Synthesis of Compounds with a Possible Ca-Antagonist and β-Blocking Activity.

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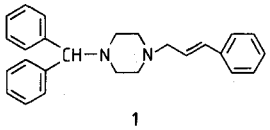
A series of new compounds structurally related to propranolol and cinnarizine were synthesized and evaluated for their possible activity as Ca-antagonist on the rabbit mesenteric artery. – In addition, the affinity of the compounds for β_1-adrenoceptors was estimated by receptor binding experiments in rat atrium membrane preparations. No Ca-antagonist activity was detected by the assay conducted on the test drugs, whereas some of them (**14** and **16**) showed affinity for atrial β_1-adrenoceptors.

Synthese von Verbindungen mit potentiell Ca-antagonistischen und β-Blocker-Eigenschaften

Einige neue Verbindungen, strukturverwandt mit Propranolol und Cinnarizin, wurden synthetisiert und auf Ca-antagonistische Wirkung an der mesenterialen Arterie des Kaninchens geprüft. Zusätzlich wurde die Affinität der Verbindungen gegenüber β_1-Adrenoceptoren durch Rezeptorbindungsexperimente mit Vorhofmembran-Präparationen von Rattenherzen untersucht. Es wurden keine Ca-antagonistischen Eigenschaften gefunden, die Verbindungen **14** und **16** zeigten jedoch Affinität gegenüber β_1-Adrenozeptoren des Atriums.

There is a growing interest in the correlations between calcium-antagonism and beta-antagonism. Calcium channel blockers are the most widely used drugs at the present time in the treatment of the variety of forms of myocardial ischemia[1]. – β-adrenergic blockers act only by reducing oxygen demand. For this reason the combination of β-adrenergic blockers and calcium-antagonists in helpful in the majority of patients with severe angina[2]. Moreover the combination Ca-antagonists and beta-blockers has been proven to be useful for antihypertensive treatment[3].

The objective of our work has been the synthesis of new compounds related to Cinnarizine **(1)**[4], a Ca-antagonist and to Propranolol **(2)**.



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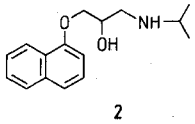

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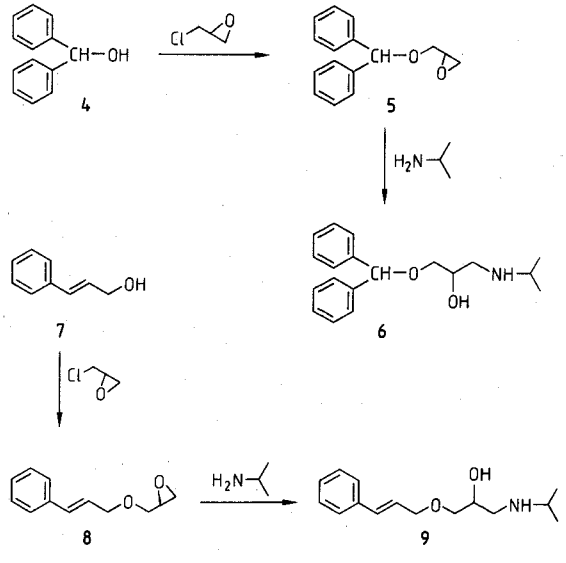
In this paper we report the synthesis and the biological activity of the new compounds **6** and **9** where one fragment of Cinnarizine was grafted with the group significant for β-blockers and of **12, 14** and **16**, in which the isopropylamino group of the propranolol was substituted by the fragments of the Cinnarizine. By this way we hoped to obtain new compounds with a β-blocking and Ca-antagonist activity that could be applied as antihypertensive drug.

The synthesis of compounds **6** and **9** was accomplished by standard procedures (Scheme 1).

Alkylation of benzhydrol **(4)** with epichlorohydrin afforded the epoxide **5**, which was treated with isopropylamine to give compound **6**. With the same procedure, starting from cinnamyl alcohol **(7)**, compound **9** was synthesized.

