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# Synthesis and pharmacological evaluation of verapamil analogs with restricted molecular flexibility

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[2]*Dipartimento di Scienze Farmaceutiche, Università di Bologna, via Belmeloro 6, 40126 Bologna, Italy;*
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**Summary** — Several analogs of verapamil, which are characterized by a reduced molecular flexibility, have been synthesized. Their pharmacological activity has been evaluated on guinea-pig atria (negative chronotropic and inotropic activities) and guinea-pig aorta strips (vasorelaxing activity). Their ability to displace the calcium antagonist (–)-desmethoxyverapamil ((–)-[$^3$H]-D888) on kitten cardiac tissue has also been evaluated. The pharmacological results are in accord with the previously reported models for negative inotropic and chronotropic activities of verapamil-like compounds, but fail to give information about the conformation(s) that act on smooth muscle.

**verapamil / calcium antagonists / chronotropic activity / inotropic activity / reduced flexibility**

## Introduction

For many years we have been engaged in a research project whose aim is to identify the active conformation(s) of verapamil, a well-known calcium antagonist with many rotational degrees of freedom, which is therefore able to take on several active conformations [1–4].

We chose the frozen analog method, involving the design of rigid or semi-rigid analogs which may represent stable conformers of the parent molecule.

In the previous paper of this series [4], we elaborated 2 molecular models for the chronotropic and inotropic action of verapamil (on the basis of several previously synthesized cyclohexane analogs [2, 3]). So far we have not been able to describe a model for smooth-muscle calcium-channel interaction, since all our compounds have been practically devoid of any activity on smooth muscles such as aorta.

All our rigid or semi-rigid analogs are characterized by some degree of bending; a U-shaped molecule is actually required for the best fit to our model for chronotropic action. We reasoned that an extended conformation of verapamil might be responsible for activity on smooth-muscle channels, and we therefore designed compounds of general structure A (fig 1) as rigid analogs for testing our working hypothesis. The triple bond inserted into the 4-carbon chain of verapamil (lipophilic side) imposes a linear structure on this part of the molecule, with the carbon atoms on the same line.

Moreover, reduction of the triple bond to the corresponding *cis*-alkene (structure B in fig 1) would transform the linear structure into a bent shape, thus allowing further checking of our models for inotropic and chronotropic activity. We also explored the consequence of simultaneously introducing elements of rigidity into the 2-methylene chain (homoveratryl side) of the molecule and for this reason we designed and synthesized derivatives in which the homoveratryl moiety was substituted by tetrahydroisoquinoline, 2-aminotetralin and 2-aminoindan moieties (fig 1).

It should be emphasized that the flexibility of the verapamil molecule in these structures has been reduced avoiding, as far as possible, the introduction of additional atoms in order to eliminate the problems that additional bulk gives in the interpretation of biological results. At this stage of the research, although all compounds contain dissymmetric carbon atoms, we did not attempt to obtain chiral compounds, and all the tests were performed on the racemates or diastereomeric mixtures.

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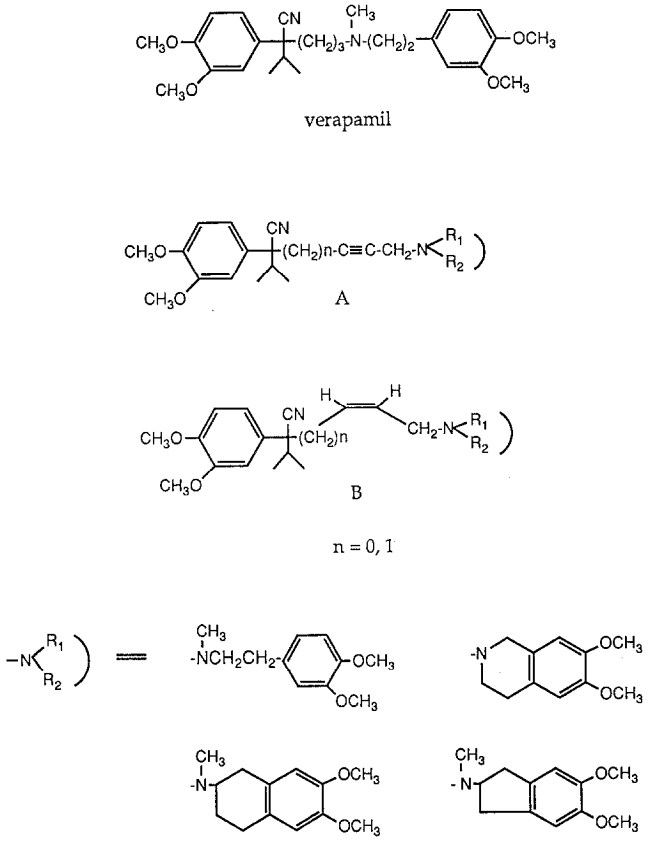



**Fig 1.** Structures of verapamil, A and B.

## Chemistry

The synthetic pathways used to synthesize the designed compounds are reported in schemes 1–3. The methods used are standard and only a few comments on the key steps of the synthesis will be necessary. For the synthesis of compounds **1–5** (scheme 1, table I), alkylation of 3,4-dimethoxybenzene acetonitrile and its α-isopropyl derivative was troublesome, in that the reaction can produce a large amount of dialkyl derivative (**15**), or proceed to the complete isomerization to the corresponding allene (**17**); the reaction conditions thus required some time to be optimized.

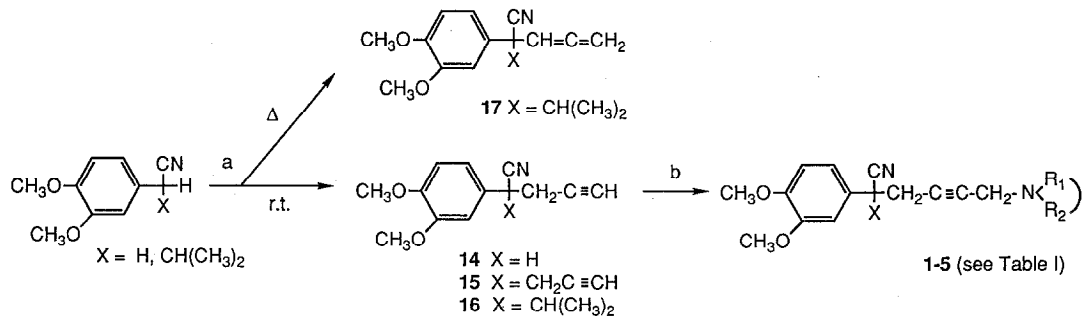
Once the propargyl derivatives **14** and **16** were obtained, the subsequent Mannich reaction went smoothly, but with low yields (40–70%) (scheme 1).

