Eur. J. Med. Chem. **23** (1988) 11—20

Original paper

Synthesis of indolyloxypropanolamines bearing the 2-(1H-indol-3yl)-1,1-dimethylethyl group: structure—activity studies and anti-hypertensive activity

Jean WAGNON, Claude PLOUZANÉ, Bernard TONNERRE, Henri DEMARNE, Martine SERRE, Colette LACOUR, Jean GOUGAT, Jean-Pierre GAGNOL and Patrick GAUTIER

Sanofi Recherche, av. du Prof. Joseph-Blayac, 34082 Montpellier Cedex, France

(Received February 11, 1987, accepted June 25, 1987)

Summary — New indolyloxypropanolamines in which the substituent at the side chain nitrogen atom was the 2-(1H-indol-3yl)-1,1-dimethylethyl group have been synthesized as putative anti-hypertensive agents. *In vitro* assays were used to measure their affinity for the β_1-, β_2-, α_1- and α_2-adrenoceptors, where a marked tendency towards β_2-adrenoceptor selectivity was observed.

The tryptamine indole ring was responsible for their α_1 and Ca^{2+} blocker properties and results compared to those obtained with the 4 stereoisomers of 1-((2-(1H-indol-3yl)-1 methylethyl)amino)-3-(2-methylphenoxy)-2 propanol **32.** Intrinsic sympathomimetric activity *(ISA)* was imparted to the oxyindole ring.

In the anesthetized dog, five analogs **(4, 5, 24, 25, 27)** antagonized the effects of isoproterenol (β-blockade) and norepinephrine (α-blockade) more than 50% at doses lower than or equal to 0.1 mg/kg i.v. The anti-hypertensive activity was confirmed in spontaneously hypertensive rats. Two compounds, **4** (CM 40441a) and **25** (SR 41717a), were selected for further pharmacological studies.

Résumé — **Synthèse d'indolyloxypropanolamines portant le groupement (1H-indolyl-3)-2-diméthyl-1,1 éthyle: études de structure—activité et activité anti-hypertensive.** *Une série d'indolyloxypropanolamines portant sur l'atome d'azote de la chaîne latérale le groupement (1H-indolyl-3)-2-diméthyl-1,1 éthyle a été synthétisée en vue d'obtenir de nouvelles molécules anti-hypertensives. Leur affinité aux récepteurs β_1, β_2, α_1, α_2 a été déterminée* in vitro *où l'on note une tendance vers une sélectivité β_2. La tryptamine induit des propriétés α_1-lytiques et antagonistes du calcium. Ces résultats sont comparés à ceux des quatre isomères du (((1H-indolyl-3)-2 méthyl-1)éthylamino)-1 (méthyl-2 phénoxy)-3 propanol-2,* **32.**

Le cycle oxyindolique ajoute une composante sympathomimétique intrinsèque.

Chez le chien anesthésié, on remarque que 5 composés ***(4, 5, 24, 25, 27)*** *antagonisent à plus de 50% les effets de l'isoprénaline (blocage β) et de la noradrénaline (blocage α) à des doses inférieures ou égales à 0,1 mg/kg i.v. L'activité anti-hypertensive a été démontrée chez le rat spontanément hypertendu. Deux composés,* ***4 (CM 40441a)*** *et* ***25 (SR 41717a)*** *ont été retenus pour des études pharmacologiques approfondies.*

indolyloxypropanolamines / tryptamines / β- and α-adrenoceptor affinities / vasodilating activity / anti-hypertensive activity

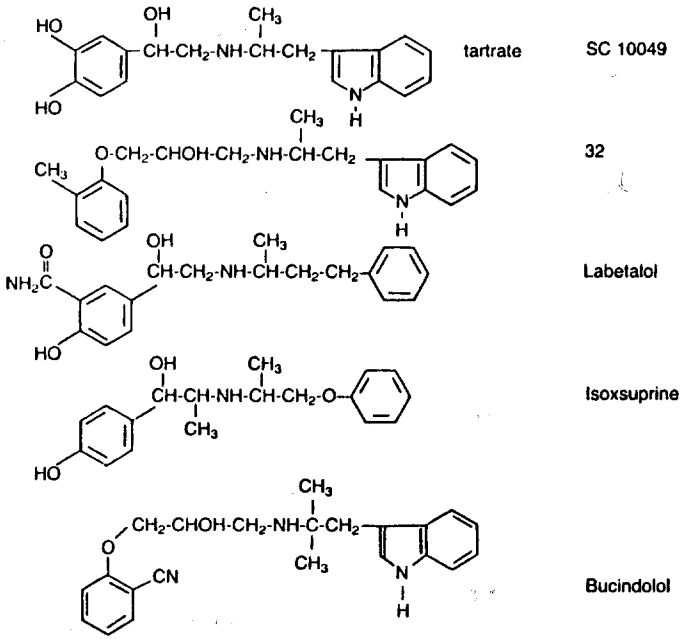
Introduction

It was demonstrated, in the treatment of hypertension, that synergistic effects could be achieved by the combination of β-blocking drugs with vasodilators [1, 2]. Labetalol [3] was the first agent to be marketed combining both α- and β-blocking properties. Some other compounds have recently been developed such as prizidolol [4], carvedilol [5] and bucindolol [6] which exhibit both β-adrenoceptor blocking and vasodilating properties, or medroxalol [7] which also has vasodilating properties in addition to α- and β-blocking effects.

Some recent reports [6, 8—11] have shown the dual effects of arylalkylamino analogs of pure β-blockers of the isopropyl- or tertiobutyl-type at β- and α_1-receptors.