Scheme 1



Subsequently, we synthesized compounds **12, 14** and **16** where the isopropylamino group of the propranolol has been substituted by fragments of cinnarizine.

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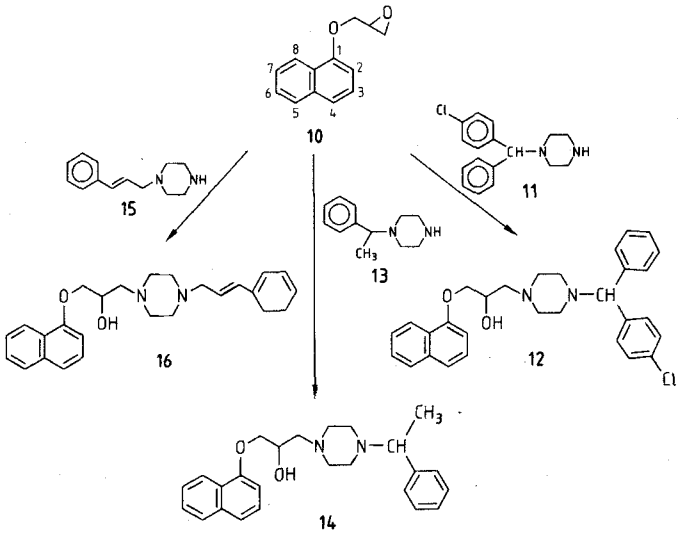
Alkylation of α-naphthol with epichlorohydrin afforded the epoxide **10**. The reactions of **10** with the p-chloro-benzhydrylpiperazine **11**, with N-(α-phenylethyl)-piperazine **(13)** and with 1-(3-phenyl-2-propenyl)-piperazine **(15)** gave **12**, **14**, and **16** respectively.



Pharmacology

Ca-antagonist activity on rabbit mesenteric artery

The Ca-antagonist activity was evaluated according to *Schümann* et al.[5], modified by using KCl 80 mM instead of KCl 40 mM. The results are reported in table 1.

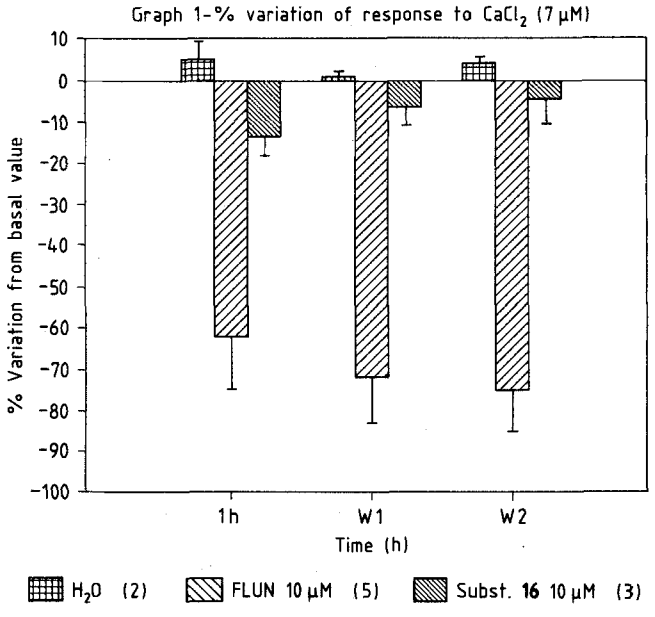
None of the substances under investigation at the two concentrations used ($1 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$ M) antagonizes $CaCl_2$-induced contractions to an extent similar to that of diltiazem.

Time course assessment of Ca-antagonist activity on rabbit mesenteric artery

In this experiment, **16** was studied for the time course of Ca-antagonist activity exerted by concentration of 10 μmol/l. The first Ca solution was introduced into the bath after 1 h contact time of the substance. The preparation was then washed and two additional solutions were added at 1 h intervals. Similar experiments were performed with flunarizine as a reference.

