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Novel halogenated 1,4-dihydropyridines: synthesis, bioassay, microsomal oxidation and structure–activity relationships*

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Summary — Nine new 1,4-dihydropyridines (DHPs) were synthesized and evaluated for their relaxant ability (rat aorta) and their antihypertensive activity in spontaneously hypertensive rats; their microsomal oxidation rate (MOR) was determined. In terms of relaxant activity, the 4-(3,5-difluorophenyl) analogues were more potent than those with 4-(4-fluorophenyl) but weaker than those with 4-(3-nitrophenyl) substituents, while in terms of antihypertensive activity the 4-(3,5-difluorophenyl) derivatives were more potent than their 4-(3-nitrophenyl) analogues. Their MOR could be explained on the basis of the electron-withdrawing effect of the substituents, and in some cases they permitted a rationalization of discrepancies noted between DHP antihypertensive and relaxant activities. A parabolic relationship was found between the size of the carboxylic ester substituents and their contributions calculated from a Free–Wilson/Fujita–Ban analysis of relaxant activity data.

1,4-dihydropyridine / aorta relaxant activity / antihypertensive activity / microsomal oxidation rate / structure–activity relationship

Introduction

1,4-Dihydropyridines (DHPs) are modulators of the transmembrane influx of extracellular calcium [1]. The nifedipine prototype and other DHPs have been approved for clinical use in the treatment of some cardiovascular diseases [2–4].

Numerous qualitative and quantitative structure–activity relationship studies (SAR and QSAR, respectively) have shown the importance of aryl ring substitution on pharmacological activity [5–8]. Substituents in the *ortho* and *meta* positions usually maintain or increase the activity, whereas *para* substitution drastically reduces it. In a study [5] involving pharmacological and binding data for a large series of mono- and polysubstituted DHPs, Coburn *et al* showed that electron-withdrawing substituents enhance activity, but that an increase of substituent size is unfavorable for it, especially for the *para* and *meta'* positions.

The purpose of this research was to examine the relaxant effect of DHPs with novel substituents (3-bromo-4-fluoro and 3,5-difluoro) on the 4-phenyl ring (3'-Br,4'-F and 3',5'-F_2, respectively), which have appropriate inductive effects and are small enough for the critical (*para* and *meta'*) positions, and a new ester function (CF_3CH_2OOC-) in various combinations (fig 1). These were compared with DHPs with established substituents (4'-F, 3'-NO_2) and ester functionalities. Some known DHPs (**1–3**, nitrendipine and nicardipine) were used as reference compounds.

The therapeutic efficacy of DHPs is, however, limited by a rapid first-pass hepatic inactivation which leads to brief activity [9], necessitating frequent dosing. Studies on their pharmacokinetics and biotransformation [10–13] have shown that the first and preponderant step in their metabolism is their oxidation to pyridines. A mechanism proposed by Guengerich and Böcker [14, 15] includes the transfer of an electron to generate a cationic radical as a first step (scheme 1), followed by deprotonation to a neutral radical, which then loses a hydrogen atom, or an electron and a proton, to give the final pyridine.

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Abbreviations: A_s: normalized surface area; DHP: dihydropyridine; DMSO: dimethyl sulfoxide; FW/FB: Free-Wilson analysis modified by Fujita and Ban; NIT: nitrendipine; NIC: nicardipine; MOR: microsomal oxidation rate; SHR: spontaneously hypertensive rat.

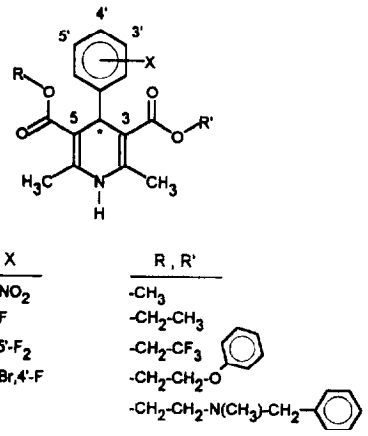
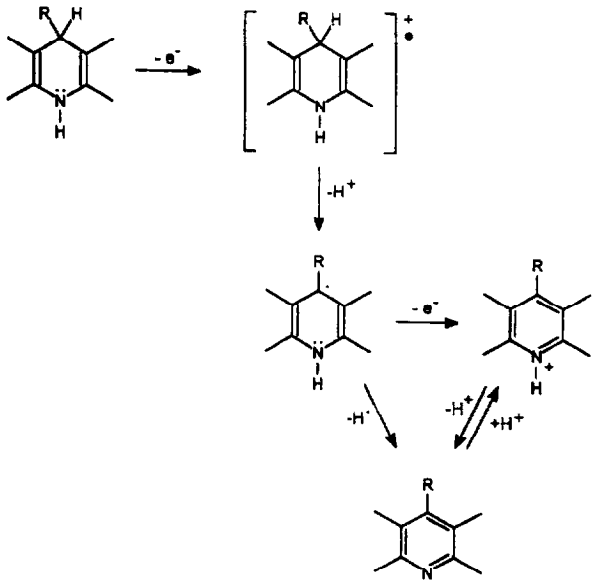


Fig 1. General structure of the studied DHPs. The asterisk identifies the chiral center when R ≠ R'.



Scheme 1.

SAR studies of the microsomal oxidation rate (MOR) [16, 17] showed that it is more influenced by the type and location of the aryl substituent than by the ester moieties. We have examined these new DHPs, together with the related reference compounds for their *in vivo* antihypertensive activity in spontaneously hypertensive rats (SHR), and their susceptibility to metabolic inactivation, quantified by their MOR. In particular we sought to relate the two parameters by deriving SARs useful in designing further DHPs with a longer duration of action.

Chemistry

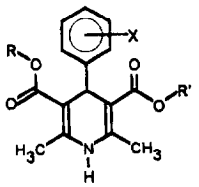
The symmetric DHPs (R = R'; table I) were synthesized by the classic Hantzsch method according to recent procedures [18–20] (scheme 2). The asymmetric DHPs (R ≠ R'; table I) were obtained as racemates as shown in scheme 3.

Results and discussion

Smooth muscle relaxing activity

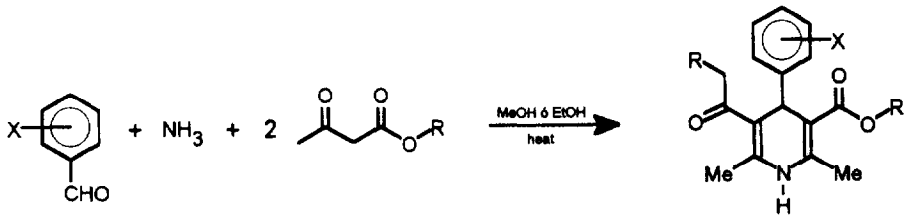
The calcium-channel-blocking activity was evaluated by the relaxation of rat aorta rings precontracted with 80 mM K^+. All tested compounds dose-dependently inhibited the K^+-induced contractions. The obtained

Table I. Structure and physical data of the DHPs.