Direct formylation of the starting material with a variety of methods to obtain compounds **6–8** was unsuccessful, and so we had to go through the time-consuming pattern shown in scheme 2 (table II). In this sequence, the crucial step is that involving DIBAL (diisobutylaluminium hydride) reduction of **18**, which gave a very low conversion of the starting material (~16%).

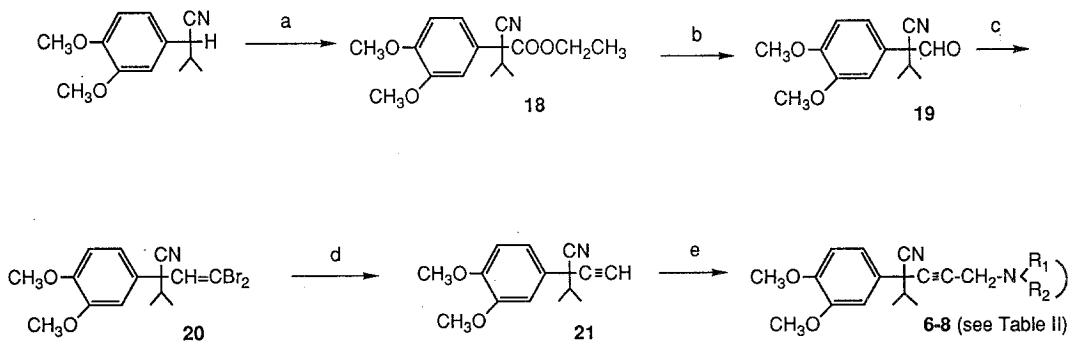
Finally, partial reduction of alkyne compounds (**2**, **5**, **6**, **8**) to the corresponding alkenes (scheme 3, table III) gave in all cases one product, which was identified as the expected *cis* isomer on the basis of $^1$H-NMR spectral data [5]. Complete reduction of **6** gave the higher verapamil homologue **13** which was necessary for comparative purposes.

## Pharmacological results and discussion

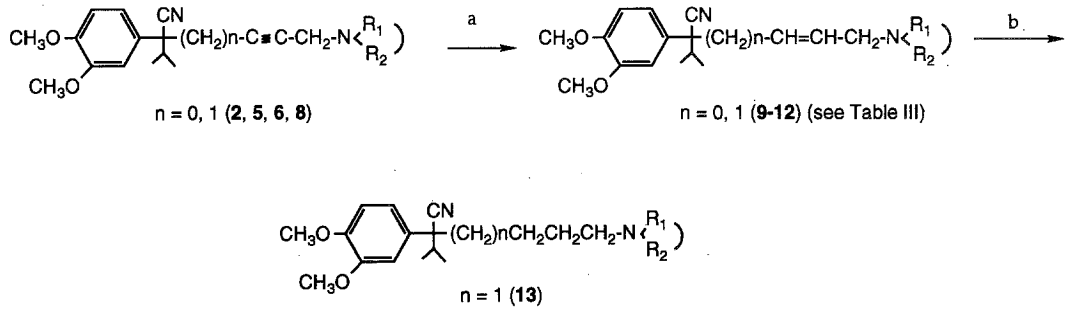
Negative inotropic and chronotropic activies were evaluated on guinea-pig atria and are reported in table IV. Vasorelaxing activity, which is a measure of calcium antagonistic action on smooth muscles, was evaluated on guinea-pig aorta strips and is reported in table V. Finally, binding to calcium channels was studied on kitten cardiac tissue as the ability to displace the calcium antagonist (–)-desmemoxyverapamil ((–)-[$^3$H]-D888) (table VI). In all cases verapamil was used as a reference drug.




**Scheme 1.** a: Br-$CH_2$-C≡CH/TEBAC; b: $CH_2O$, *N*-methyl-homoveratrylamine for **1** and **2**, tetrahydroisoquinoline for **3**, 2-methylaminotetralin for **4**, and 2-methylaminoindan for **5**.



**Scheme 2.** a: BuLi, ClCOOEt; b: DIBAL; c: $Ph_3P$, $CBr_4$; d: BuLi, $NH_4Cl$; e: $CH_2O$, *N*-methyl-homoveratrylamine for **6**, tetrahydroisoquinoline for **7**, and 2-methylaminoindan for **8**.



**Scheme 3.** a: $H_2$, $Pd/CaCO_3$; b: $H_2$, Pd/C.

The analysis of the data was quite difficult and the results defy any complete explanation. However, quite a clear trend can be identified starting from the binding data. For the sake of clarity, it will be useful to separate the discussion concerning the compounds in which the flexibility has been reduced on one side (**1**, **2**, **6**, **9**, **10**) from those in which it has been reduced on both sides (**3–5**, **7**, **8**, **11**, **12**).

*Compounds modified on one side **1**, **2**, **6**, **9**, **10***

A first observation that complies with the known SARs in the verapamil series [6] regards the importance of the isopropyl group. In fact compound **1** had less affinity for calcium channels than the isopropyl derivative **2** and at the same time had lower negative chronotropic and inotropic activity.

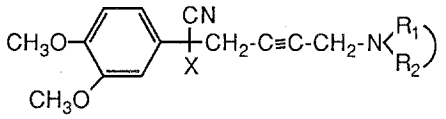
Binding data are quite informative as to the influence of the rigidity imposed on the molecule by triple and double bonds. The most rigid compound **6** had the lowest affinity ($pK_D$ = 5.29) followed by **2** ($pK_D$ = 5.75). Both had some 100–200-fold lower affinity than verapamil ($pK_D$ = 7.04) or the higher homologue **13** ($pK_D$ = 7.09).

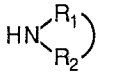
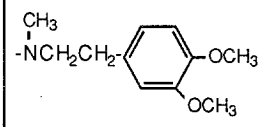
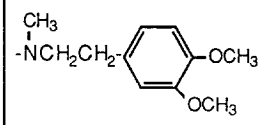
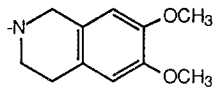
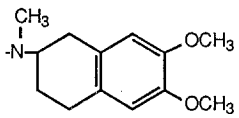
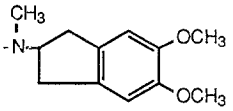
When these compounds were partially reduced to the corresponding *cis* alkenes **9** and **10** the binding affinity increased, to a lesser extent for **9** ($pK_D$ = 5.98), and to a much greater extent for **10**, which had an affinity ($pK_D$ = 6.89) that was practically identical to that of its fully flexible parent compound **13**.

