, 111320-53-7; **23** (free base), 111407-17-1; **24**, 111237-98-0; **24** (free base), 111320-55-9; Z-Trp-OH, 7432-21-5; D-Arg-OMe·HCl, 111237-99-1; Z-D-Trp-OH, 2279-15-4; Arg-OMe·HCl, 18598-71-5; N^{ϵ}-Z-Lys-OMe·HCl, 27894-50-4; Nps-Cl, 7669-54-7.

Supplementary Material Available: Tables of ^{1}H NMR data containing proton chemical shifts of **15–24** and coupling constants of amide protons in **20–24** (2 pages). Ordering information is given on any current masthead page.

Crystal Structures and Pharmacologic Activities of 1,4-Dihydropyridine Calcium Channel Antagonists of the Isobutyl Methyl 2,6-Dimethyl-4-(substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Nisoldipine) Series

R. Fossheim,*† A. Joslyn,‡,⊥ A. J. Solo,§ E. Luchowski,‡ A. Rutledge,‡ and D. J. Triggle*‡

Department of Chemistry, University of Oslo, Blindern 0315, Oslo 3, Norway, and Departments of Biochemical Pharmacology and Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.
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A series of isobutyl methyl 2,6-dimethyl-4-(X-substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates (X = H, 2-NO_2, 3-NO_2, 3-CN, 3-MeO, 4-F, 2-CF_3, 3-CF_3, and 4-Cl) related to and including nisoldipine (X = 2-NO_2) has been synthesized, their solid-state structures determined by X-ray analysis (X = H, 2-NO_2, 3-NO_2, 3-CN, 3-MeO, and 4-F), and their pharmacologic activities determined, as the racemic compounds, against [^{3}H]nitrendipine binding and K^+-depolarization-induced tension responses in intestinal smooth muscle as measures of Ca^{2+} channel antagonist activity. Comparisons of structure are presented to previously analyzed 1,4-dihydropyridines. The degree of 1,4-dihydropyridine ring puckering is dependent on the nature and position of the phenyl ring substituent and the adopted interring conformation. Different ester substituents affect 1,4-dihydropyridine ring puckering to a small extent in most cases. Pharmacologic and radioligand binding activities for the nine compounds studied show a parallel dependence on phenyl ring substituent, but the compounds are approximately 10-fold more active in the radioligand binding assay than in the pharmacologic assay. Consistent with a previous report for the nifedipine series (Fossheim et al. *J. Med. Chem.* **1982**, *25*, 126), pharmacologic activity increases with increasing 1,4-dihydropyridine ring planarity.

The Ca^{2+} channel antagonists are a heterogeneous group of agents possessed of a common property, the ability to block current through potential-dependent Ca^{2+} channels.[1-4] Verapamil, diltiazem, and nifedipine, as representatives of the phenylalkylamine, benzothiazepine, and 1,4-dihydropyridine categories of Ca^{2+} channel drugs, enjoy substantial use in cardiovascular medicine.[2,5,6] Because of their potent and generally selective activities, these drugs are of increasing prominence as molecular tools with which to probe Ca^{2+} channel structure and function.[6-8]

The 1,4-dihydropyridines have assumed particular importance in the analysis of Ca^{2+} channel structure and function because of the availability of many analogues of nifedipine and the existence of both activator and antagonist ligands. Pharmacologic and radioligand binding studies are consistent with actions at specific membrane sites. Published structure–activity correlations indicate that the nature and position of the substituent in the aryl ring of the 1,4-dihydropyridine antagonists are very important determinants of the antagonist activity, which increases with minimum Sterimol width of the ortho or meta substituent and with the electron-withdrawing capacity of the meta substituent.[3,6,9-12] Our previous X-ray structural studies of phenyl ring substituted analogues of nifedipine indicate that the degree of puckering of the 1,4-dihydropyridine ring is related to pharmacologic activity, the latter increasing with increasing ring planarity.[13,14] However, only a limited and homogeneous series

† University of Oslo.
‡ Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo.
§ Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo.
⊥ Present address: Smith Kline and French Laboratories, Swedeland, PA.

(1) Fleckenstein, A. *Annu. Rev. Pharmacol. Toxicol.* **1977**, *17*, 149.
(2) Fleckenstein, A. *Calcium Antagonism in Heart and Smooth Muscle. Experimental Facts and Therapeutic Prospects*; Wiley-Interscience: New York, NY, 1983.
(3) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1985**, *26*, 775.
(4) Triggle, D. J.; Janis, R. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 347.
(5) Bühler, F. R., Distler, A., Gross, F., Eds. *J. Cardiovas. Pharmacol.* **1984**, *6*, Suppl. 7.
(6) Janis, R. A.; Triggle, D. J. *Drug Dev. Res.* **1984**, *4*, 257.
(7) Glossmann, H.; Ferry, D. R.; Goll, A.; Streissnig, J.; Zernig, G. *Arzneim.-Forsch.* **1985**, *35*, 1917.
(8) Hess, P.; Fox, A. P.; Lansman, J. B.; Nilius, B.; Nowycky, M. C.; Tsien, R. W. *Ion Channels in Neural Membranes*; Ritchie, J. M., Keynes, R. D., Bolis, L., Eds.; Alan R. Liss: New York, 1986; pp 227–252.
(9) Loev, B.; Goodman, M. M.; Snader, K. M.; Tedeschi, R.; Macko, E. *J. Med. Chem.* **1974**, *17*, 956.
(10) Rodenkirchen, R.; Bayer, R.; Steiner, R.; Bossert, F.; Mayer, H.; Moller, E. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1978**, *310*, 69.
(11) Bolger, G. T.; Gengo, P.; Klockowski, R.; Luchowski, E.; Siegel, H.; Janis, R. A.; Triggle, A. M.; Triggle, D. J. *J. Pharmacol. Exptl. Therap.* **1983**, *225*, 291.
(12) Mahmoudian, M.; Richards, G. W. *J. Pharm. Pharmacol.* **1988**, *38*, 272.