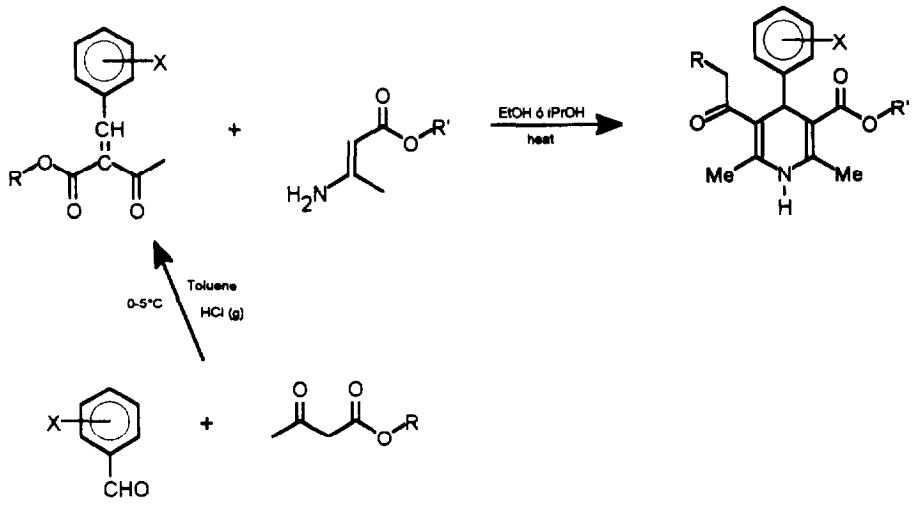


DHP	X	R	R'	Mp (°C)	Formula[a]	Cryst solvent[b]	Yield (%)
0	H	C_2H_5	C_2H_5	154-156[c]	$C_{19}H_{23}O_4$	A	31
1	3'-NO_2	CH_3	CH_3	206-208[d]	$C_{17}H_{18}N_2O_6$	A	44
2	3'-NO_2	C_2H_5	C_2H_5	165-166[e]	$C_{19}H_{22}N_2O_6$	A	56
3	4'-F	CH_3	CH_3	170-171[f]	$C_{17}H_{18}NO_4F$	A	45
4	4'-F	C_2H_5	C_2H_5	148-149	$C_{19}H_{22}NO_4F$	A	64
5	3',5'-F_2	CH_3	CH_3	185-187	$C_{17}H_{17}NO_4F_2$[g]	A	55
6	3',5'-F_2	CH_3	C_2H_5	151-153	$C_{18}H_{19}NO_4F_2$	B	35
7	3',5'-F_2	CH_3	CH_2CF_3	162-164	$C_{19}H_{16}NO_4F_6$[h]	A	46
8	3',5'-F_2	CH_3	$CH_2CH_2OC_6H_5$	139-141	$C_{24}H_{25}NO_5F_2$	A	29
9	3',5'-F_2	CH_3	EtN(Me)Bz[i]	92-94	$C_{28}H_{28}N_2O_4F_2$	C	37
10	3',5'-F_2	C_2H_5	C_2H_5	181-183	$C_{19}H_{21}NO_4F_2$	A	51
11	3'-Br,4'-F	CH_3	CH_3	165-167	$C_{17}H_{17}NO_4FBr$	A	55
12	3'-Br,4'-F	CH_3	CH_2CF_3	146-149	$C_{18}H_{16}NO_4F_4Br$[j]	A	42
NIT[k]	3'-NO_2	CH_3	C_2H_5	158-159[l]	$C_{18}H_{20}N_2O_6$	D	—
NIC[m]	3'-NO_2	CH_3	EtN(Me)Bz[i]	107-108[n]	$C_{26}H_{29}N_3O_6$	E	—

[a]The analyses of DHPs **4–12** for C, H, N and halogens were within ±0.4% of the theoretical values, except as noted; [b]A = methanol, B = ethanol/hexane, C = isopropyl ether, D = ethanol, E = isopropyl ether/hexane; [c]lit mp 156–157°C [34]; [d]lit mp 207°C [20]; [e]lit mp 168°C [20]; [f]lit mp 171°C [35]; [g]F: calc 11.27, found 10.80; [h]F: calcd 23.44, found 22.67; [i]*N*-benzyl-*N*-methyl-2-aminoethyl; [j]F: calcd, 16.30, found 15.41; [k]nitrendipine; [l]see reference [20]; [m]nicardipine; [n]see reference [41].



Scheme 2.



Scheme 3.

pIC_{50} values are shown in table II. Except for **3**, all the tested DHPs were more potent than verapamil (pIC_{50} = 6.78 (6.65–6.90)).

The novel substitution 3',5'-F_2 results in highly potent DHPs, only slightly less active than the 3'-NO_2 derivatives, but more active than the 4'-F analogues. The relatively low potency of the 4'-F DHPs is clearly evidence for the strict steric requirements of the receptor in the *para* position. This is confirmed by the weak activity obtained for DHP **11** (3'-Br,4'-F-substituted) in comparison to the excellent activity reported by Coburn *et al* for its 3'-Br analogue [5].

As regards the ester moieties, where direct comparisons were possible (**1** *vs* **2**, **3** *vs* **4**, **5** *vs* **10**), the diethyl esters were more potent than the dimethyl compounds. Mixed esters (R = Me, R' = Et) were intermediate, while the trifluoroethyl substituent conferred no remarkable enhancement. Although it has been claimed that asymmetric DHPs are in general more potent [19, 21, 22], our results contradict this, since within the 3'-NO_2, 4'-F and 3',5'-F_2 series, the diethyl ester analogues (**2**, **4**, and **10**) were the most potent.

Antihypertensive activity

The effects of the DHPs on the systolic blood pressure and cardiac frequency of SHRs are listed in table III and shown in figures 2 and 3. All the DHPs displayed significant antihypertensive activity. For most, the maximum drop was seen 1 h after administration. Except for NIC, the 3',5'-F_2 DHPs were more potent than their 3'-NO_2 analogues, while the 4'-F analogues were less potent and had a shorter time of action. Most of the new DHPs showed relatively good antihypertensive activity, considering their intermediate potency relative to the reference compounds (NIT and NIC). DHPs **6** and **7** were still active after 4 h, whereas **5** showed a significant reduction over 8 h.

It was of interest to compare the rank order of potencies obtained in this bioassay with that found by us for the *in vitro* aorta relaxant bioassay. The DHPs with dimethyl esters were more potent *in vivo* than those with diethyl esters for both 3'-NO_2 and 3',5'-F_2 series, whereas this order was reversed *in vitro*. The 3',5'-F_2 DHPs were more potent *in vivo* than their 3'-NO_2 analogues (except for NIC), while they were less

Table II. Relaxant activity and physicochemical parameters of DHPs.

DHP	pIC_{50} (95% CI)	R_M	Log P		
			LH[a]	calcd[b]	calcd[c]
1	8.22 (8.06 - 8.38)	-0.586	0.46	0.85	0.50
2	9.22 (9.12 - 9.34)	-0.431	1.54	1.66	1.60
3	6.67 (6.60 - 6.74)	-0.591	0.86	0.82	0.99
4	7.22 (7.03 - 7.40)	-0.447	1.94	1.58	1.66
5	7.25 (7.10 - 7.40)	-0.528	1.00	1.15	1.28
6	7.77 (7.68 - 7.86)	-0.491	1.54	1.35	1.46
7	7.73 (7.67 - 7.80)	-0.251	2.37	2.60	2.58
8	7.78 (7.70 - 7.87)	-0.145	2.91	3.15	3.08
9	7.34 (7.24 - 7.45)	-0.152	3.37	3.12	3.05
10	8.03 (7.84 - 8.22)	-0.348	2.08	2.09	2.13
11	7.18 (7.07 - 7.30)	-0.486	1.72	1.37	1.48
12	7.24 (7.14 - 7.33)	-0.124	3.09	3.26	3.18
NIT[d]	8.30 (8.23 - 8.36)	-0.528	1.00	1.15	0.91
NIC[e]	8.26 (8.19 - 8.34)	-0.259	2.83	2.56	2.82

[a]Calculated by the Leo-Hansch fragmental system; [b]calculated by eq 1; [c]calculated by eqs 2 or 3; [d]nitrendipine; [e]nicardipine.