These data clearly show that introduction of rigidity elements into the verapamil molecule is detrimental for calcium-channel affinity, at least as far as cat cardiac tissues are concerned. Nevertheless it is remarkable that when such rigidity results in an overall U-shaped molecule, the affinity of the flexible molecule is practically restored.

A good correlation between the degree of rigidity and the activity is only partly substantiated by functional data as far as negative inotropic and chronotropic activities are concerned. The data reported in table IV show that negative chronotropic activity increased nearly to the level of the reference compound (verapamil $ED_{30}$ = 0.07 μmol/l) when passing from the rigid linear alkyne derivative **2** ($ED_{30}$ = 0.28) to the U-shaped *cis* alkene counterpart **10** ($ED_{30}$ = 0.14). Likewise, **6** was devoid of any remarkable activity, while **9** was only 10 times less potent than verapamil ($ED_{30}$ = 0.88). The trend suggested by the binding data seems confirmed even if the differences in potency are much less impressive than those observed in binding.

**Table I.** Data for compounds **1** to **5**.



| N | X |  | salt (abs. EtOH) | Formula (salt) | M.p. °C | $^1$H NMR ($CDCl_3$) δ ppm[a] |
|---|---|---|---|---|---|---|
| 1 | H |  | oxalate | $C_{27}H_{32}N_2O_8$ | 142-144 | 2.30 (s, 3H, $NCH_3$); 2.55-2.69 (m, 4H, $2CH_2$); 2.71-2.90 (m, 2H, $CH_2$); 3.35 (s, 2H, $NCH_2$); 3.80-3.90 (m, 13H, $4OCH_3$ and CH); 6.70-6.90 (m, 6H, aromatics) |
| 2 | $CH(CH_3)_2$ |  | oxalate[b] | $C_{30}H_{38}N_2O_8$ | 50-51 | 0.85 (d, J=6.7Hz, 3H); 1.19 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.20 (s, 3H, $NCH_3$); 2.22-2.32 (m, 1H, $CH_3$-CH); 2.45-2.70 (m, 4H, $2CH_2$); 2.75-3.10 (m, 2H, $CH_2$); 3.30 (s, 2H, $NCH_2$); 3.80 (s, 3H, $OCH_3$), 3.84 (s, 3H, $OCH_3$), 3.95 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$); 6.70-7.05 (m, 6H, aromatics) |
| 3 | $CH(CH_3)_2$ |  | hydrochloride | $C_{28}H_{35}ClN_2O_4$ | 199-201 | 0.80 (d, J=6.7Hz, 3H); 1.15 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.15-2.28 (m, 1H, $CH_3$-CH); 2.40-2.52 (m, 2H, $CH_2$); 2.65-2.70 (m, 2H, $CH_2$); 2.88 (q, 2H, $CH_2$); 3.35 (d, 4H, $2CH_2$); 3.70 (s, 2H, $CH_2$); 3.70 (s, 3H, $OCH_3$); 3.77 (s, 6H, $2OCH_3$); 3.81 (s, H, $OCH_3$); 6.40 and 6.55 (s, 2H, aromatics) ; 6.70-6.95 (m, 3H, aromatics) |
| 4 | $CH(CH_3)_2$ |  | hydrochloride | $C_{30}H_{39}ClN_2O_4$ | 181-183 | 0.82 (d, J=6.7Hz, 3H); 1.15 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 1.40-1.58 (m, 2H, $CH_2$); 1.95-2.05 (m, 1, CH); 2.15-2.25 (m, 1H, $CH_3$-CH); 2.28 (d, 3H, $NCH_3$); 2.40-2.80 (m, 4H, $2CH_2$); 2.91 (q, 2H, $CH_2$); 3.41 (s, 2H, $NCH_2$); 3.78 (s, 3H, $OCH_3$); 3.84 (s, 6H, $2OCH_3$); 3.88 (s, 3H, $OCH_3$); 6.55 (s, 2H, aromatics); 6.65-6.95 (m, 3H, aromatics) |
| 5 | $CH(CH_3)_2$ |  | hydrochloride | $C_{29}H_{37}ClN_2O_4$ | | 0.85 (d, J=6.7Hz, 3H); 1.18 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.15-2.30 (m, 4H, $NCH_3$ and CH-$CH_3$); 2.60-3.10 (m, 7H, $3CH_2$ and CH); 3.38 (s, 2H, $NCH_2$); 3.81 (s, 3H, $OCH_3$); 3.83 (s, 3H, $OCH_3$); 3.85 (s, 3H, $OCH_3$); 3.88 (s, 3H, $OCH_3$); 6.75-6-99 (m, 5H, aromatics) |

[a]Of the free base; [b]the salt was not recrystallized.

These results are in complete accordance with the model for negative chronotropic activity that we have developed on the basis of other semi-rigid analogs of verapamil [4]; in particular the U-shaped compound **10** fits the model reasonably well as does the fully flexible **13** (fig 2).

As far as negative inotropic activity is concerned, our previously derived model [4] favors a more extended conformation of verapamil; as a consequence, **2** ($ED_{50}$ = 1.9) should possess higher potency than its bent *cis* alkene analog **10** ($ED_{50}$ = 3.5). However the differences found are not statistically significant, for

example, this trend was reversed in the case of **6** ($ED_{50}$ = 1.1), which was less effective than the *cis* alkene derivative **9** ($ED_{50}$ = 0.73).

No correlation was found for vasorelaxing activity, since none of the compounds showed any remarkable action on guinea-pig aorta. Once again [1–4], any element of rigidity introduced into the verapamil molecule resulted in a dramatic reduction of vasorelaxing activity. Therefore, the introduction of a functional group that 'linearizes' the verapamil molecule does not produce compounds with better activity on smooth-muscle calcium channels, as was expected from our working hypothesis. However, our model of chronotropic and inotropic action of verapamil-like molecules seem to be validated by the results obtained with these new compounds.