0022-2623/88/1831-0300$01.50/0

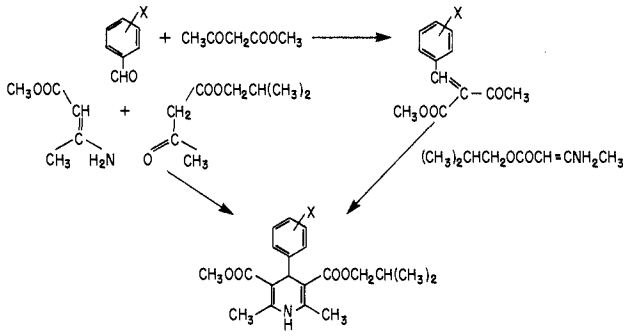

Figure 1. 1,4-Dihydropyridine synthesis.

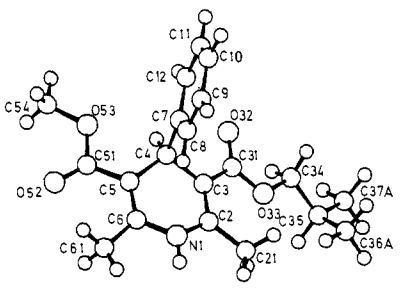

Figure 2. Solid-state structure of isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-phenyl-3,5-pyridinedicarboxylate (I).

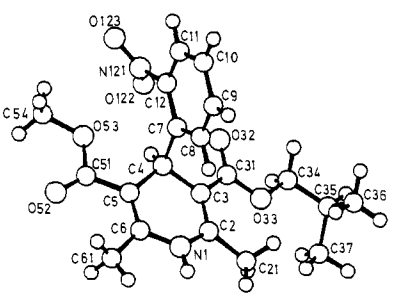

Figure 3. Solid-state structure of isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (II).

has been studied, and it was felt necessary to extend this to additional compounds. Because of suggestions[3,4,6] that the ester substituents at C3 and C5 of the 1,4-dihydropyridine ring influence tissue selectivity, a series of phenyl-substituted derivatives was synthesized bearing different ester groups, CO_2Me and $CO_2CH_2CHMe_2$ (nisoldipine analogues), to permit comparison of solid-state features and biological activities with those of the previously determined nifedipine series, which possesses CO_2Me groups at the C3 and C5 positions. This newly synthesized series has been examined as the racemates.

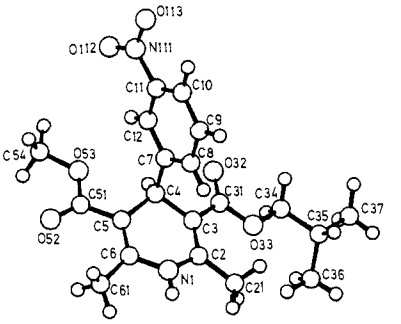

Figure 4. Solid-state structure of isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (III).

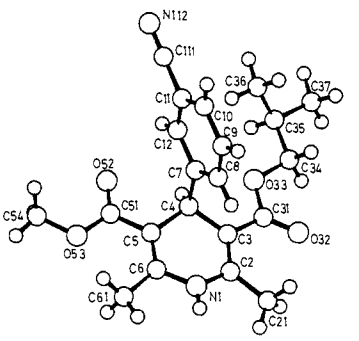

Figure 5. Solid-state structure of isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-cyanophenyl)-3,5-pyridinedicarboxylate (IV).

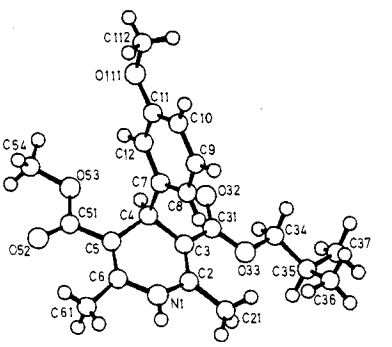

Figure 6. Solid-state structure of isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-methoxyphenyl)-3,5-pyridinedicarboxylate (V).

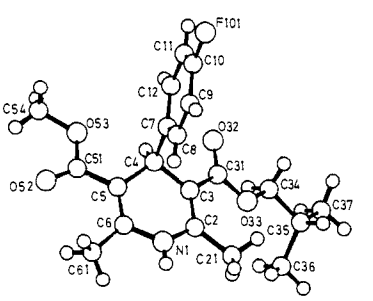

Figure 7. Solid-state structure of isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(4-fluorophenyl)-3,5-pyridinedicarboxylate (VI).

Chemistry

Nine members of the nisoldipine 1,4-dihydropyridine series were synthesized by the Hantzsch procedure. Condensation of methyl-substituted benzylideneacetoacetates, prepared from methyl acetoacetate and the corresponding substituted benzaldehyde, in ethanol with isobutyl aminocrotonate yielded the desired 1,4-dihydropyridine in 10–54% yields. The 2- and 3-(trifluoromethyl)phenyl derivatives were prepared by a mixed