Tab. 2

	n of preparations	% variation of Ca dependent response compared to base: after 1 h	after 1st washing	after 2nd washing
		control		
m ± ES	2	5.00 ± 5.00	1.00 ± 1.00	4.00 ± 1.00
		substance **16** (10 μmol/l)		
m ± ES	3	5.00 ± 5.00	−6.33 ± 4.37	−4.33 ± 5.81
		flunarizin (10 μmol/l)		
	1	−70.00	−76.00	−77.00



1h = effect at 1 hour contact time
W1 = effect following 1st washing
W2 = effect following 2nd washing

The results are summarized in graph 1. While 10 μmol/l flunarizine could significantly antagonize Ca^{++} after 1h, the antagonism being increasingly greater with subsequent Ca^{++} additions following removal of the substance, **16** showed no effect either after 1 h contact time or subsequent Ca^{++} additions following their removal from the bath.

Tab. 1

Compound	n. of preparations	$CaCl_2$ 7 mmol/l basal (mg) (m ± S.E.)	% Variation (m ± S.E.) of Ca^{++} dependent response as caused by the following substance concentrations: 1 μM	10 μM
6	2	1236 ± 246	−5 ± 3	−4 ± 0.5
9	2	902 ± 265	+4 ± 1.5	+5 ± 5
12	3	1048 ± 148	−0.6 ± 0.6	−2 ± 2
14	2	865 ± 4.5	−18 ± 1	−25 ± 2
16	3	1078 ± 295	−5 ± 2	−28 ± 6
Diltiazem	2	1110 ± 202	−54 ± 6.5	−84 ± 5.3
H_2O	4	1175 ± 254	+2 ± 1	

Assessment of the affinity for β_1-adrenoceptors

The affinity of test drugs for β_1-adrenergic receptors was estimated by measuring their ability to inhibit ^{3}H-dihydroalprenolol (^{3}H-DHA) specific binding in rat atrium membrane preparations. Binding experiments were performed as described by *Baker* and *Potter*[6] using ^{3}H-DHA of a specific activity of 84 Ci/mmole (Amersham). The concentration able to induce a 50 % inhibition of 1nM ^{3}H-DHA specific binding (IC_{50}, nmol/l) was calculated from displacement curves obtained by using 10 increasing concentrations of each compound. The affinity was expressed as inhibitory constant value (Ki, nmol/l) calculated from the relationship:

$$Ki = \frac{IC_{50}}{1 + \frac{[^3H\ DHA]}{Kd}}$$

where [^{3}H-DHA] is the concentration of ^{3}H-DHA and Kd is the dissociation constant of the labelled ligand obtained by *Scatchard* analysis[7]. Atenolol, propranolol and practolol were used as reference drugs. The results obtained are shown in table 3.

Tab. 3

Compound	3H-DHA Binding Ki, nM (m ± S.E.)
6	3579 ± 688
9	11404 ± 180
12	2311 ± 749
14	1001 ± 87
16	231 ± 23
(±) Atenolol	745 ± 102
(±) Propranolol	3.5
(±) Practolol	1950 ± 520

This evaluation of new derivatives structurally related to propranolol and cinnarizine showed that none of the tested compounds is endowed with an appreciable Ca-antagonist activity. Nevertheless, some of them showed a good affinity to β_1-adrenoceptors.

Among the assayed drugs, compound **16** showed the highest affinity towards β_1-adrenoceptors, being about three times as potent as atenolol and eight times as potent as practolol.

Compounds **12** and **14** showed displacing activities very close to that of practolol and slightly higher than that of compound **6**. Finally, negligible displacement activity was shown by compound **9**.

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Experimental Part

MP: Reichert microhotstage, uncorr.-IR-spectra: Beckman Acculab 5 (CCl_4). – ^{1}H-NMR-spectra: Varian EM 390, 90 MHz $CDCl_3$; TMS int. stand.-MS: Varian Matt 311, all compounds show parent peaks corresponding to theoretical values. – Purity was checked by TLC.