*Compounds modified on both sides* ***3–5, 7, 8, 11, 12***

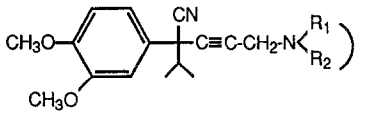
In a previous paper [4] we showed that limiting the flexibility of the homoveratryl sides of the verapamil molecule does not substantially alter the affinity for cardiac tissues. It had different effects on negative inotropic and chronotropic activity and was also detrimental for vasorelaxing activity on smooth muscles.

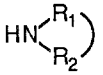
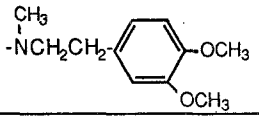
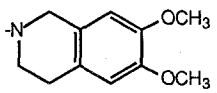
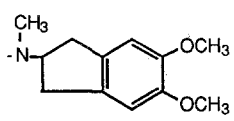
When the 2 modifications were combined, the results appeared be generally controlled by the change in flexibility of the lipophilic part of the molecule. In fact binding affinity, vasorelaxing and negative chronotropic activity were all lower, but comparable with those of the corresponding homoveratryl compounds.

Negative inotropic activity, on the other hand, was increased by further reducing the flexibility of the molecule. As a matter of fact, with only one exception (**4**), all the compounds (**3**, **5**, **7**, **8**, **11**, **12**) showed a higher activity than that of their homoveratryl counterpart (**2**, **6**, **9**, **10**), comparable with that of verapamil.

Finally, a very intriguing result was obtained in this study for compound **13**, the higher homologue of verapamil. In our experiments, this compound showed low negative inotropic activity, no action on smooth muscles but the same affinity for cardiac tissues as verapamil. It is in fact only 4-fold less potent than verapamil as a negative chronotropic agent and (see above) much more selective than the parent compound, appearing as a nearly pure negative chronotropic agent.

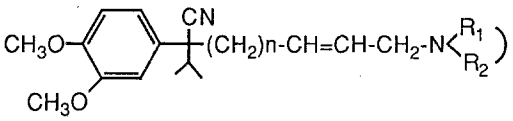
**Table II.** Data for compounds **6** to **8**.

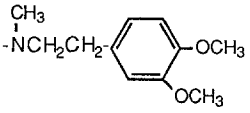
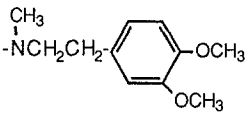
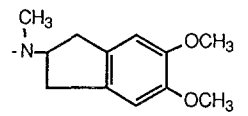
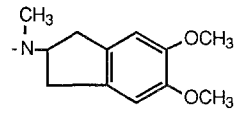



| N |  | Formula | $^1$H NMR ($CDCl_3$) δ ppm |
|---|---|---|---|
| 6 |  | $C_{27}H_{34}N_2O_4$ | 1.02 (d, J=6.7Hz, 3H); 1.12 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.15-2.30 (m, 1, $CH_3$-CH); 2.42 (s, 3H, $NCH_3$); 2.70 (s, 4H, $2CH_2$); 3.55 (s, 2H, $CH_2$); 3.84 (s, 9H, $3OCH_3$); 3.88 (s, 3H, $OCH_3$); 6.71-7.18 (m, 6H, aromatics) |
| 7 |  | $C_{27}H_{32}N_2O_4$ | 1.01 (d, J=6.7Hz, 3H); 1.08 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.15-2.30 (m, 1H, $CH_3$-CH); 2.85 (s, 4H, $2CH_2$); 3.63-3.75 (m, 4H, $2CH_2$); 3.81 (s, 3H, $OCH_3$); 3.84 (s, 6H, $2OCH_3$); 3.91 (s, 3H, $OCH_3$); 6.55 (d, 2H, aromatics); 6.71-7-18 (m, 3H, aromatics) |
| 8 |  | $C_{28}H_{34}N_2O_4$[a] | 1.02 (d, J=6.7 Hz, 3H); 1.09 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.15-2.30 (m,1H,$CH_3$-CH); 2.42 (s, 3H, $NCH_3$); 2.75-3.10 (m, 4H, $2CH_2$); 3.32-3.52 (m, 1H, CH); 3.87 (s, 3H, $OCH_3$); 3.90 (s, 6H, $2OCH_3$); 3.95 (s, 3H, $OCH_3$); 6.71-6.88 (m, 3H, aromatics); 7.05-7.15 (m, 2H, aromatics) |

[a]As oxalate; mp 139–141°C from ethyl acetate.

**Table III.** Data for compounds **9** to **12**.



| N | n | $HN(R_1)(R_2)$ | salt (abs. EtOH) | Formula (salt) | M.p. °C | $^1$H NMR ($CDCl_3$) δ ppm[a] |
|---|---|---|---|---|---|---|
| 9 | 0 |  | | $C_{27}H_{36}N_2O_4$[a] | | 0.83 (d, J=6.7Hz, 3H); 1.14 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.09 (s, 3H, $NCH_3$); 2.05-2.20 (m, 1, $CH_3$-CH); 2.38-2.65 (m, 4H, $2CH_2$); 2.75-2.88 (m, 1H, CH); 3.21-3.35 (m,1, CH); 3.85 (s, 6H, $2OCH_3$); 3.86 (s, 6H, $2OCH_3$); 5.78-5.85 (m, 2H, 2CH); 6.61-6.99 (m, 6H, aromatics) |
| 10 | 1 |  | oxalate | $C_{30}H_{40}N_2O_8$ | 173-174 | 0.85 (d, J=6.7Hz, 3H); 1.25 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.10-2.22 (m, 1H, $CH_3$-CH); 2.25 (s, 3H, $NCH_3$); 2.48-2.78 (m, 4H, $2CH_2$); 2.90-3.05 (m, 4H, $CH_2$ and $NCH_2$); 3.82 (s, 3H, $OCH_3$); 3.85 (s, 6H, $2OCH_3$); 3.92 (s, 3H, $OCH_3$); 5.25-5.40 (m,1H, CH); 5.48-5.65 (m,1H, CH); 6.70-6.95 (m, 6H, aromatics) |
| 11 | 0 |  | oxalate | $C_{30}H_{38}N_2O_8$ | 167-169 | 0.83 (d, J=6.7Hz, 3H); 1.17 (d,J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.04 (s, 3H, $NCH_3$); 2.05-2.20 (m, 1H, $CH_3$-CH); 2.60-2.95 (m, 5H, CH-CH= and $2CH_2$); 3.10-3.15 (m, 2H,CH-CH= and CH-N ); 3.83 (s, 6H, $2OCH_3$); 3.86 (s, 3H, $OCH_3$); 3.88 (s, 3H, $OCH_3$); 5.79-5.86 (m, 2H, CH=CH); 6.60-7.01 (m, 5H, aromatics) |
| 12 | 1 |  | hydrochloride | $C_{29}H_{39}ClN_2O_4$ | 220-222 | 0.82 (d, J=6.7Hz, 3H); 1.15 (d, J=6.7Hz, 3H) ($CH_3$-C-$CH_3$); 2.05-220 (m, 1H, $CH_3$-CH); 2.29 (s, 3H, $NCH_3$); 2.75-3.10 (m, 8H, $4CH_2$); 3.25-3.40 (M, 1H, CH); 3.81 (s, 6H, $2OCH_3$); 3.85 (s, 3H, $OCH_3$); 3.91 (s, 3H, $OCH_3$); 5.25-5.41 (m, 1H, CH); 5.54-5.69 (m, 1H, CH); 6.72 (s, 2H, aromatics); 6.79-6-95 (m, 3H, aromatics) |