(13) Triggle, A. M.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1980**, *23*, 1442.
(14) Fossheim, R.; Svarteng, K.; Mostad, A.; Rømming, C.; Shefter, E.; Triggle, D. J. *J. Med. Chem.* **1982**, *25*, 126.
(15) Fossheim, R. *Acta Chem. Scand., Ser. B.* **1985**, *B39*, 785.
(16) Fossheim, R. *J. Med. Chem.* **1986**, *29*, 305.
(17) Fossheim, R. *Acta Chem. Scand., Ser. B* **1986**, *B40*, 777.
(18) Hempel, A.; Gupta, M. P. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1978**, *B34*, 3815.
(19) Tacke, R.; Bentlage, A.; Sheldrick, W. S.; Ernst, L.; Towart, R.; Stoepel, K.; *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1982**, *B37*, 443.
(20) Krajewski, J.; Urbanczyk-Lipowska, Z.; Gluzinski, P. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1977**, *B33*, 2967.
(21) Krajewski, J.; Urbanczyk-Lipowska, Z.; Gluzinski, P. *Cryst. Struct. Commun.* **1977**, *6*, 787.
(22) Schauer, C. K.; Anderson, O. P.; Natale, N. R.; Quincy, D. A. *Acta Crystallogr. Sect. C: Cryst. Struct. Commun.* **1986**, *C42*, 884.
(23) Bundule, M. F.; Keeme, A. A.; Bleidelis, J. J.; Biseniece, E. A.; Uldrikis, J. R.; Duburs, G. J. *Zh. Strukt. Khim.* **1981**, *22*, 124.

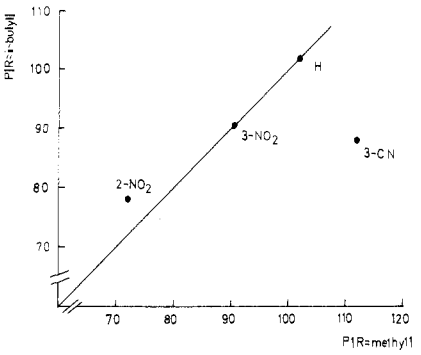

Figure 8. A plot of the puckering (P) of the 1,4-dihydropyridine ring in the nifedipine series (R = Me) against that in the nisoldipine series (R = isobutyl). Indicated are the substituents on the phenyl ring of the two series of compounds. Data for the nifedipine series from Triggle et al.[13] and Fossheim et al.[14]

Hantzsch synthesis from the aldehyde, methyl aminocrotonate, and isobutyl acetoacetate (Figure 1).

X-ray Results

The structures of six of the synthesized 1,4-dihydropyridine molecules showing the adopted atomic labeling scheme are given in Figures 2–7 (Table I, supplementary material). The 1,4-dihydropyridine ring is labeled so that C3 always bears the (isobutyloxy)carbonyl group. With one exception (compound IV) discussed below, this is consistent with the convention that C3 is the position bearing the carbonyl group antiperiplanar to the ring double bond in compounds where the carbonyl groups are twisted in opposite directions. The molecular conformations adopted by these six nisoldipine derivatives are best discussed with respect to the relatively large number of published 1,4-dihydropyridine structures. Selected torsion angles for the six compounds may be found in Table II (supplementary material). Conformational values relevant to the discussion presented here are summarized in Table III for a number of 1,4-dihydropyridines.

The magnitudes of the 1,4-dihydropyridine ring puckering parameter P found in nisoldipine derivatives and their counterparts with symmetric ester groups may be compared by reference to Figure 8. In this figure, P for the nifedipine series is plotted against P for the nisoldipine series of compounds. Three points fall close to a line of unit slope, illustrating that the different ester substituents and the different crystalline environments in these cases affect the adopted conformation of the 1,4-dihydropyridine ring to a small extent only. The m-cyano compounds provide an exception for which there may be several reasons. The isobutoxycarbonyl group of IV is synperiplanar rather than antiperiplanar; in addition, the hydrogen bonding pattern is different since the cyano group acts as the H-bond acceptor. Usually the carbonyl group(s) are H-bond acceptors in these compounds (Table IV, supplementary material). It may also be related to the fact that two different orientations of the phenyl ring are found in the 3-cyano compounds. In the nisoldipine derivative IV the cyano substituent points away from the 1,4-dihydropyridine ring, corresponding to the substituent being on C11. This phenyl ring orientation is preferred in all meta phenyl substituted derivatives investigated thus far except for the 3-cyano nifedipine derivative (compound X), where the substituent points toward the 1,4-dihydropyridine ring. Electronic effects of the cyano substituents are thus not equal in these two compounds.

The extent to which carbonyl groups are torsionally distorted from their respective anti- or synperiplanar values in the various 1,4-dihydropyridine structures is depicted in Figure 9. There is a general trend for both carbonyl groups to be twisted to approximately the same extent. However, in the majority of the compounds, the deviations from synperiplanar or antiperiplanar conformations are small, the carbonyl groups being twisted less than 10° from 0 to 180°. These results can be compared to recent theoretical calculations on the conformational preference of the carboxyl groups in calcium antagonists and agonists[24] that suggest that in antagonists the low-energy conformation corresponds to synperiplanar carbonyl groups with the plane of the carboxy group intersecting the plane of the 1,4-dihydropyridine ring at an angle between 30 and 60°. It is somewhat surprising that this putative low-energy conformation has not been found in a single reported structure. Even in compounds with

Table III. Selected Conformational Parameters in DHP Structures[a]