Table III. Antihypertensive activity and microsomal oxidation rates of the DHPs.

DHP	AHA[a]		ε[b]	MOR(±SD)[c]
	E_{max} (mmHg)	T (h)	$mM^{-1}cm^{-1}$	nmol/mg prot·min
0	---	---	4.55	1.37 (±0.26)
1	-20	6	4.76	0.66 (±0.11)
2	-13	4	4.16	0.76 (±0.04)
3	-10	2	4.83	0.90 (±0.15)
4	-15	2	4.78	0.99 (±0.14)
5	-32	8	4.55	0.57 (±0.05)
6	-30	6	4.80	0.58 (±0.03)
7	-22	6	---	---
8	-21	2	---	---
9	-23	6	4.62	0.54 (±0.04)
10	-18	2	---	---
11	-14	2	4.52	0.59 (±0.08)
12	-25	6	---	---
NIT[d]	-15	4	4.36	0.71 (±0.11)
NIC[e]	-48	6	---	---

[a]Antihypertensive activity (n = 6), where E_{max} and T are the maximum effect and the time during which the reduction was significantly different from t_0 and the control ($p < 0.05$–0.01); [b]molar extinction coefficient of DHP at 360 nm; [c]microsomal oxidation rate (n = 5–7); [d]nitrendipine; [e]nicardipine.

potent *in vitro*. This indicates that even if the antihypertensive activity results from the relaxing effect, the latter does not completely account for the former.

Cardiac frequency

Of all the compounds tested only three had a significant effect on cardiac frequency (see figure 3); NIC showed a peak increase between 0 and 2 h, while **1** and **3** diminished it significantly after 2 h. For NIC, the increase in cardiac frequency could be explained as a compensatory response to the drop in arterial pressure. However, for DHPs **1** and **3**, we assume that the effect on cardiac frequency is due to their direct effect on cardiac muscle.

Microsomal oxidation rate

The MOR results are shown in table III. Our proposal that introduction of two small electron-attracting substituents, *ie* 3',5'-F_2, would retard oxidation, was confirmed, since their MOR was consistently smaller than those of the corresponding 3'-NO_2 analogues. The singly substituted 4'-F derivatives (**3** and **4**) were the most susceptible to oxidation (except for **0**), while the MOR for the only 3'-Br,4'-F was intermediate to that of the 3',5'-F_2 and 3'-NO_2 analogues. This MOR order is consistent with their inductive effect. According to published values [23], the inductive components of σ (σ_I) for 3',5'-F_2, 3'-Br,4'-F, 3'-NO_2, 4'-F and H are 1.04, 0.96, 0.60, 0.52 and zero, respectively (although the σ_I values for 4'-F and 3'-Br,4'-F might be smaller than those indicated, this would not change their relative order due to the variation in the location of the F). These results indicate that the presence of electron-attracting substituents or atoms on the phenyl ring protects the DHP from oxidation. This would support the DHP oxidation mechanism proposed by Guengerich and Böcker [14, 15], since an electron-attracting substituent would destabilize the formation of a cationic radical [24], reducing the MOR.

Studies on the effect of substitution on the rates of ferricyanide-mediated chemical oxidation of model 3-substituted 1,4-dihydropyridines [25] showed that electron-withdrawing 3-substituents made them less susceptible to oxidation. In this work, the methyl derivative was less oxidizable than that with ethyl, in accord with our results.

Examination of the MOR results also shows that they could resolve to a large extent the discrepancies between the *in vitro* and *in vivo* effects. For example, the diethyl DHPs had a greater relaxant effect than the corresponding dimethyl analogues, but also had a greater MOR, implying faster metabolic deactivation, and thus, less *in vivo* activity. Similarly, the 3',5'-F_2 DHPs, which had a good relaxant effect, show a reduced MOR and therefore a stronger and longer *in vivo* activity. These considerations also apply to the 4'-F derivatives.

Determination of molecular descriptors

In table II have been gathered some molecular descriptors for use in QSAR. We chose to calculate their log *P* by the Leo-Hansch fragmental system [26] or to estimate their hydrophobicity from their thin-layer chromatographic index (R_M) [27].

The Leo-Hansch fragmental method was applied as described in the *Experimental protocols*. It might be noted that the log *P* calculated for **1** of 0.46 is close to the reported [28] value for nifedipine of 0.42.

In general the order of the hydrophobic substituent contribution derived from the R_M values for the aryl (3'-Br,4'-F > 3',5'-F_2 > 3'-NO_2 ≥ 4'-F) and ester substituents (2-phenoxyethyl > *N*-benzyl, *N*-methyl-2-aminoethyl > 2,2,2-trifluoroethyl > ethyl > methyl) is in agreement with the various fragmental-derived values [26, 29]. Only the 3'-NO_2 analogues show discrepancies. It has frequently been noted that the R_M values of chemically different families are affected not only by their hydrophobicity but also by electronic factors [30].

We compared the R_M and log *P* values (fig 4) and obtained the following correlations. When all points are included in a single regression, line A is obtained:

$$\log P = 5.22\, R_M + 3.91 \qquad [1]$$

$n = 14$, $r^2 = 0.93$, $s = 0.26$, $p < 0.0001$

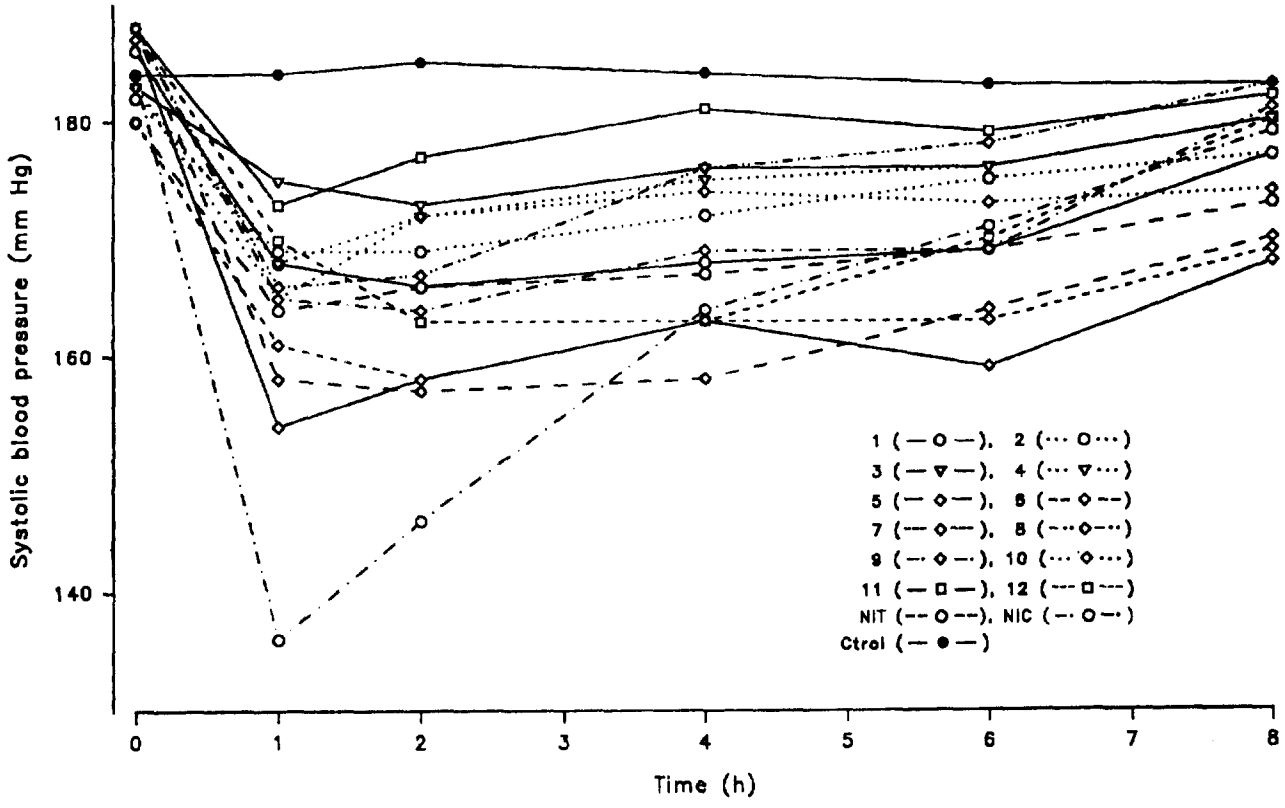


Fig 2. Effects of the DHPs on the systolic blood pressure in spontaneously hypertensive rats. Each point represents the mean for six rats.

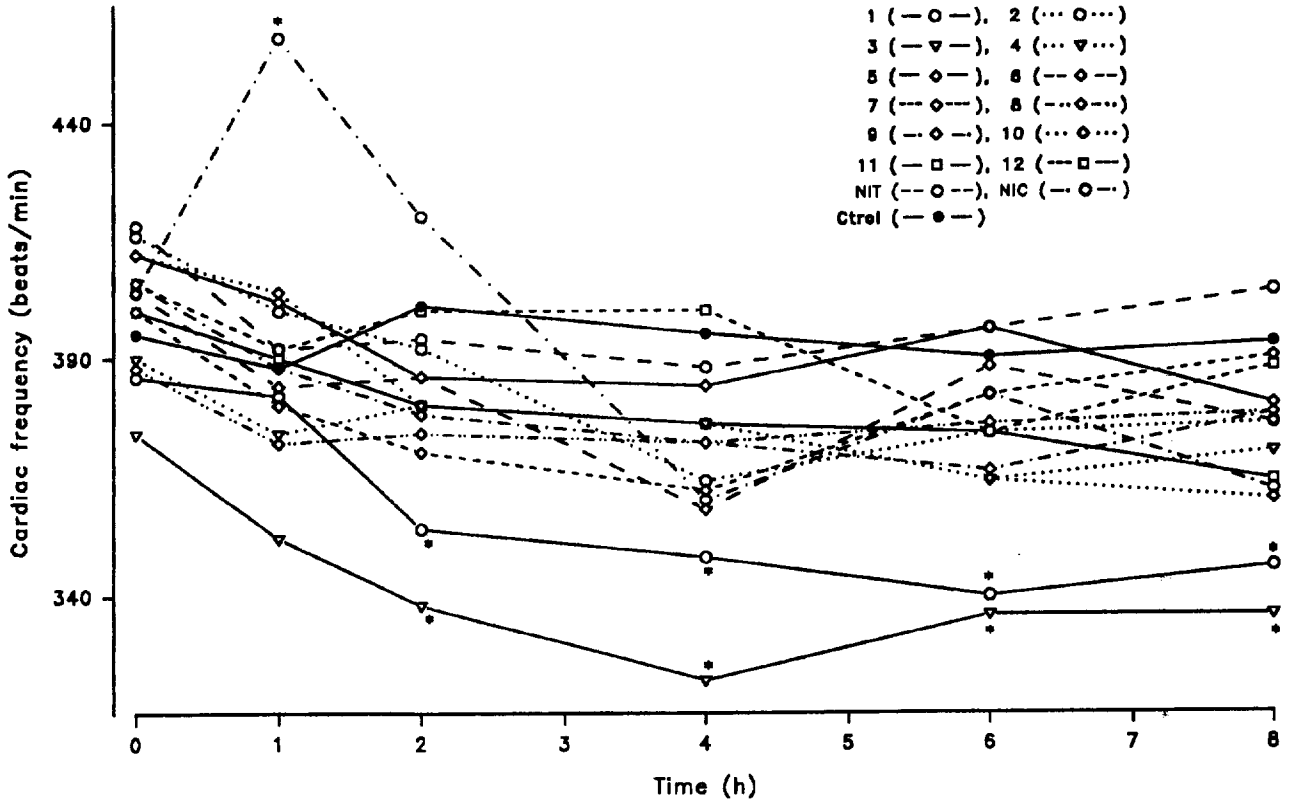


Fig 3. Effect of the DHPs on the cardiac frequency in spontaneously hypertensive rats. Each point represents the mean for six rats. * Difference statistically significant ($p < 0.05$) in comparison with t_0 (Dunnett's test) and the control (t-test: grouped data).

When they are split into logical subgroups, the non-3'-NO_2 series (line B) gives:

$$\log P = 4.68\, R_M + 3.76 \quad [2]$$

$n = 10$, $r^2 = 0.94$, s = 0.23, $p < 0.0001$

while the 3'-NO_2 derivatives (line C) clearly lie on a different line:

$$\log P = 7.08\, R_M + 4.65 \quad [3]$$

$n = 4$, $r^2 \sim 1.0$, $s = 0.08$, $p < 0.005$

The slopes of lines B and C differed significantly ($p < 0.05$). This shows that the DHPs with 3'-NO_2 and those with halogens (4'-F, 3'-Br,4'-F or 3',5'-F_2) interact differently with the chromatographic system used.

The size of the various ester substituents was estimated by their surface area (calculated with the PC-Model program). The normalized surface areas, relative to methyl = 1, are shown in table IV.

Structure–activity relationships

A Free–Wilson/Fujita–Ban (FW/FB) QSAR was made of the relaxant activity data. Since we have some chiral DHPs, whose ester positions were not fixed, we combined them into a fictitious substituent R(3,5) [31]. The substituent contributions obtained from this analysis are given in table V (we chose DHP **1** as the reference compound). We made an initial FW/FB analysis, leaving out DHP **8** (single-point determination for 2-phenoxyethyl in C(3,5)) [32]. The results of this analysis (coefficient contributions and statistical parameters not shown) are the same or similar to those obtained in the FW/FB analysis with all DHPs.

The FW/FB analysis explains more than 95% of the biological variance. It show that the variations in the aryl ring led to a 100-fold potency difference, whereas for changes in the ester groups it was only 10-fold. Moreover, the contribution order obtained from this analysis, for aromatic substituents, is the same as that found by Coburn *et al* [5].