[a]Of the free base.

## Experimental protocols

*Chemistry*

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin–Elmer 681 spectrophotometer in a Nujol mull for solids or neat for liquids. Mass spectra were measured on a Perkin–Elmer 8420 capillary gas chromatograph connected to a Perkin–Elmer Ion Trap detector. Unless otherwise stated, NMR spectra were measured on a Gemini 200 spectrometer. Chromatographic separations were performed on a silica-gel column by gravity chromatography (Kieselgel 40, 0.063–0.200 mm, Merck) or flash chromatography (Kieselgel 40, 0.040–0.063 mm, Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4 % of the theoretical values. The conformational analysis was calculated using Insight II and Discover (Biosym) running on a Silicon Graphic Personal Iris. For other experimental details see [4].

*2-(3,4-Dimethoxyphenyl)-4-pentynenitrile* ***14***

$NaNH_2$ (1 g, 22.5 mmol) was added to a solution of 3,4-dimethoxybenzeneacetonitrile (2 g, 11.2 mmol) in dry THF (30 ml). The mixture was stirred at room temperature for 0.5 h then a solution of propargylbromide (0.65 ml, 5.6 mmol) was added.

**Table IV.** Negative inotropic and chronotropic activities of compounds **1–13**.

| *Compound* | *Negative inotropic activity*[a] | *$ED_{50}$ of negative inotropic potency*[b] | *95% Confidence limit (x $10^{-6}$)* | *Negative chronotropic activity*[c] | *$ED_{30}$ of negative chronotropic potency*[b] | *95% Confidence limit (x $10^{-6}$)* |
|---|---|---|---|---|---|---|
| **1**[h] | 79 ± 2.8 | 49 | 35–72 | 72 ± 3.5[d] | 4.90 | 4.10–5.80 |
| **2**[h] | 63 ± 3.5 | 1.90 | 1.40–2.50 | 95 ± 4.2 | 0.28 | 0.23–0.35 |
| **3** | 67 ± 1.3[e] | 0.31 | 0.25–0.40 | 80 ± 2.9 | 0.64 | 0.50–0.80 |
| **4** | 37 ± 2.0[e] | – | – | 44 ± 1.3[d] | – | – |
| **5** | 58 ± 2.3[d] | 0.49 | 0.40–0.60 | 62 ± 3.4[e] | 1.10 | 0.80–1.50 |
| **6** | 71 ± 2.5 | 1.10 | 0.7–1.4 | 38 ± 1.8[d] | – | |
| **7** | 70 ± 3.0 | 0.93 | 0.85–1.03 | 75 ± 5.2[e] | 0.41 | 0.37–0.45 |
| **8**[h] | 82 ± 2.3 | 1.30 | 0.90–1.80 | 22 ± 1.8[g] | – | |
| **9** | 61 ± 3.5[e] | 0.73 | 0.68–0.81 | 73 ± 4.0[e] | 0.88 | 0.75–1.00 |
| **10**[h] | 90 ± 5.5 | 3.50 | 2.4–4.8 | 85 ± 5.7[f] | 0.14 | 0.11–0.17 |
| **11**[h] | 75 ± 3.0 | 0.65 | 0.58–0.75 | 90 ± 4.5 | 0.49 | 0.43–0.54 |
| **12** | 86 ± 4.4[e] | 0.63 | 0.58–0.70 | 100 | 0.40 | 0.37–0.44 |
| **13** | 88 ± 4.9 | 28 | 22–35 | 92 ± 4.7 | 0.24 | 0.18–0.32 |
| Verapamil | 84 ± 2.1[e] | 0.61 | 0.40–0.80 | 94 ± 3.4[f] | 0.07 | 0.05–0.10 |

[a]Decrease in developed tension in isolated guinea-pig left atrium at $10^{-4}$ M, expressed as percent changes from the control ± SEM ($n$ = 5–6). The left atria were driven at 1 Hz. The $10^{-4}$ M concentration gave the maximum effect for most compounds; [b]calculated from log conc/response curves (probit analysis according to Litchfield and Wilcoxon [13] with $n$ = 6–8). Expressed as μM; [c]decrease in atrial rate on guinea-pig spontaneously beating isolated right atrium at 5 x $10^{-6}$ M, expressed as percent changes from the control ± SEM ($n$ = 7–8). Pretreatment ranged from 170–185 beats/min. The 5 x $10^{-6}$ M concentration gave the maximum effect for most compounds; [d]at 5 x $10^{-5}$ M; [e]at $10^{-5}$ M; [f]at $10^{-6}$ M; at this concentration **10** and verapamil produced a complete standstill of spontaneously beating right atria (5 out of 7 experiments); [g]at $10^{-4}$ M; [h]this compound was tested as an oxalate. We checked that equimolecular amounts of oxalic acid had no effect on our *in vitro* models (Chiarini A, unpublished results).