compd	P[b]	τ(C3–C4–C7–C8)	τ(C2–C3–C31–O32)	τ(C6–C5–C51–O52)
I	101.9	−72.3	−175.0 (ap)[c]	−4.9 (sp)
II	78.2	−53.8	−177.7 (ap)	−2.7 (sp)
III	90.9	−39.1	178.2 (ap)	7.8 (sp)
IV	88.1	−38.1	−2.5 (sp)	−179.4 (ap)
V	108.9	−36.5	176.9 (ap)	4.0 (sp)
VI	86.8	−69.8	−176.9 (ap)	8.2 (sp)
VII[d]	52.1	−56.7	−9.4 (sp)	13.3 (sp)
VII	59.4	−62.8	8.1 (sp)	5.3 (sp)
VIII	104.5	−90.1	175.4 (ap)	12.0 (sp)
IX	72.1	−73.5	−169.9 (ap)	9.1 (sp)
X	112.5	−96.3	174.2 (ap)	4.3 (sp)
XI	102.7	−80.4	178.4 (ap)	5.2 (sp)
XII	93.7	−58.2	173.5 (ap)	11.4 (sp)
XIII	86.9	−42.8	157.8 (ap)	15.9 (sp)
XIV	90.6	−57.8	−177.8 (ap)	7.1 (sp)
XV	85.8	−39.5	165.7 (ap)	13.0 (sp)
XVI	55.0	−77.0	−174.2 (ap)	5.5 (sp)
XVII	62.7	−64.0	−174.7 (ap)	4.0 (sp)
XVIII	51.7	−61.3	0.9 (sp)	−0.3 (sp)
XIX	82.9	−73.9	−176.8 (ap)	8.2 (sp)
XX	121.8	−29.3	169.3 (ap)	2.0 (sp)
XXI	83.2	−69.6	−14.6 (sp)	16.2 (sp)
XXII	80.1	−64.4	−11.2 (sp)	2.9 (sp)
XXIII	86.9	−66.4	179.2 (ap)	7.3 (sp)
XXIV	65.2	−66.8	−8.0 (sp)	−5.0 (sp)
XXV	90.2	−54.4	−2.9 (sp)	−2.4 (sp)

[a] I–VI are defined in footnote a of Table I. VII–X = 2,6-dimethyl-3,5-bis(methoxycarbonyl)-4-(2,3,4,5,6-pentafluoro-, 4-(dimethylamino)-, 2-nitro-, and 3-(cyanophenyl)-1,4-dihydropyridine[13] (compounds VII–X, respectively). XI–XVI = 2,6-dimethyl-3,5-bis(methoxycarbonyl)-4-(unsubstituted)-, 3-methyl-, 4-methyl-, 3-nitro-, 4-nitro-, and 2,4-bis(nitrophenyl)-1,4-dihydropyridine[14] (compounds XI–XVI, respectively). XVII = 2,6-dimethyl-3,5-bis(methoxycarbonyl)-4-[2-(trifluoromethyl)phenyl]-1,4-dihydropyridine.[15] XVIII = ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate.[16] XIX = 2,6-dimethyl-3,5-bis(methoxycarbonyl)-4-(2-aminophenyl)-1,4-dihydropyridine.[17] XX = 2,6-dimethyl-3,5-bis(ethoxycarbonyl)-4-phenyl-1,4-dihydropyridine.[18] XXI = neopentyl (trimethylsilyl)methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate.[19] XXII = 2,6-dimethyl-3,5-bis(ethoxycarbonyl)-4-β-pyridyl-1,4-dihydropyridine.[20] XXIII = 2,6-dimethyl-3,5-diacetyl-4-β-pyridyl-1,4-dihydropyridine.[21] XXIV = diethyl 2,6-dimethyl-4-(5-methyl-3-phenylisoxazol-4-yl)-1,4-dihydropyridine-3,5-dicarboxylate.[22] XXV = 2,6-dimethyl-1,4-diphenyl-3,5-bis(ethoxycarbonyl)-1,4-dihydropyridine.[23] [b] P is the DHP ring puckering parameter, defined as the sum of the numeric values of the six intraring torsion angles. [c] The ester conformation is designated ap when the carbonyl group is antiperiplanar with respect to the ring double bond and sp when the carbonyl group is synperiplanar with respect to the ring double bond. [d] In this compound, there are two molecules in the asymmetric unit.

(24) Mahmoudian, M.; Richards, G. W. J. Chem. Soc., Chem. Commun. 1986, 739.

Table V. Pharmacologic and Radioligand Binding Activities of Isobutyl Methyl 2,6-Dimethyl-4-(substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates in Intestinal Smooth Muscle

no.	Ph substit	binding[a] IC_{50}, M (95% CL)	n_H[b]	pharmacology[c] IC_{50}, M (95% CL)
I	H	2.5×10^{-10} (0.7–8.7)	0.85	3.3×10^{-9} (1.9–5.7)
II	2-NO_2	1.5×10^{-11} (0.4–5.9)	0.89	2.2×10^{-10} (0.8–5.9)
III	3-NO_2	8.1×10^{-12} (2.5–26.1)	0.81	5.9×10^{-10} (3.9–8.9)
IV	3-CN	1.3×10^{-11} (0.5–3.6)	0.80	2.7×10^{-10} (1.5–4.7)
V	3-MeO	2.3×10^{-9} (1.0–5.1)	0.98	7.1×10^{-9} (3.5–14.6)
VI	4-F	1.5×10^{-9} (0.7–3.3)	1.07	6.8×10^{-9} (4.4–10.4)
XXVI	2-CF_3	2.7×10^{-11} (1.2–6.2)	1.08	2.1×10^{-9} (1.3–3.3)
XXVII	3-CF_3	1.4×10^{-10} (0.7–3.1)	1.00	1.5×10^{-8} (1.0–2.2)
XXVIII	4-Cl	2.8×10^{-9} (1.3–6.3)	0.82	2.7×10^{-8} (1.7–4.2)

[a] Inhibition of specific [^{3}H]nitrendipine binding in guinea pig ileal longitudinal smooth muscle. [b] Pseudo Hill coefficient for displacement of bound [^{3}H]nitrendipine.[30] [c] Inhibition of tonic (slow) component of K^+-depolarization response in guinea pig ileal longitudinal muscle.