3-Diphenylmethoxy-1,2-epoxypropane **(5)**

To a solution of $NaOCH_3$, preparated from 0.46 g (20 mmole) of Na in anhydrous MeOH(60 ml), 3.6 g (20 mmole) of benzhydrol **(4)** were added. The mixture was refluxed for 1 h. Evaporation in vacuo gave the sodium salt to which 35 ml of anhydrous DMF was added, followed by the addition of 1.6 g (20 mmole) of epichlorohydrin. – The solution was stirred at room temp. for 5 h during which a precipitate of NaCl was formed. The mixture was poured into 300 ml of water and extracted 3 × with 100 ml of $CHCl_3$. – The $CHCl_3$ phase was washed twice with water, dried on Na_2SO_4 and evaporated in vacuo. The crude epoxide residue was purified by chromatography on silica gel with hexane/benzene 1:1; yield 50 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.6–2.8 (m, 2H; CH_2O-epoxide), 3.2–3.3 (m, 1H; CH_2-C<u>H</u>-CH_2), 3.5–3.6 (m, 2H; CH-O-C<u>H</u>$_2$), 5.6 (s, 1H; C<u>H</u>-C_6H_5), 7.0–7.5 (m, 10 H-aromat.).

1-Diphenylmethoxy-3-isopropylamino-propan-2-ol **(6)**

A mixture of **5**, 1.42 g (6.2 mmole) and 1.4 g (24 mmole) of isopropylamine was heated at 100 °C for 4 h. The reaction mixture evaporated in vacuo gave an oily residue which was purified on alumina Brockmann II with Et_2O-CH_2Cl_2. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.0 (d, 6H; J = 6 Hz, 2 CH_3), 1.9 (s, 1H; NH), 2.5 (s, 1H; OH), 2.6–2.8 (m, 3H; -C<u>H</u>$_2$-N, C<u>H</u>$(CH_3)_2$), 3.4 (d, 2H; J = 6 Hz, O-C<u>H</u>$_2$CH), 3.6–3.9 (m, 1H; C<u>H</u>-OH), 5.2 (s, 1H; C<u>H</u>$(C_6H_5)_2$), 7.0–7.4 (s, 10 H aromat.). – $C_{19}H_{25}NO_2$ (299) Calc. C 76.2 H 8.3 N 4.5 Found C 76.6 H 8.5 N 4.9. The corresponding hydrochloride shows m.p. 165–170 °C.

3-(3-Phenyl-2-propen-1-oxy)-1,2-epoxypropane **(8)**

To a solution of $NaOCH_3$, prepared from 0.46 g (20 mmole) of Na in anhydrous MeOH (100 ml), 2.7 g (20 mmole) of cinnamyl alcohol **(7)** were added. The mixture was refluxed for 1 h. Evaporation in vacuo gave the sodium salt, to which 60 ml of anhydrous DMF was added, followed by 1.6 g (20 mmole) of epichlorohydrin. The mixture was stirred at room temp. for 12 h (precipitation of NaCl). The mixture was poured into 300 ml of water and extracted 3 × with 100 ml of $CHCl_3$. Removal of solvent after drying gave on oil that was purified on silica gel with hexane and hexane/ether 2:1; yield 50 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.5–2.7 (m, 2H; CH_2O-epoxide), 3.1–3.3 (m, 1H; CH_2-C<u>H</u>-CH_2), 3.5–3.7 (m, 2H; CH_2OC<u>H</u>$_2$), 4.2 (d, 2H; J = 7 Hz, C<u>H</u>$_2$CH = CH), 6.1–6.6 (m, 2H; C<u>H</u> = C<u>H</u>CH_2), 7.1–7.4 (m, 5H-aromat.).