**Table V.** Vasorelaxing activity of compounds **1–13**.

| *Compound* | *Vasorelaxation*[a] | *$IC_{50}$ of vasorelaxing potency*[b] | *95% Confidence limit (x $10^{-6}$)* |
|---|---|---|---|
| **1** | 44 ± 2.7 | | |
| **2** | 32 ± 2.2 | | |
| **3** | 52 ± 2.3 | 18 | 14–23 |
| **4** | 29 ± 1.6 | | |
| **5** | 40 ± 2.5 | | |
| **6** | 42 ± 2.5 | | |
| **7** | 30 ± 1.0 | | |
| **8** | 27 ± 1.8 | | |
| **9** | 31 ± 2.0 | | |
| **10** | 39 ± 2.5 | | |
| **11** | 48 ± 3.7 | | |
| **12** | 44 ± 2.0[c] | | |
| **13** | 32 ± 1.5 | | |
| Verapamil | 95 ± 1.7[d] | 0.38 | 0.20–0.70 |

[a]Percent inhibition of calcium-induced contraction on $K^+$-depolarized guinea-pig aortic strips at $10^{-4}$ M. Values are means ± SEM ($n$ = 6–7). The $10^{-4}$ M concentration gave the maximum effect for most compounds; [b]calculated from log conc/response curves (probit analysis according to Litchfield and Wilcoxon [13] with $n$ = 6–8). Expressed as μM; [c]at 5 x $10^{-5}$ M; [d]at $10^{-5}$ M.

**Table VI.** (–)-[$^3$H]-D888 competition on kitten heart ventricle membranes[a].

| *Compound* | *$pK_D$* | *Compound* | *$pK_D$* |
|---|---|---|---|
| **1** | 5.41 ± 0.07 | **8** | nt |
| **2** | 5.75 ± 0.12 | **9** | 5.98 ± 0.16 |
| **3** | 5.52 ± 0.11 | **10** | 6.89 ± 0.08 |
| **4** | 5.94 ± 0.10 | **11** | nt |
| **5** | 5.59 ± 0.10 | **12** | nt |
| **6** | 5.29 ± 0.10 | **13** | 7.09 ± 0.12 |
| **7** | 5.16 ± 0.07 | Verapamil | 7.04 ± 0.08 |

[a]The pharmacological profile of verapamil analogs was evaluated by employing 9 drug concentrations (each in quadruplicate) under standard assay conditions with 1.5–2.2 nM (–)-[$^3$H]-D888 and 17.9–26.5 mg/75ml of protein. Data from 2–3 independent experiments were computer-fitted separately to the general dose-response equation according to Ehle *et al* [11]. $pK_D$ values are given as means ± asymptotic SD estimated by the 'nlin' procedure (Gauss–Newton algorithm) from the SAS software package. For the calculation of $pK_D$-values (–log mol/l) a dissociation constant was used as reported by Goll *et al* [12]. Apparent Hill coefficients range from 0.91–1.07. nt = not tested.

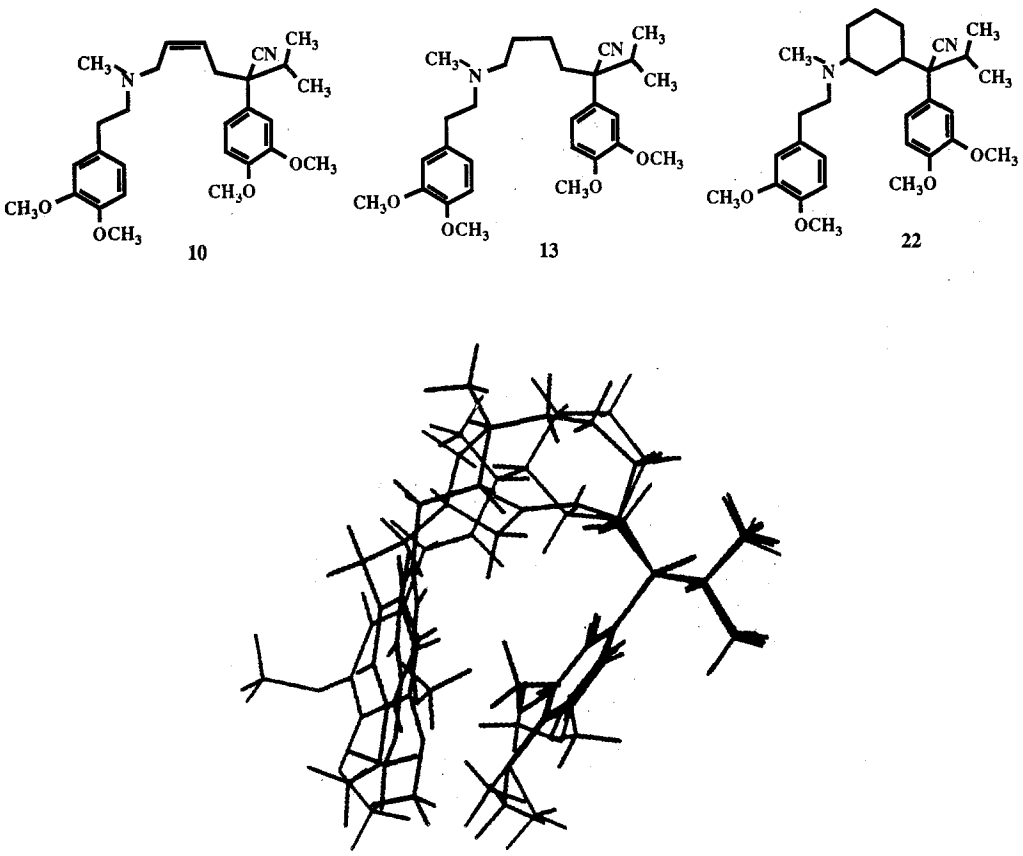


**Fig 2.** Overlap of **10**, **13** and **22** ((α*R**, 1*R**, 3*R**)-α[1-[3-[*N*-[1-[2-(3,4-dimethoxyphenyl)-ethyl]]-*N*-methylamino] cyclohexyl]]-α-isopropyl-3,4-dimethoxybenzene-acetonitrile [3]) in the putative active conformation to produce negative chronotropic activity. For each conformation, the quaternary carbon atom and its substituents have been superimposed; the aromatic rings of the phenylethylamino groups of the compounds show a good overlap. Compound **22** has been taken as a template for this model as reported in [4].

The mixture was heated to reflux for 12 h, poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography using a mixture of ethyl acetate/cyclohexane (50:50) as eluent. The first fraction, about 20% of the chromatographed mixture, was an oily compound identified as the bialkylated compound 2-(3,4-dimethoxyphenyl)-2-(3-(1-propinyl))-4-pentynenitrile **15**.