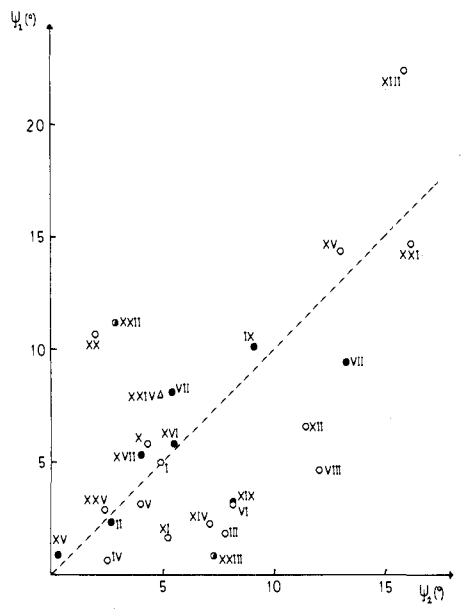

Figure 9. A plot of the torsional distortions of the C3 and C5 substituents. ψ_1 is defined as the absolute difference of the C2–C3–C31–C32 torsion angle and its ideal value 0 or 180°. ψ_2 is defined as the absolute value of the C6–C5–C51–O52 torsion angle and its ideal value 0 or 180°. In order to make the description of compound IV consistent, ψ_1 and ψ_2 have been interchanged for this compound. This is equivalent to reflection across the dashed line ($\psi_1 = \psi_2$). The numbers describe the 1,4-dihydropyridines in Tables I and III.

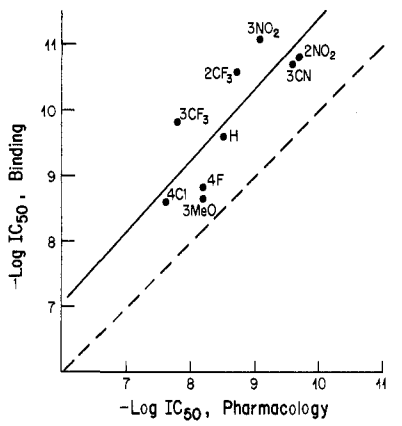

Figure 10. Correlation between the abilities of isobutyl methyl 2,6-dimethyl-4-(substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates (nisoldipine series) to inhibit specific [^{3}H]nitrendipine binding and K^+-depolarization-induced tension response (tonic component) in guinea pig ileal longitudinal smooth muscle. The dashed lines represents 1:1 equivalency, and the filled line represent the regression where: log IC_{50} (bind) = 1.08 log IC_{50} (pharmacol) + 0.58 (r = 0.80, SE regression 0.64, F = 12.28, significant at the 0.05 level).

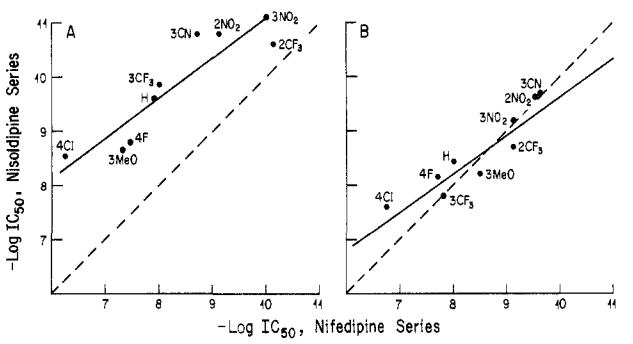

Figure 11. Correlations between inhibitory activities of phenyl-substituted nifedipine and nisoldipine analogues. (A) Inhibition of specific [^{3}H]nitrendipine binding in guinea pig ileal longitudinal smooth muscle. (B) Inhibition of K^+-depolarization-induced tension response (tonic component) in guinea pig ileal longitudinal smooth muscle. The dashed lines represent 1:1 equivalency, and the filled lines represent the regression where: (A) log IC_{50} (nisoldipine series) = 0.71 log IC_{50} (nifedipine series) + 3.99 (r = 0.91, SE regression = 0.46, F = 31.96, significant at 0.05 level). (B) log IC_{50} (nisoldipine series) = 0.70 log IC_{50} (nifedipine series) + 2.66 (r = 0.91, SE regression = 0.33, F = 35.60, significant at the 0.05 level).

relatively bulky ortho phenyl substituents, the carbonyl groups do not deviate more than a few degrees from ideal synperiplanar or antiplanar values.

Biological Results and Discussion

The pharmacological and radioligand binding activities of the complete nisoldipine series were evaluated in guinea pig intestinal smooth muscle by measuring their antagonistic effects against K^+-depolarization-induced tension responses and specific high-affinity [^{3}H]nitrendipine binding. The data are summarized in Table V and the correlation between these two activities shown in Figure 10. These data and the derived correlation reinforce the conclusion drawn previously for a series of nifedipine analogues in this and other smooth muscles that binding and pharmacologic affinities reflect the same process.[4,11,25] For the nifedipine series, the correlation was 1:1, whereas in this smaller series of nisoldipine analogues, binding activities exceed by an approximately 10-fold factor the corresponding pharmacologic activities. In principle, several reasons may underlie this discrepancy, most notably the comparison of two sets of activities obtained with different cellular states, temperatures, and ionic strength.[4,6,11]

Comparisons may be made between the binding and pharmacologic data reported here for the nisoldipine series and those reported previously for a more extensive nifedipine series.[11] The satisfactory correlations (Figure

(25) Yousif, F. B.; Bolger, G. T.; Ruzycky, A.; Triggle, D. J. *Can. J. Physiol. Pharmacol.* **1985**, *63*, 453.