A parabolic dependence was found between the (normalized) ester moiety surface area, A_s, and the respective FW/FB contribution (fig 5):

$$\text{FW/FB contr} = 0.769\ (\pm 0.063)\, A_s - 0.137\ (\pm 0.011)\, A_s^2 - 0.650\ (\pm 0.071) \quad [4]$$

$n = 5$, $r^2 = 0.99$, $s = 0.03$, $F = 78.4$, $p < 0.05$

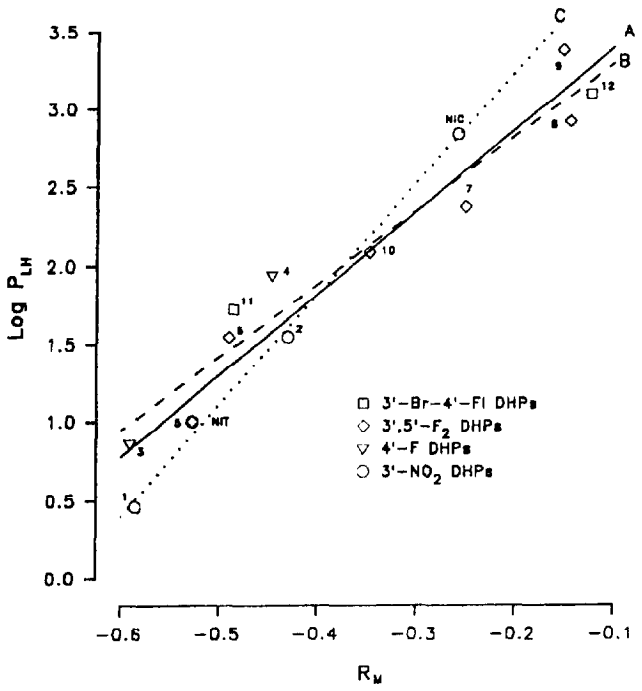


Fig 4. Plots of R_M *vs* calculated log P_{LH}. The regression lines drawn are: **A)** all 14 DHPs; **B)** DHPs with non-3'-NO_2; and **C)** 3'-NO_2 DHPs (for numbers see table I).

The dependence is clear and shows an optimum value of 2.81 (corresponding to 110 Å^2). The optimal surface area suggests that substituents such as *iso*-pentyl, *iso*-propoxyethyl or others of similar size might give potent DHPs. It is interesting to note that in a report by Dubur *et al* [33], the most potent DHPs in smooth muscle relaxation were some with 2-ethoxyethyl and 2-*n*-propoxyethyl substituents. In the above equation we used only the ester combinations with methyl groups.

Table IV. Surface area of the ester substituents.

Substituent	*Surface area*[a] *(A²)*	A_s[b]
CH_3	39	1.00
C_2H_5	60	1.54
CH_2CF_3	68	1.74
$CH_2CH_2OC_6H_5$	135	3.46
EtN(Me)Bz[c]	180	4.62

[a]Calculated with the PC-Model program; [b]normalized values of the surface area, CH_3 = 1; [c]*N*-benzyl-*N*-methyl-2-amino-ethyl.

Table V. FW/FB analysis of relaxant activity.

Fragment	*N*[a]	*Contribution (±SD)*
μ	---	8.27 (±0.14)[b]
R(C4)[c]:		
3-$NO_2C_6H_4$	4	0.00[d]
4-FC_6H_4	2	-1.70 (±0.18)*
3-Br-4-FC_6H_3	2	-1.18 (±0.20)*
3,5-$F_2C_6H_3$	5	-0.88 (±0.13)*
R(C3,C5)[e]:		
CH_3, CH_3	4	0.00[d]
CH_3, C_2H_5	2	0.21 (±0.18)
CH_3, CH_2CF_3	2	0.25 (±0.18)
CH_3, $CH_2CH_2OC_6H_5$	1	0.39 (±0.23)
CH_3, $CH_2CH_2N(CH_3)CH_2C_6H_5$	2	-0.03 (±0.18)
C_2H_5, C_2H_5	3	0.75 (±0.15)*

[a]Frequency of occurrence of the substituents in DHP set; [b]relaxant activity calculated for DHP **1** (reference compound); [c]substituent on C4; [d]by definition; [e]ester substituents at C3 and C5. *Value significantly different ($p < 0.05$) from zero (coefficient's significance in FW/FB does not have the same meaning as in the Hansch model, but depends on the choice of the reference substituents [32]). n = 14, r^2 = 0.97; s = 0.19; F = 18, $p < 0.005$.

We did not find any correlation (linear or parabolic) between relaxant activity and hydrophobic parameters (log P or R_M) for the entire DHP set. It was only possible for the 3',5'-F_2 DHP subset, where a parabolic relationship (r^2 = 0.88) was found with log P, but we believe that this is due to the high correlation (r^2 = 0.87) between log P and A_s.

In conclusion, the 3',5'-F_2 substitution is a good option for this class of compounds in order to obtain long-acting DHPs. The relative MOR of DHPs clarifies some discrepancies between their *in vitro* (aortic ring) potency and *in vivo* (antihypertensive potency in the intact SHR) effects.

Experimental protocols

Chemistry

All reagents and solvents were analytical or HPLC grade obtained from Aldrich (Milwaukee, WI, USA) or Baker

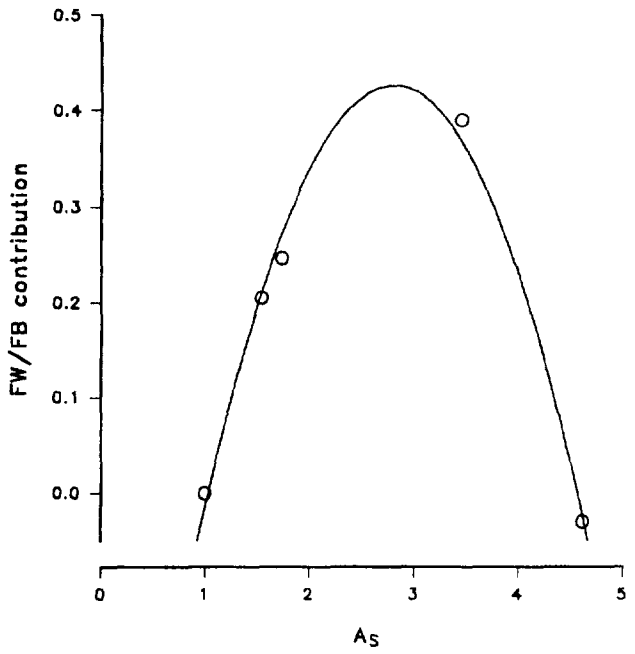


Fig 5. Parabolic dependence between the relative ester moiety surface area (A_s) and its FW/FB contribution.

(Xalostoc, Edo de Mexico, Mexico). Nitrendipine and nicardipine used as standards were obtained from Bayer (Wuppertal, FRG) and Sigma (Saint Louis, MO, USA). The purity of the DHPs was established by thin-layer chromatography with various eluents. Melting points were determined on a Kofler hot stage and are uncorrected. NMR spectra were obtained on Varian EM-390 or EM-360 instruments in deuterated chloroform or acetone. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN, USA). The quantitative elemental analyses for all new compounds (**4–12**) were within ±0.4% of the theoretical values except where noted.

The required acetoacetates (2,2,2-trifluoroethyl, 2-phenoxyethyl and *N*-benzyl-*N*-methyl-2-aminoethyl) were obtained by reacting the appropriate alcohol with diketene in tetrahydrofuran with heating for various times and partially purifying the product by passage through silica gel. The 3-nitrobenzylidine and 3,5-difluorobenzylidine ethyl acetoacetates were obtained by reacting the appropriate aldehyde and ethyl acetoacetate in toluene with gaseous HCl; recrystallization from isopropanol or hexane gave materials melting at 108–109 and 58–60°C, respectively. DHPs **0**, **1**, **2** and **3** were obtained according to synthetic procedures described in the literature [20, 34, 35].