IR (neat): ν 3300 (C≡CH), 2240 (CN), 2120 (C≡C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.15–2.21 (m, 2H, 2CH); 2.90–3.10 (m, 4H, $2CH_2$); 3.85 (s, 3H, $OCH_3$); 3.90 (s, 3H, $OCH_3$); 6.80–7.10 (m, 3H, aromatics) ppm. Ms: m/e 253 ($M^+$). Anal ($C_{16}H_{15}NO_2$): C, H, N.

The second fraction, **14**, was 35% of the chromatographed mixture. IR (neat): ν 3300 (C≡CH), 2240 (CN), 2120 (C≡C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 2.12–2.22 (m, 1H, CH); 2.75 (d, $J$ = 6.7 Hz, 2H, $CH_2$); 3.85 (s, 3H, $OCH_3$); 3.90 (s, 3H, $OCH_3$); 3.92 (t, $J$ = 6.7 Hz, 1H, CH); 6.80–7.05 (m, 3H, aromatics) ppm. Ms: m/e 215 ($M^+$). Anal ($C_{13}H_{13}NO_2$): C, H, N.

The third fraction, about 45% of the chromatographed mixture, was the starting product.

*2-(3,4-Dimethoxyphenyl)-2-isopropyl-4-pentynenitrile* ***16***

A solution of propargyl bromide (2 ml of a 80% solution in toluene, 9.1 mmol), powdered KOH (3.6 g) and benzyltriethylammonium chloride (0,1 g) was added to a solution of α-isopropyl-3,4-dimethoxybenzeneacetonitrile [8] (2 g, 9.1 mmol) in 30 ml dry THF. The vigorously stirred mixture was left at room temperature for 3 h. The solid was filtered off and the solvent was removed. The residue was then dissolved in ethyl acetate, washed with water, dried and evaporated under vacuum to give 2.0 g (88% yield) of an oil which was suitably pure for the following reaction.

IR (neat): ν 3300 (C≡C-H), 2240 (CN), 2120 (C≡C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 0.85 (d, $J$ = 6.7 Hz, 3H); 1.20 (d, $J$ = 6.7 Hz, 3H) ($CH_3$-C-$CH_3$); 2.00–2.10 (m, 1H, C≡CH); 2.25–2.45 (m, 1H, $CH_3$-CH); 2.75–3.05 (m, 2H, $CH_2$); 3.88 (s, 3H, $OCH_3$); 3.92 (s, 3H, $OCH_3$); 6.80–7.10 (m, 3H, aromatics) ppm. Ms: m/e 257 ($M^+$). Anal ($C_{16}H_{19}NO_2$): C, H, N.

When the reaction mixture was refluxed for 48 h to complete the reaction there was complete conversion to the corresponding allene **17**, which was isolated in 51% yield.

IR (neat): ν 2240 (CN), 1970 (C=C=C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 0.95 (d, $J$ = 6.7 Hz, 3H); 1.10 (d, $J$ = 6.7 Hz, 3H) ($CH_3$-C-$CH_3$); 2.10–2.30 (m, 1H, $CH_3$-CH); 3.88 (s, 3H, $OCH_3$), 3.92 (s, 3H, $OCH_3$); 4.95–5.05 (m, 2H, $CH_2$); 5.35–5.45 (m, 1H, CH); 6.80–7.15 (m, 3H, aromatics) ppm. Ms: m/e 257 ($M^+$). Anal ($C_{16}H_{19}NO_2$): C, H, N.

*Ethyl-2-(3,4-dimethoxyphenyl)-2-cyano-3-methylbutanoate* ***18***
A solution of butyllithium (36 ml of a 1.6 M solution in hexane, 57.6 mmol) was added to a solution of α-isopropyl-3,4-dimethoxybenzeneacetonitrile [8] (9 g, 41.0 mmol) in anhydrous diethylether at –78°C under $N_2$. The mixture turned yellow, and was kept at –78°C for 3 h. Ethyl chloroformate (4 ml, 41.8 mmol) was added and the reaction left to reach room temperature overnight. A saturated aqueous $NH_4Cl$ solution was added to the crude reaction mixture, the layers were separated and the organic extracts washed with water and dried over $Na_2SO_4$. The solvent was removed under vacuum and the crude product was purified by flash chromatography, using cyclohexane/ethyl acetate (70:30) as the eluent. **18** (6 g, 50.2% yield) was obtained as an oil.

IR (neat): ν 2240 (CN), 1750 (CO) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 1.02 (d, 6H, $2CH_3$); 1.28 (t, 3H, $CH_3$); 1.98–2.18 (m, 1H, CH); 3.85 (s, 3H, $OCH_3$); 3.87 (s, 3H, $OCH_3$); 4.18 (q, 2H, $CH_2$); 6.73–6.86 (d, 3H, aromatics) ppm. Ms: m/e 291 ($M^+$). Anal ($C_{16}H_{21}NO_4$): C, H, N.

*2-(3,4-Dimethoxyphenyl)-2-formyl-3-methylbutanenitrile* ***19***
A solution of DIBAL (24 ml of a 1.0 M solution in toluene, 24 mmol) was added to a solution of **18** (6 g, 20.6 mmol) in 60 ml dry toluene at –96°C under $N_2$. After 45 min, 90 ml of a solution of $NaHSO_3$ (35 g in 70 ml $H_2O$ and 20 ml EtOH) was added and the reaction was left to reach room temperature. $CHCl_3$ was then added and the layers separated. The solvent was removed under vacuum and the crude product purified from starting material by flash chromatography using cyclohexane/ethyl acetate (70:30) as the eluent. **19** (0.9 g, 16% yield) was obtained as an oil.

IR (neat): ν 2240 (CN), 1730 (CHO) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 0.88 (d, $J$ = 6.7 Hz, 3H); 1.18 (d, $J$ = 6.7 Hz, 3H) ($CH_3$-C-$CH_3$); 2.60–2.81 (m, 1H, $CH_3$-CH); 3.85 (s, 6H, $2OCH_3$); 6.80–7.05 (m, 3H, aromatics); 9.43 (s, 1H, CHO) ppm. Ms: m/e 247 ($M^+$). Anal ($C_{14}H_{17}NO_3$): C, H, N.