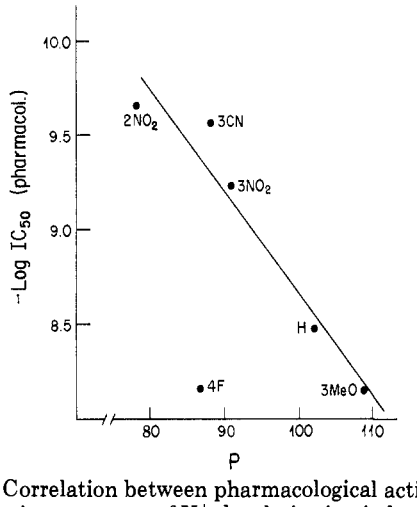

Figure 12. Correlation between pharmacological activities (inhibition of tonic component of K^+-depolarization-induced tension response in ileal longitudinal muscle) and 1,4-dihydropyridine ring puckering (P, Table III) in the nisoldipine series (compounds I–V). The line of regression is plotted: $-\log IC_{50} = -0.05P + 14.02$ ($r = 0.98$, SE regression = 0.15, $F = 75.1$, significant at the 0.05 level).

11A,B) show that the effects of aromatic ring (phenyl) substitution are expressed independent of the nature of the C3- and C5-ester substituents (similar conclusions have been drawn from a series of nicardipine derivatives, Joslyn, A.; Solo, A. J.; Triggle, D. J., unpublished observations). The nisoldipine series is consistently some 10-fold more active than the corresponding nifedipine series in the binding assay, but the two series are essentially equiactive in the pharmacologic assay (Figure 11A,B). A similar discrepancy is observed in the comparison of binding and pharmacologic activities in the nisoldipine series (Figure 10), which may indicate a diffusion limitation to access by the bulkier nisoldipine derivatives to binding sites in the intact tissue.

Despite these quantitative discrepancies, the data show that the C4 and C3/C5 substituents make independent contributions to the activity of 1,4-dihydropyridines, at least over the comparatively limited range studied. This is revealed also in the data of Figure 12, correlating the puckering of the 1,4-dihydropyridine ring with the pharmacologic activities of the nisoldipine series. As described for the nifedipine series[14] a good linear correlation is found between this structural parameter and the pharmacologic activities of ortho- and meta-substituted derivatives. The low activity of the 4-fluoro analogue accords with previous reports of the detrimental effects of 4-substituents, presumably due to steric limitations of the binding site.[3,10,11]

The parameters describing the puckering of the 1,4-dihydropyridine ring obviously constitute a small selection of the structural parameters (i.e., internal coordinates) describing these structures. The observed correlation may be criticized from a statistical point of view, since all possible relevant parameters are not considered, and Berntsson and Wold[26] have indicated that an alternative structure–activity relationship based on conventional physicochemical parameters is also satisfactory. The data presented here support the claim that the puckering of the 1,4-dihydropyridine ring is a significant structural parameter in describing the pharmacologic activity of a series of ortho and meta phenyl substituted nisoldipine derivatives. This structure–activity correlation is similar to that found for the nifedipine series.[14] Resolution of the relative importance of the geometrical and electronic factors awaits the analysis of more extensive series. Since different ester substituents lead to minor changes in 1,4-dihydropyridine ring puckering, this aspect of the structure–activity relationship cannot explain why asymmetric esters are usually more potent than their symmetrically substituted counterparts.[6,10,27] Other molecular features must be important, and this is illustrated by observations that 3-nitro-5-ester-substituted 1,4-dihydropyridines show remarkable enantiomeric selectivity where S and R enantiomers activate and block, respectively, Ca^{2+} channels.[28,29] This has not been observed with 3,5-diester-substituted compounds, but the present series of compounds were evaluated only as racemates, a potential limitation to the interpretation of their structure–activity relationships.

Table VI. Physical and Analytical Data for Isobutyl Methyl 2,6-Dimethyl-4-(X-substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates

no.	X	yield, %	mp, °C	formula	anal.
I	H	13	131–134	$C_{20}H_{26}NO_4$	C, H, N
III	3-NO_2	10	127–129	$C_{20}H_{24}N_2O_6$	C, H, N
IV	3-CN	54	137–138	$C_{21}H_{24}N_2O_6$	C, H, N
V	3-OMe	32	99–101	$C_{21}H_{27}NO_5$	C, H, N
VI	4-F	30	117–120	$C_{20}H_{24}FNO_4$	C, H, N
XXVI	4-Cl	51	132–134	$C_{20}H_{24}ClNO_4$	C, H, N
XXVII	2-CF_3	5	118–119	$C_{21}H_{24}F_3NO_4$	C, H, N
XXVIII	3-CF_3	10	88–90	$C_{21}H_{24}F_3NO_4$	C, H, N

Experimental Section

Synthetic Methods. Melting points were determined with a Meltemp apparatus (Laboratory Devices) and are reported uncorrected. Infrared spectra were obtained with a Nicolet FT-IR spectrophotometer. NMR spectra were determined with a Varian T60A or Varian EM390 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Commercially available reagents and solvents were used without additional purification unless otherwise stated. The purity of literature compounds was determined by boiling points, melting points, NMR spectroscopy, FT-IR spectroscopy, and thin-layer chromatography.

General Procedure for Isobutyl Methyl 2,6-Dimethyl-4-(X-substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates. Isobutyl Methyl 2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (III). A solution of methyl (3-nitrobenzylidene)acetoacetate (4 g, 16 mmol) and isobutyl aminocrotonate (2.75 mL, 16 mmol) was refluxed in 50 mL of ethanol for 96 h. After the ethanol was removed in vacuo, the residue was purified by column chromatography [silica gel (60–200 mesh), 75 g (dry weight), methylene chloride]; the starting materials were separated from the desired product, III, in this solvent system, followed by elution of the product from the column by increasing the polarity of the elution solvent with ethyl acetate/chloroform (1:1). Crystallization from ethyl acetate yielded III as yellow crystals (0.63 g, 10%): mp 127–129 °C; NMR ($CDCl_3$/TMS) δ 0.8, 0.9 (2 d, 6 H), 1.5–2.1 (m, 1 H), 2.3 (s, 3 H), 2.35 (s, 3 H), 3.6 (s, 3 H), 3.8 (d, 2 H), 5.1 (s, 1 H), 6.2 (br s, 1 H), 7.2–8.1 (m, 4 H); IR (KBr) 3341, 2964, 1700, 1531, 1349 cm^{-1}.