1-Isopropylamino-3-(3-phenyl-2-propen-1-oxy)-propan-2-ol **(9)**

A mixture of epoxide **8**, 0.95 g (4.9 mmole) and 1.4 g (24 mmole) of isopropylamine was heated at 100 °C for 4 h. The reaction mixture, evaporated in vacuo, gave an oily residue which was purified on alumina Brockmann II with CH_2Cl_2/MeOH 9:1; yield 40 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.1 (d, 6H; J = 6 Hz, 2 CH_3), 2.5–2.9 (m, 3H; N-C<u>H</u>$_2$-CH, C<u>H</u>$(CH_3)_2$), 3.2 (s, 1H; OH), 3.5 (d, 2H; J = 6 Hz, O-C<u>H</u>$_2$-CH), 3.7–3.9 (m, 1H; C<u>H</u>-OH), 4.1 (d, 2H; J = 7 Hz, C<u>H</u>$_2$-CH = CH), 6.0–6.7 (m, 2H; C<u>H</u> = C<u>H</u>-CH_2), 7.1–7.4 (m, 5H-aromat.). – $C_{15}H_{23}NO_2$ (249) Calc. C 72.2 H 9.2 N 5.6 Found C 72.0 H 9.0 N 5.0.
The corresponding oxalate shows m.p. 95–100 °C.

1-(p-Chlorobenzhydryl)-4-[2-hydroxy-3-(1-naphthoxy)-propyl-1]-piperazine **(12)**

The epoxide **10**[8], 3 g (13 mmole) was added to a solution of N-(p-chlorobenzhydryl)-piperazine **(11)**, 4 g (14.5 mmole) in 50 ml of anhydrous EtOH. The mixture was refluxed for 2 h. The cold reaction mixture was evaporated under reduced pressure. The crude residue was crystallized from MeOH: m.p. 72–75 °C.
– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.2–2.7 (m, 8H; H-piperazine ring), 3.8–4.0 (m, 4H; O-C<u>H</u>$_2$-CH-C<u>H</u>$_2$-N), 4.1–4.3 (m, 1H; CH_2-C<u>H</u>-CH_2), 4.5 (s, 1H; OH), 5.0 (s, 1H; C<u>H</u>$(C_6H_5)_2$), 6.4–6.6 (m, 1H; H-4 naphthalene), 6.9–7.4 (m, 13 H; H-5, 6, 7, 8 naphthalene, H-benzhydryl), 7.4–7.5

(m, 1H; H-3 naphthalene), 8.0–8.1 (m, 1H; H-2 naphthalene). – $C_{30}H_{31}ClN_2O_2$ (487) Calc. C 74.0 H 6.36 N 5.7 Found C 74.02 H 6.45 N 5.69.
The corresponding hydrochloride shows m.p. 144–145 °C.

1-(α-Phenylethyl)-piperazine **(13)**

A mixture of 4.8 g (0.03 mole) of α-phenylethyl bromide and 3.4 g (0.03 mole) of 1-formylpiperazine in 35 ml toluene containing 4 g of anhydrous Na_2CO_3 was heated at 100 °C for 24 h to give the corresponding 1-(α-phenylethyl)-4-formylpiperazine, which was directly hydrolyzed by heating with conc. HCl for 3 h. The aqueous solution was made basic with N NaOH and extracted with CH_2Cl_2. Removal of the dried solvent gave an oil that was chromatographed on alumina Brockmann II, using Et_2O-CH_2Cl_2 1:1, yield 50 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.3 (d, 3H; J = 6 Hz, $C\underline{H}_3$-CH), 1.5 (s, 1H; NH), 2.2–2.4 (m, 4H; CH_2-N-CH_2), 2.6–2.8 (m, 4H; CH_2-N-CH_2), 3.25 (q, 1H; J = 6 Hz, C$\underline{H}$-CH_3), 7.1 (s, 5H aromat.).