2,6-Dimethyl-3,5-diethoxycarbonyl-4-(4-fluorophenyl)-1,4-dihydropyridine 4

A solution of 4-fluorobenzaldehyde (1.1 g, 9 mmol), ethyl acetoacetate (2.3 g, 18 mmol) and ammonium hydroxide (0.7 ml 40% aq, equivalent to 0.15 g, 9 mmol NH_3) in ethanol (2.5 ml) was heated under reflux with stirring during 10 h. Upon pouring over cold water a precipitate formed, which was filtered off, washed with water and recrystallized from methanol to yield *ca* 2 g (64%) of **4**, mp 148–149°C. Anal C, H, N, F. ^{1}H-NMR (acetone-d_6, 90 MHz): δ 1.2 (t, J = 7 Hz, 6H), 2.4 (s, 6H), 4.1 (q, J = 7 Hz, 4H), 5.1 (s, 1H), 6.8–7.5 (m, 4H), 8.1 (s, 1H).

2,6-Dimethyl-3,5-dimethoxycarbonyl-4-(3,5-difluorophenyl)-1,4-dihydropyridine 5

A solution of 3,5-difluorobenzaldehyde (0.9 g, 6.5 mmol), methyl acetoacetate (1.5 g, 13 mmol) and ammonium hydroxide (6.5 mmol NH_3) in methanol (2 ml) was heated under reflux 24 h with stirring and then refrigerated for 5 h. The product formed was filtered off, washed with cold methanol and recrystallized from methanol giving 1.2 g (55%) of **5**, mp 185–187°C. Anal C, H, N; F, found 10.80, calc 11.27. ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 2.4 (s, 6H), 3.7 (s, 6H), 5.1 (s, 1H), 6.1 (s, 1H), 6.5–7.0 (m, 3H).

D,L-2,6-Dimethyl-3-ethoxycarbonyl-4-(3,5-difluorophenyl)-5-methoxycarbonyl-1,4-dihydropyridine 6

A solution of methyl 3-aminocrotonate (0.46 g, 4 mmol) and 2-ethyl-2-(3,5-difluorobenzylidene)acetoacetate (1 g, 4 mmol) in ethanol (5 ml) was heated under reflux with stirring for 15 h. The solvent was removed under reduced pressure and the residue was recrystallized from ethanol/hexane to furnish 0.5 g (35%) of **6**, mp 151–153°C. Anal C, H, N, F. ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.2 (t, J = 7 Hz, 3H), 2.4 (s, 6H), 3.7 (s, 3H), 4.1 (q, J = 7 Hz, 2H), 5.1 (s, 1H), 6.0 (s, 1H), 6.5–7.0 (m, 3H).

D,L-2,6-Dimethyl-3-(2,2,2-trifluoroethoxycarbonyl)-4-(3,5-difluorophenyl)-5-methoxycarbonyl-1,4-dihydropyridine 7

A solution of 3,5-difluorobenzaldehyde (0.72 g, 5 mmol), methyl 3-aminocrotonate (0.58 g, 5 mmol) and 2,2,2-trifluoromethyl acetoacetate (0.92 g, 5 mmol) in isopropanol (2 ml) was heated under reflux with stirring for 20 h. The solid obtained was filtered off and recrystallized from methanol to furnish 0.8 g (46%) of **7**, mp 162–164°C. Anal C, H, N; F, found 22.67, calc 23.44. ^{1}H-NMR (acetone-d_6, 90 MHz): δ 2.4 (s, 6H), 3.7 (s, 3H), 4.3–4.8 (m, 2H), 5.1 (s, 1H), 6.6–7.0 (m, 3H), 8.3 (s, 1H).

D,L-2,6-Dimethyl-3-(2-phenoxyethoxycarbonyl)-4-(3,5-difluorophenyl)-5-methoxycarbonyl-1,4-dihydropyridine 8

A solution of 3,5-difluorobenzaldehyde (1 g, 8 mmol), 2-phenoxyethyl acetoacetate (1.8 g, 8 mmol) and piperidine (0.5 ml) in benzene (50 ml) was placed in a Dean–Stark apparatus and heated under reflux for 5 h. The solvent was removed under reduced pressure and the product passed through a 5 cm silica-gel column using ethyl acetate/hexane (1:1) as the eluent. After partitioning between H_2O/CH_2Cl_2, the organic phase was dried over $MgSO_4$ and evaporated to dryness. This product and methyl 3-aminocrotonate (0.6 g, 5 mmol) in isopropanol (10 ml) were heated under reflux with stirring for 24 h. The product was recrystallized from methanol yielding *ca* 1 g (29%) of **8**, mp 139–141°C. Anal C, H, N, F. ^{1}H-NMR (acetone-d_6, 90 MHz): δ 2.4 (s, 6H), 3.7 (s, 3H), 4.2 (t, J = 5 Hz, 2 H), 4.5 (t, J = 5 Hz, 2H), 5.2 (s, 1H), 6.6–7.6 (m, 2H), 8.2 (s, 1H).

D,L-2,6-Dimethyl-3-(N-benzyl-N-methyl-2-aminoethoxycarbonyl)-4-(3,5-difluorophenyl)-5-methoxycarbonyl-1,4-dihydropyridine 9

A solution of 3,5-difluorobenzaldehyde (1 g, 7 mmol), 2-(*N*-benzyl-*N*-methylamino)ethyl acetoacetate (2 ml) and piperidine (0.5 ml) in benzene (40 ml) were placed in a Dean–Stark apparatus and heated under reflux for 3 h. The solvent was removed and the product passed through a silica-gel column, eluting with ethyl acetate/hexane (1:1). A solution of the above product and methyl 3-aminocrotonate (0.7 g, 6 mmol) in isopropanol (10 ml) was heated under reflux with stirring for *ca* 17 h. The solvent was removed and the product was passed through a silica-gel column eluting with ethyl acetate/hexane (1:1). The product was crystallized in isopropyl ether to yield 1.2 g (37%)

of **9**, mp 92–94°C. Anal C, H, N, F. ^{1}H-NMR ($CDCl_3$, 60 MHz): δ 2.2 (s, 3H), 2.4 (s, 6H), 2.7 (t, J = 6 Hz, 2H), 3.55 (s, 2H), 3.7 (s, 3H), 4.3 (t, J = 6 Hz, 2H), 5.1 (s, 1H), 6.0 (s, 1H), 6.5–7.1 (m, 3H), 7.4 (s, 5H).

2,6-Dimethyl-3,5-diethoxycarbonyl-4-(3,5-difluorophenyl)-1,4-dihydropyridine ***10***

A solution of 3,5-difluorobenzaldehyde (1 g, 7 mmol), ethyl acetoacetate (1.8 g, 14 mmol) and ammonium hydroxide (7 mmol NH_3) in ethanol (3 ml) was heated under reflux with stirring for 10 h. The solid product was filtered off and recrystallized from methanol giving 1.3 g (51%) of **10**, mp 181–183°C. Anal C, H, N, F. ^{1}H-NMR (acetone-d_6, 90 MHz): δ 1.2 (t, J = 7 Hz, 6H), 2.4 (s, 6H), 4.1 (q, J = 7 Hz, 4 H), 5.1 (s, 1H), 6.6–7.1 (m, 3H), 8.1 (s, 1H).