This method was used to prepare isobutyl methyl 4-(X-substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates (I, IV–VI, XXVI; X = 3-CN, 3-MeO, H, 4-F, and 4-Cl, Table VI).

Isobutyl Methyl 2,6-Dimethyl-4-[2-(trifluoromethyl)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (XXVII). A solution of 2-(trifluoromethyl)benzaldehyde (2.9 g, 16.5 mmol), methyl acetoacetate (2.1 g, 16.5 mmol), and isobutyl amino-

(26) Berntsson, P.; Wold, S. *Quant. Struct.-Act. Relat. Pharmacol., Chem. Biol.* **1986**, *5*, 45.
(27) Towart, R.; Wehinger, E. E.; Meyer, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *317*, 183.
(28) Hof, P. R.; Ruegg, U. T.; Hof, A.; Vogel, A. *J. Cardiovas. Pharmacol.* **1985**, *7*, 689.
(29) Franckowiak, G.; Bechem, M.; Schramm, M.; Thomas, G. *Eur. J. Pharmacol.* **1985**, *114*, 223.
(30) Limbird, L. E. *Cell Surface Receptors: A Short Course on Theory and Methods*; Martinus Nijhoff: Boston, 1985.

crotonate (2.6 g, 16.5 mmol) was refluxed in 50 mL of ethanol for 96 h. The ethanol was removed in vacuo, and the residue was purified by column chromatography [silica gel (60–200 mesh), 100 g (dry weight), ethyl acetate/hexanes (1:2)] and crystallized from ethyl acetate/hexanes: NMR ($CDCl_3$/TMS) δ 0.8 and 0.9 (2 d, 6 H), 1.6–2.1 (m, 1 H), 2.3 (s, 3 H), 2.35 (s, 3 H), 3.6 (s, 3 H), 3.8 (d, 2 H), 5.6 (s, 1 H), 5.8 (br s, 1 H), 7.1–7.6 (m, 4 H); IR (KBr) 3329, 2975, 1695, 1308 cm^{-1}.

Isobutyl Methyl 2,6-Dimethyl-4-[3-(trifluoromethyl)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (XXVIII). This was prepared similarly to VIII from 3-(trifluoromethyl)-benzaldehyde (2.9 g, 16.5 mmol), methyl acetoacetate (2.1 g, 16.5 mmol), and isobutyl aminocrotonate (2.6 g, 16.5 mmol): NMR ($CDCl_3$/TMS) δ 0.8 and 0.9 (2 d, 6 H), 1.5–2.1 (m, 1 H), 2.3 (s, 3 H), 2.35 (s, 3 H), 3.6 (s, 3 H), 3.8 (d, 2 H), 5.1 (s, 1 H), 5.7 (br s, 1 H), 7.2–7.6 (m, 4 H); IR (KBr) 3360, 2973, 1703, 1311 cm^{-1}.

Crystallographic Methods. Crystallographic and experimental data for the six compounds are given in Table I (supplementary material). Single crystals were obtained by slow evaporation from methanol (Compounds I, II, and IV–VI) and acetonitrile (Compound III) solutions. Data were collected on an automatic four-circle diffractometer (SYNTEX P1) with graphite-monochromatized Mo Kα radiation (λ = 0.71069 Å) and the $\theta/2\theta$ scan procedure. During data collection, the crystals were maintained at approximately −150 °C by means of nitrogen cooling. The unit-cell parameters for each crystal were obtained by a least-squares fit of the diffractometer settings for 15 high-order reflections. The measured intensities were corrected for Lorentz and polarization effects but not for absorption.

All structures were solved with the direct methods program MULTAN.[31] They were refined by difference Fourier and least-squares techniques. The weights in least squares were calculated from the standard deviations in intensities ($\sigma(I)$, taken as $\sigma(I) = \sqrt{[C_1 + (0.02C_2)^2]}$, where C_1 is the total number of counts and C_2 the net peak counts). Non-hydrogen atoms were refined anisotropically; hydrogen atoms were refined isotropically with a common thermal parameter for H atoms in methyl groups. Fractional occupancy numbers were introduced in order to describe a statistical disorder of the isobutyl group in compound I. The positions of the hydrogen atoms in the disordered fragment were calculated. Final R values are given in Table I (supplementary material). The computer programs used are described by Groth.[32] Final positional parameters for the six structures are given in Table VII (supplementary material). Intramolecular bond distances and bond angles for compounds I–VI are given in Tables VIII and IX (supplementary material). Thermal parameters and tables of observed and calculated structure factors are available from the authors.

Pharmacologic Methods. Tissue Preparation. Male albino guinea pigs (Buckberg, Tomkins Cove, NY) weighing from 300 to 500 g were killed by decapitation. The terminal ileum was quickly removed and placed into ungassed buffered saline (Tyrode's solution) at 37 °C having the composition (millimolar): NaCl, 137; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.36; $NaHCO_3$, 11.9; dextrose, 5.55. Longitudinal muscle strips were prepared according to Rosenberger et al.[33]