1-(α-Phenylethyl)-4-[2-hydroxy-3-(1-naphthoxy)-propyl-1]-piperazine **(14)**

The epoxide **10**, 2.6 g (13 mmole) was added to 2.6 g (14 mmole) of 1-(α-phenylethyl)-piperazine **(13)** in 40 ml of anhydrous EtOH and the mixture was refluxed for 3 h. The mixture, evaporated in vacuo, gave a solid residue, which was chromatographed on alumina Brockmann II with Et_2O, m.p. 40–45 °C; yield 60 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.3 (d, 3H; J = 6 Hz CH-C$\underline{H}_3$), 2.3–2.6 (m, 10 H; H-piperazine, CH-C$\underline{H}_2$-N), 3.2 (s, 1H; OH), 3.3 (q, 1H; J = 6 Hz, C$\underline{H}$-CH_3),), 4.1–4.3 (m, 3H; O-C$\underline{H}_2$-C$\underline{H}$OH), 6.7–6.8 (m, 1H; H-4 naphthalene), 7.1–7.4 (m, 9H; H-5, 6, 7, 8 naphthalene, 5H-aromat.), 7.6–7.7 (m, 1H; H-3 naphthalene), 8.1–8.2 (m, 1H; H-2 naphthalene). $C_{25}H_{30}N_2O_2$ (390) Calc. C 76.9 H 7.6 N 7.1 Found C 76.5 H 7.3 N 7.5. The corresponding hydrochloride shows m.p. 115–120 °C.

1-(3-Phenyl-2-propenyl)-4-[2-hydroxy-3-(1-naphthoxy)-propyl-1]-piperazine **(16)**

A mixture of **10**, 5.7 g (26 mmole) and 5.8 g (29.5 mmole) of 1-(3-phenyl-2-propenyl)-piperazine[9] **(15)** in 60 ml of anhydrous EtOH was refluxed for 3 h. The mixture, evaporated in vacuo, gave a solid residue that was crystallized from hexane/Et_2O, m.p. 93–95 °C, yield 50 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.4–2.7 (m, 10H; H-piperazine ring, CH-C$\underline{H}_2$-N), 3.2 (d, 2H; J = 7 Hz, C$\underline{H}_2$CH = CH), 3.5 (s, 1H; OH), 4.1–4.4 (m, 3H; O-C$\underline{H}_2$-C$\underline{H}$-OH), 6.0–6.5 (m, 2H; C$\underline{H}$=C$\underline{H}$-CH_2), 6.6–6.8 (m, 1H; H-4 naphthalene), 7.1–7.5 (m, 9H; H-5, 6, 7, 8 naphthalene, 5H-aromat.), 7.7–7.8 (m, 1H; H-3 naphthalene), 8.1–8.2 (m, 1H; H-2 naphthalene). – $C_{26}H_{30}N_2O_2$ (402) Calc. C 77.5 H 7.46 N 6.9 Found C 77.2 H 7.86 N 6.9. – M.p. of the corresponding hydrochloride 220–225 °C.

References

1 P. R. Lichtlen, Recent Aspect in Calcium Antagonism, Stuttgart, New York 1985.

2a A. Strano, S. Novo, and L. Adamo, Effect of calcium blockers and of betablockers on exercise tolerance in patient of different age suffering from effort angina pectoris, Int. Sym. Calcium Antagonists, New York, February 1987.

2b S. K. Atwall, C. B. O'Reilly, E. P. Ruby, F. C. Turek, G. Aberg, M. M. Asaad, L. J. Bergey, S. Moreland, and R. J. Powell, J. Med. Chem. *30*, 627 (1987).

3 J. J. Baldwin, R. Hirschmann, E. L. Engelhardt, G. S. Ponticello, C. S. Sweet, and A. Scriabine, J. Med. Chem. *24*, 628 (1981).

4 R. A. Jonis and D. J. Triggle, J. Med. Chem. *26*, 775 (1983).

5 H. J. Schumann, B. D. Gorlitz, and J. Wagner, Naunyn-Schmiedeberg's Arch. Pharmakol., *289*, 409 (1975).

6 S. P. Baker and L. T. Potter, Br. J. Pharmacol. *68*, 8 (1980).

7 G. A. Weiland and B. B. Molinoff, Life Sci. *29*, 313 (1981).

8 G. Shtacher, M. Erez, and S. Cohen, J. Med. Chem. *16*, 516 (1973).

9 S. Corsano, G. Strappaghetti, and L. Brasili, Arch. Pharm. (Weinheim) *321*, 171 (1988).

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