2,6-Dimethyl-3,5-dimethoxycarbonyl-4-(3-bromo-4-fluorophenyl)-1,4-dihydropyridine ***11***

A solution of 3-bromo-4-fluorobenzaldehyde (1 g, 5 mmol), methyl acetoacetate (1.2 g, 10 mmol) and ammonium hydroxide (5 mmol NH_3) in methanol (2 ml) was heated under reflux with stirring for 9 h. After partitioning in H_2O/CH_2Cl_2, the organic phase was dried over $MgSO_4$ and the solvent removed. The residue was recrystallized from methanol yielding *ca* 1.1 g (55%) of **11**, mp 165–167°C. Anal C, H, N, F, Br. ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 2.4 (s, 6H), 3.7 (s, 6H), 5.0 (s, 1H), 5.9 (s, 1H), 7.0–7.5 (m, 3H).

D,L-2,6-Dimethyl-3-(2,2,2-trifluoroethoxy)carbonyl-4-(3-bromo-4-fluorophenyl)-5-methoxycarbonyl-1,4-dihydropyridine ***12***

A solution of 3-bromo-4-fluorobenzaldehyde (1 g, 5 mmol), methyl 3-aminocrotonate (0.6 g, 5 mmol) and 2,2,2-trifluoroethyl acetoacetate (0.9 g, 5 mmol) in isopropanol (2 ml) was heated under reflux with stirring for 15 h. After partitioning in H_2O/CH_2Cl_2, the organic phase was dried over $MgSO_4$ and the solvent removed. The product was recrystallized from methanol, yielding 1.0 g (42%) of **12**, mp 146–149°C. Anal C, H, N, Br; F, found 15.41, calc 16.30. ^{1}H-NMR ($CDCl_3$, 90 MHz): δ 2.3 (s, 6H), 3.6 (s, 3H), 4.2–4.7 (m, 2H), 5.0 (s, 1H), 5.9 (s, 1H), 6.9–7.5 (m, 3H).

Pharmacology

Rat aorta relaxation bioassay

Male Wistar rats (*ca* 300 g) were decapitated after ether anesthesia and the thoracic artery was extirpated and cleaned. Then 5-mm-wide rings were obtained and suspended by Nichrom hooks in 10 ml organ baths containing standard Krebs solution (pH 7.4, 37°C) with the following (mM) composition: NaCl 118, $NaHCO_3$ 25, glucose 11.6, KCl 4.7, $MgSO_4$ 1.17, KH_2PO_4 1.17, EDTA 0.026, $CaCl_2$ 2.5, and bubbled continuously with O_2/CO_2 (95:5). One of the hooks was affixed to the bottom of the bath and the other to a tension transducer (Grass FT03C) connected to a polygraph recorder (Grass 7D). The rings were stabilized for 30 min with an initial tension of 5 g, after which their integrity was tested by their contractile responses to noradrenaline (10^{-7} M final concentration) every 30 min, with intermediate washing, until maximum sensitivity was developed. After developing tension they were washed with a Krebs solution high in potassium (NaCl 42.7 mM, KCl 80 mM) but free of calcium. After a further 30 min, the rings were again washed. Immediately afterwards aqueous $CaCl_2$ was added to a final concentration of 2.5 mM, and after the rings had developed maximum tension, the DHP (dissolved in aqueous DMSO) was added in cumulative fashion. The pIC_{50} was determined from the corresponding dose–response curve [36].

Antihypertensive activity

Spontaneously hypertensive rats (24–32 weeks old, 250–300 g) were tested and only those which at t = 0 had an arterial blood pressure of 160–200 mm Hg were used. The DHPs, suspended in aqueous 5% methylcellulose, were administered orally to groups of 6 rats at a single dose of 10 mg/kg, and the systolic arterial blood pressure was measured at t = 0, 1, 2, 4, 6 and 8 h. A control group (n = 6) received vehicle only. The pressure was determined indirectly by registering the caudal artery with a sensor (IITC) and a sphygmomanometer (Narco Biosystems, INC), both connected to a polygraph. The measurements were made in a room with a controlled temperature of 28°C, while the rats were warmed with an IR bulb. The antihypertensive effect was quantified by the maximum observed reduction compared to base levels (Dunnett's test) and the time during which the reduction was significantly different from the control (t-test: grouped data). Cardiac frequency was registered simultaneously.

Microsomal oxidation rate

Hepatic microsomes from Wistar rats (220–270 g), obtained by the method of Kamath *et al* [37], were placed in tubes containing phosphate buffer (0.1 M, pH 7.85) together with an NADPH-generating system ($NADP^+$ 0.25 mM, glucose-6-phosphate 0.5 mM, glucose-6-phosphate dehydrogenase 0.17 IU/ml, final concentrations). The tubes were equilibrated 3 min at 37°C and the reaction was initiated by adding the DHP (final concentration 20 μM from the addition of a 4 mM ethanol solution). Controls were included without the NADPH-generating reagents. Upon addition of the DHP, the contents were mixed and two aliquots (500 μl) were added to vials containing 250 μl of 10% aqueous trichloroacetic acid. Further aliquots were taken at t = 10 min. Both aliquots were centrifuged at 15000 rpm, and the supernatant placed in a spectrophotometer (Beckman 26) for determination of the non-reacted DHP at 360 nm. The MOR was calculated from the difference in absorbance between t = 0 and t = 10 min samples, using the molar extinction coefficient (ε, table III), and adjusted for protein concentration, determined by the method of Lowry *et al* [38]. The MOR (table III) is expressed as nanomoles DHP consumed per minute and per milligram of protein.

Measurement of the chromatographic index, R_M

Silica-gel plates (Sigma T-7270 with a 254 nm fluorescence indicator, 20 × 5 cm) were placed in developing chambers maintained at 37 ± 1°C. The DHPs (1 μl of a 3 mg/ml methanol solution) were applied 1.5 cm from the bottom, and made to migrate with a mobile phase consisting of phosphate buffer (pH 7.4, 0.07 M) and acetone (3:2). When the solvent front had reached some 17 cm, the plates were removed, dried and examined under UV light at 254 nm. The chromatographic index was calculated from $R_M = \log [(1/R_F) - 1]$.

Calculation of log P values

The octanol/water partition coefficients were calculated by the fragmental method of Leo-Hansch [26], in which $\log P = \Sigma a_i f_i + \Sigma b_j F_j$, where a_i and f_i are the number and fragment values, and b_j and F_j the number and value of the correction factors. The particular procedure, for each DHP, was as follows: DHP **1**: $\log P_{NIT} - f_{CH_3} + f_H - F_b = 1.00 - 0.89 + 0.23 - (-0.12) = 0.46$; DHP **2**: $\log P_{NIT} - f_H + f_{CH_3} + F_b = 1.00 - 0.23 + 0.89 + (-0.12) = 1.54$; DHP **3**: $\log P_{DHP1} - f_{NO_2(ar)} + f_{F(ar)} = 0.46 - (-0.03) + 0.37 = 0.86$; DHP **4**: $\log P_{DHP2} - f_{NO_2(ar)} + f_{F(ar)} = 1.54 - (-0.03) + 0.37 = 1.94$; DHP **5**: $\log P_{DHP1} - f_{NO_2(ar)} - f_H + 2f_{F(ar)} = 0.46 - (-0.03) - 0.23 + 2(0.37) = 1.00$; DHP **6**: $\log P_{NIT} - f_{NO_2(ar)} - f_H + 2f_{F(ar)} = 1.00 - (-0.03) - 0.23 + 2(0.37) = 1.54$; DHP **7**:

$\log P_{DHP6} - 3f_H + 3f_{F(al)} + 3F_{mhG3} + F_{H/SP3F} = 1.54 - 3(0.23) + 3(-0.38) + 3(0.53) + 1.07 = 2.37$; DHP **8**: $\log P_{DHP6} - f_H + f_{O(ar)} + f_{C_6H_5} + F_{P_4(CO_2/O)} + 2F_b = 1.54 - 0.23 + (-0.61) + 1.90 + [-0.26(-1.49 - 0.61)] + 2(-0.12) = 2.91$; DHP **9**: $\log P_{NIC} - f_{NO_2(ar)} - f_H + 2f_{F(ar)} = 2.83 - (-0.03) - 0.23 + 2(0.37) = 3.37$; DHP **10**: $\log P_{DHP2} - f_{NO_2(ar)} - f_H + 2f_{F(ar)} = 1.54 - (-0.03) - 0.23 + 2(0.37) = 2.08$: DHP **11**: $\log P_{DHP3} - f_H + f_{Br(ar)} = 0.86 - 0.23 + 1.09 = 1.72$; DHP **12**: $\log P_{DHP7} - f_{F(ar)} + f_{Br(ar)} = 2.37 - 0.37 + 1.09 = 3.09$; NIT: $\log P = 1$ (value taken from Pang and Sperelakis [28]); NIC: $\log P_{NIT} - f_H + f_{N(bz)} + f_{CH_3} + f_{CH_2} + f_{C_6H_5} + 4F_b + F_{P_4(CO_2/N)} = 1.00 - 0.23 + (-1.76) + 0.89 + 0.66 + 1.90 + 4(-0.12) + [-0.26(-1.49 - 1.76)] = 2.83$.

Substituent steric descriptors

These were obtained with the PC-Model program (Serena Software) which furnishes information on the steric characteristics of a molecule such as the van-der-Waals volume and surface area, as well as its conformational energy, dipole moment, solvation energy and other parameters.

Free–Wilson/Fujita–Ban analysis

For the QSAR analysis we used the method of Free and Wilson [39] as modified by Fujita and Ban [40]. The FW/FB procedure is based on the equation:

$$\log (1/C)_i = \mu + \sum_{j=1}^{r} \sum_{k=1}^{m_j} b_{ijk} z_{jk}$$

where $\log (1/C)_i$ is the biological activity of the ith compound and μ is that calculated by regression for the reference compound, r is the number of substitution sites, m_j is the number of variable fragments or substituents at site j, z_{jk} is the contribution of fragment jk (substituent k at position j), and b_{ijk} is an indicator variable which takes the value of 1 in the presence and 0 in the absence of substituent jk in compound i. The computer program has been used previously [31] in this kind of analysis.

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References

1 Rampe D, Triggle DJ (1993) *Prog Drug Res* 40, 191–238
2 Baky SH (1985) *In: New Cardiovascular Drugs* (Scriabine A, Ed), Raven Press, New York, USA, 153–172
3 Salerno SM, Zugibe Jr FT (1994) *Postgrad Med* 95, 181–190
4 Scriabine A, Battye R, Hoffmeister F *et al* (1985) *In: New Cardiovascular Drugs* (Scriabine A, Ed), Raven Press, New York, USA, 197–218
5 Coburn RA, Wierzba M, Suto MJ, Solo AJ, Triggle AM, Triggle DJ (1988) *J Med Chem* 31, 2103–2107
6 Mahmoudian M, Richards WG (1986) *J Pharm Pharmacol* 38, 272–276
7 Rodenkirchen R, Bayer R, Steiner R, Bossert F, Meyer H, Möller E (1979) *Naunyn-Schmiedeberg's Arch Pharmacol* 310, 69–78
8 Triggle DJ, Langs DA, Janis RA (1989) *Med Res Rev* 9, 123–179
9 Janis RA, Triggle DJ (1983) *J Med Chem* 26, 775–785
10 Beresford AP, Macrea PV, Alker D, Kobylecki RJ (1989) *Arzneim Forsch/Drug Res* 39, 201–209
11 Guengerich FP, Peterson LA, Böcker RH (1988) *J Biol Chem* 263, 8176–8183
12 Ogawa N, Mizuno K, Fukushima K, Suwa T, Satoh T (1993) *Xenobiotica* 23, 747–759
13 Regårdh CG, Bäärnhielm C, Edgar B, Hoffmann KJ (1990) *Prog Drug Metab* 12, 41–86
14 Guengerich FP, Böcker RH (1988) *J Biol Chem* 263, 8168–8175
15 Guengerich FP, Macdonald TL (1990) *FASEB J* 4, 2453–2459
16 Bäärnhielm C, Westerlund C (1986) *Chem-Biol Interactions* 58, 277–288
17 Kurfurst A, Ludvik J, Rauch P, Marek M (1981) *Coll Czech Chem Commun* 46, 1141–1147
18 Bossert F, Meyer H, Wehinger E (1981) *Angew Chem Int Ed Engl* 20, 762–769
19 Iwanami M, Shibanuma T, Fujimoto M *et al* (1979) *Chem Pharm Bull* 27, 1426–1440
20 Meyer VH, Bossert F, Wehinger E, Stoepel K, Vater W (1981) *Arzneim Forsch/Drug Res* 31, 407–409
21 Goldmann S, Stoltefuss J (1991) *Angew Chem Int Ed Engl* 30, 1559–1578
22 Kojda G, Klaus W, Werner G, Fricke U (1991) *Naunyn-Schmiedeberg's Arch Pharmacol* 344, 488–494
23 Van de Waterbeemd H, Testa B (1987) *Adv Drug Res* 16, 85–225
24 Viehe HG, Janousek Z, Merenyi R (1985) *Acc Chem Res* 18, 148–154
25 Brewster ME, Simay A, Czako K, Winwood D, Farag H, Bodor N (1989) *J Org Chem* 54, 3721–3726
26 Hansch C, Leo A (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, USA, 18–43
27 Biagi GL, Barbaro AM, Guerra MC, Forti GC, Fracasso ME (1974) *J Med Chem* 17, 28–33
28 Pang DC, Sperelakis N (1984) *Biochem Pharmacol* 33, 821–826
29 Rekker RF (1977) *The Hydrophobic Constant. Its Derivation and Applications. A Means of Characterizing Membrane Systems*, Elsevier, Amsterdam, The Netherlands
30 Hulshoff A, Perrin J (1976) *J Chromatogr* 129, 263–276
31 Hernández-Gallegos Z, Lehmann-F PA (1990) *J Med Chem* 33, 2813–2817
32 Kubinyi H (1988) *Quant Struct-Act Relat* 7, 121–133
33 Dubur GJ, Veveris MM, Weinheimer G *et al* (1989) *Arzneim Forsch/Drug Res* 39, 1185–1189
34 Loev B, Goodman MM, Snader KM, Tedeschi R, Macko E (1974) *J Med Chem* 17, 956–965
35 Bolger GT, Gengo P, Klockowski R *et al* (1983) *J Pharmacol Exp Ther* 225, 291–309
36 Tallarida RJ, Murray RB (1981) *Manual of Pharmacologic Calculations With Computer Programs*, Springer-Verlag, New York, USA, 14–19
37 Kamath SA, Kummerow FA, Ananth-Narayan K (1971) *FEBS Lett* 17, 90–92
38 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) *J Biol Chem* 193, 265–275
39 Free Jr SM, Wilson JW (1964) *J Med Chem* 7, 395–399
40 Fujita T, Ban T (1971) *J Med Chem* 14, 148–152
41 Shibanuma T, Iwanami M, Okuda K, Takenaka T, Murakami M (1980) *Chem Pharm Bull* 28, 2809–2812