Radioligand Binding. Microsomal membranes were prepared from guinea pig ileal longitudinal smooth muscle according to the description by Bolger et al.[11] Binding of [^{3}H]nitrendipine and its competition by the 1,4-dihydropyridine series was carried out according to Bolger et al.[11] In brief, membrane protein (20–80 μg) was incubated in a total volume of 5 mL of 50 mM tris[(hydroxymethyl)amino]methane (Tris) buffer (pH 7.2) for 60 min at 25 °C with 10^{-10} M [^{3}H]nitrendipine and varying concentrations of specific compounds (where indicated). Equilibrium binding is reached during this incubation period. Nonspecific binding was defined in a duplicate set of tubes by the addition of 10^{-7} M unlabeled nitrendipine. The reaction was terminated by rapid filtration through Whatman GF/B filters with a Brandel cell harvester (Model M-24R, Biomedical Research Lab., Gaithersburg, MD) followed by two washes of 5 mL each, under vacuum, with ice-cold buffer. The filters were placed in individual 10-mL glass vials and dissolved in 5 mL of "scintillation fluor" that consisted of 4.5 g of PPO, 0.3 g of dimethyl POPOP, and 7.5 mL of BTS-450 (Beckman) per liter of toluene. The filters were allowed to stand a minimum of 3 h before counting was performed in a Packard liquid scintillation counter (Model 3255) at an efficiency, determined by external standards, of 40–45%. Binding experiments were performed in subdued light and 4-(2-nitrophenyl) compounds were handled exclusively under sodium light. Because many of the drugs were dissolved in ethanol as 10^{-3}–10^{-5} M stock solutions, control experiments were included to determine the effect of the ethanol on [^{3}H]nitrendipine binding. Concentrations of ethanol up to 0.2% (v:v) did not affect specific [^{3}H]nitrendipine binding.

[^{3}H]Nitrendipine (2,6-dimethyl-3-ethoxy-5-[^{3}H]methoxy-4-(3-nitrophenyl)-1,4-dihydropyridinedicarboxylate) with a specific activity of 79.5 Ci $mmol^{-1}$ (1 Ci = 3.7 × 10^{10} bq) was purchased from Du Pont–New England Nuclear (Boston, MA).

Contractile Studies. Longitudinal muscle strips of guinea pig ileum were prepared to record mechanical responses.[11,23] In brief, tissue pieces, approximately 2 cm long, were suspended in 10-mL jacketed glass tissue baths connected to a recirculating heated reservoir maintained at 37 °C. Each tissue was fixed to the bottom of the tissue bath with a stainless steel hook, and the other end was attached to the writing lever of a smoked drum kymograph. The physiologic saline (PSS) had the following composition (millimolar): NaCl, 118; KCl, 4.7; $MgCl_2$, 1.2; $CaCl_2$, 1.8; $NaHCO_3$, 84; KH_2PO_4, 1.2; dextrose, 5.5 and was aerated with O_2/CO_2 (95:5).

Isotonic recordings were made at a magnification ratio of 9:1 against 500 mg of resting tension. The contraction height was measured from the base-line tension attained by each tissue and recorded as millimeters contraction. Longitudinal smooth muscle strips were allowed to equilibrate for 90–120 min.[11,23] During this time, the bathing solutions were replaced with fresh PSS every 15 min, and the tissues were twice exposed at 60-min intervals to a depolarizing saline solution (isotonic substitution of 80 mM KCl for NaCl) to determine the tissue viability and the reproducibility of the response. After the initial equilibration period, a control maximal response to depolarizing saline was recorded followed by washing and a 30-min reequilibration. The antagonist was then equilibrated for 30 min, and a response to the depolarizing (80 mM KCl) saline was measured. Only one drug concentration was used on any given tissue.

Statistics. Statistical analysis of pharmacologic and radioligand binding data employed standard pharmacology programs or ligand binding programs implemented on an IBM PC.[34]

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Registry No. (±)-I, 111556-83-3; (±)-II, 86189-67-5; (±)-III, 111634-72-1; (±)-IV, 111556-84-4; (±)-V, 111556-85-5; (±)-VI, 111556-86-6; (±)-XXVI, 111556-87-7; (±)-XXVII, 111556-88-8; (±)-XXVIII, 111556-89-9; C_6H_5CHO, 100-52-7; 3-$O_2NC_6H_4CHO$, 99-61-6; 3-NCC_6H_4CHO, 24964-64-5; 3-$H_3COC_6H_4CHO$, 591-31-1; 4-FC_6H_4CHO, 459-57-4; 2-$F_3CC_6H_4CHO$, 447-61-0; 3-$F_3CC_6H_4CHO$, 454-89-7; 4-ClC_6H_4CHO, 104-88-1; $H_3CCOCH_2CO_2CH_3$, 105-45-3; $H_3CO_2CCH{=}C(NH_2)CH_3$, 14205-39-1; $(H_3C)_2CHCH_2O_2CCH_2COCH_3$, 7779-75-1; $(H_3C)_2CHCH_2OCOCH{=}C(NH_2)CH_3$, 52937-90-3; $C_6H_5CH{=}C(COCH_3)CO_2CH_3$, 15768-07-7; 3-$O_2NC_6H_4CH{=}C(COCH_3)CO_2CH_3$, 39562-17-9; 3-$NCC_6H_4CH{=}C(COCH_3)CO_2CH_3$, 39562-37-3; 3-$H_3COC_6H_4CH{=}C(COCH_3)CO_2CH_3$, 92565-02-1; 4-$FC_6H_4CH{=}C(COCH_3)CO_2CH_3$, 111556-82-2; 4-$ClC_6H_4CH{=}C(COCH_3)CO_2CH_3$, 103069-52-9; (±)-[^{3}H]nitrenedipine, 94365-95-4.

Supplementary Material Available: Tables I, II, IV, VII, VIII, and IX (14 pages). Ordering information is given on any current masthead page.

(31) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1971**, *A27*, 368.
(32) Groth, P. *Acta Chem. Scand.* **1973**, *27*, 1837.
(33) Rosenberger, L. B.; Ticku, M. K.; Triggle, D. J. *Can. J. Physiol. Pharmacol.* **1979**, *57*, 333.
(34) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations*; Springer-Verlag: New York, 1981.