*2-(3,4-Dimethoxyphenyl)-2-isopropyl-4,4-dibromo-3-butene-nitrile* ***20***
A solution of **19** (0.9 g, 3.64 mmol) in 5 ml dry $CH_2Cl_2$ was added to a solution of triphenylphosphine (1.9 g, 7.28 mmol) in 5 ml dry $CH_2Cl_2$ at –15°C under $N_2$. A solution of $CBr_4$ (1.36 g, 4.11 mmol) in 5 ml dry $CH_2Cl_2$ was then slowly added. The mixture was left at –15°C for 30 min and at room temperature for 30 min; during this period a yellow solid was formed. The solvent was evaporated under vacuum and the residue purified by flash chromatography using a mixture of cyclohexane/ethyl acetate (70:30) as the eluent. **20** (0.56 g, 38% yield) was obtained as an oil.

IR (neat): ν 2240 (CN) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 0.85 (d, $J$ = 6.7 Hz, 3H); 1.16 (d, $J$ = 6.7 Hz, 3H) ($CH_3$-C-$CH_3$); 2.12–2.30 (m, 1H, $CH_3$-CH); 3.88 (s, 6H, $2OCH_3$); 6.80–6.95 (m, 4H, CH and aromatics) ppm. Ms: m/e 403 ($M^+$). Anal ($C_{15}H_{17}Br_2NO_2$): C, H, N.

*2-(3,4-Dimethoxyphenyl)-2-isopropyl-3-butynenitrile* ***21***
A solution of butyllithium (1.7 ml of a 1.6 M solution in hexane, 2.6 mmol) was added to a solution of **20** (0.56 g, 1.3 mmol) in 40 ml of dry THF at –78°C under $N_2$. The mixture was kept at –78°C for 1 h. Saturated aqueous $NH_4Cl$ solution (20 ml) was then added and the mixture left at room temperature for 30 min. Diethylether was added and the organic layer separated. The solvent was dried over $Na_2SO_4$ and removed under vacuum; the crude product was purified by column chromatography using cyclohexane/ethyl acetate (60:40) as eluent. **21** (0.23 g, 78% yield) was obtained as an oil.

IR (neat): ν 3270 (C≡C-H), 2240 (CN), 2120 (C≡C) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 1.03 (d, $J$ = 6.7 Hz, 3H) ($CH_3$-C-$CH_3$); 1.11 (d, $J$ = 6.7 Hz, 3H); 2.18–2.35 (m, 1H, $CH_3$-CH); 2.64 (s, 1H, C≡CH); 3.89 (s, 3H, $OCH_3$); 3.90 (s, 3H, $OCH_3$); 6.84–7.28 (m, 3H, aromatics) ppm. Ms: m/e 243 ($M^+$). Anal ($C_{15}H_{17}NO_2$): C, H, N.

*General procedure for the Mannich reaction* ***1–8***
A solution of formaldehyde (0.25 ml, 40% solution in water), the appropriate secondary amine (3.0 mmol) and $CuSO_4$ (0.05 g) was added to a solution of the appropriate alkyne (2.3 mmol) in 4 ml EtOH/$H_2O$ (1:1). The pH of the solution was adjusted to 8 with 50% sulfuric acid. The mixture was heated to reflux for 24 h, then 15 ml $NH_4OH$ were added and the solution was extracted with diethylether. The organic layer was dried over $Na_2SO_4$ and evaporated under reduced pressure. If necessary the residue was purified by column chromatography using petrol ether/diethylether/dichloromethane/absolute ethanol/$NH_4OH$ (10:4:4:2:0.11) as the eluting system. The reaction products were generally obtained as thick oils that were transformed into the salts reported in tables I and II.

Of the amines used, *N*-methyl-homoveratrylamine and tetrahydroisoquinoline are commercially available, and 2-methylaminotetralin [9] and 2-methylaminoindan [10] were obtained according to the literature.

*2-(3,4-Dimethoxyphenyl)-2-isopropyl-6-[N-[1-[2-(3,4-dimethoxyphenyl)ethyl]]-N-methylamino]-4-hexenenitrile* ***10***
**2** (0.35 g, 0.75 mmol) was dissolved in 4 ml anhydrous methanol and was hydrogenated at room temperature and atmospheric pressure over 30 mg Lindlar's catalyst. Hydrogen absorption almost stopped after 1 equivalent was taken up. The catalyst was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography using chloroform/methanol (95:5) as the eluting system. **10** (0.15 g, 43% yield) was obtained as a thick oil. The compound was transformed into the salt (table III). The compounds **9**, **11** and **12** were obtained in the same way.

*2-(3,4-Dimethoxyphenyl)-2-isopropyl-6-[N-[1-[2-(3,4-dimethoxyphenyl)ethyl]]-N-methylamino]-n-hexanenitrile* ***13***
**2** (0.35 g, 0.75 mmol) in 5 ml absolute ethanol was hydrogenated over 75 mg 10% palladium on charcoal at room temperature under a hydrogen atmosphere for 5 h. The catalyst was filtered off and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography using chloroform/methanol (95:5) as the eluting system. **13** (0.18 g, 60%) was obtained as a thick oil.

IR (neat): ν 2240 (CN) $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ: 0.78 (d, $J$ = 6.7 Hz, 3H, $CH_3$-C-$CH_3$); 0.80–1.10 (m, 2H, $CH_2$); 1.18 (d, $J$ = 6.7 Hz, 3H, $CH_3$-C-$CH_3$); 1.22–1.55 (m, 3H, $CH_2$); 1.65–1.89 (m, 1H, CH); 1.98; 2.14 (m, 2H, $CH_2$); 2.10 (s, 3H, $NCH_3$); 2.15–2.34 (m, 2H, $CH_2$); 2.44–2.74 (m, 4H, $2CH_2$); 3.82 (s, 3H, $OCH_3$); 3.84 (s, 3H, $OCH_3$); 3.87 (s, 3H, $OCH_3$); 3.90 (s, 3H, $OCH_3$); 6.65–6.95 (m, 6H, aromatics) ppm.

The oxalate recrystallized from absolute ethanol/anhydrous diethylether and melted at 88–91°C. Anal ($C_{30}H_{42}N_2O_8$): C, H, N.

*Pharmacology*

*In vitro assays*
Inotropic and chronotropic activities were tested on guinea-pig isolated atrial preparations and vasorelaxing activity was tested on guinea-pig aortic strip preparations following standard procedures, details of which have been reported previously [7].

*Binding assay*
(–)-[$^3$H]-D888 ((–)-desmethoxyverapamil) with a specific activity of 74 Ci/mmol was obtained from Amersham Radiochemical Center, UK. Details on the protocol used have been reported elsewhere [3].

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