Since it has already been shown that selective α_1 antagonism could be gained with extended substituents at the side chain nitrogen atom (4-phenyl-2-butyl as in labetalol, 3-phenoxy-2-propyl as in isoxsuprine), we looked for new arylalkylamines which could interact strongly at the β-receptor. We came upon compound **SC10049** [12], a tryptamine analog of the biogenic amines epinephrine and norepinephrine, a bronchodilator 10 times as potent as isoproterenol.

Consequently, the use of 2-(1H-indol-3-yl)-1-methylethylamine (α-methyltryptamine) as the amine partner in



classes of β-blockers was aimed at designing compounds with synergistic activities at the α_1- and β-receptors and this was initially verified with compound **32** [13] whose valuable anti-hypertensive activity prompted us to synthesize its four stereoisomers ([14], manuscript in preparation).

Since it is also currently recognized that a limited contribution of β-adrenergic stimulation reduces side effects of the β-blocker treatment: *i.e.*, bronchospasm, bradycardia, increased peripheral resistance [15] and plasma triglyceride levels [16], we focused more particularly on 4-hydroxy indole derivatives. It is known that such a pharmacophore confers partial agonist properties [17] to β-blockers which contain it. These β-blockers with partial intrinsic sympathomimetic activity *(ISA)* might be therapeutically advantageous when compared to purely sympatholytic compounds [18].

The aim of this article is to present preliminary biochemical and pharmacological studies on a series of bis-indole propanolamines with anti-hypertensive properties. In order to simplify the analysis of biological data, achiral amines (α,α-dimethyl tryptamines) **3b—h** partly related to the bucindolol series were used in this study so that these new amino alcohols were obtained as racemates.

Chemistry

Most of the amino alcohols mentioned in this study (Tables I, II) were synthesized as outlined in Scheme 1 by refluxing equimolar amounts of an indolyloxymethyloxirane with the requisite amine in a hydroxylic solvent. Purification was achieved by gel chromatography, followed in most instances by salt formation. Intermediates **1** and **2** were known, or obtained by standard literature procedures. It appeared highly advantageous, whenever possible, to purify the epoxide **2** by chromatography or crystallization in order to achieve a clean conversion into the amino alcohol (yield 60—66%) and easy salt formation. By this procedure, the hemifumarate salts obtained were freed from contaminating amino alcohol hydrochlorides. For structure—activity purposes, we synthesized amine **3g** by nucleophilic displacement by the cyanide ion on bromo amine **3e** ($(CuCN_2)$ in *N*-methylpyrrolidone) according to a procedure previously described for the synthesis of 5-cyanoindole from 5-bromoindole [19]. Amine **3h** was obtained from 5,6-dimethoxygramine by routine procedures.

Biology

In vitro *studies*

The interactions of the compounds with the adrenergic system were studied *in vitro* in order to compare their affinity on α- and β-receptors. The affinity for β_1-, β_2-, α_1- and α_2-receptors was assessed by ligand binding techniques using [^{3}H]dihydroalprenolol on dog heart and rat lung membranes, [^{3}H]WB.4101 on rat brain membranes and [^{3}H]clonidine on rat brain membranes respectively (Table III).

In vitro rat tail artery studies were performed, using phenylephrine as an α-agonist. Some compounds were also tested against the vasoconstriction induced by a hyperpotassic solution on denervated artery, a property shared by calcium entry blockers [20] (Table IV).

Pharmacology

The *in vivo* anti-adrenergic effects were tested in the anesthetized dog on isoproterenol-induced tachycardia and hypotension and on norepinephrine (NE)-induced hypertension. The hypotensive effect of some compounds was confirmed in the conscious, spontaneously hypertensive rat (SHR) (Table V). The intrinsic sympathomimetic activity *(ISA)* was analyzed on reserpinized anesthetized rats (Table VI). The commercially available propranolol (avlocardyl®), pindolol (visken®) and labetalol (trandate®) were studied as reference compounds.

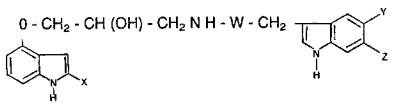
Results and Discussion

β-Adrenergic receptor affinity

As compared to propranolol, compound **4** and its fluoro-analog, compound **5,** were found to possess high, non-cardioselective affinities for β-adrenergic receptors with IC_{50} values in the 10^{-9}—10^{-10} M range. The closest isostere, the indolizine analog **28,** displayed much weaker affinity for the β_1-receptor.

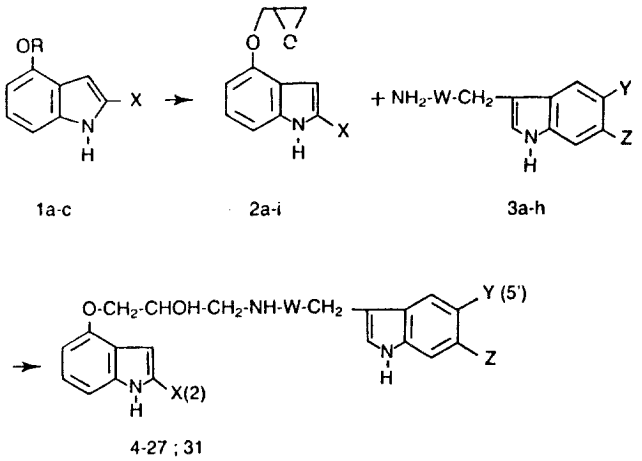
When various monosubstituted indolealkylamines were introduced as amino components, large shifts in binding constants (3 log units) were evenly observed at both β_1- and β_2-adrenoceptor sites. It turned out that slightly hydro-

Table I. Substituted 4-indolyloxypropanolamines.



Cp	X	Y	Z	W[a]	FORMULA	Mp, °C[b]	Recrystallisation solvent
4	H	H	H	A	$C_{23}H_{27}N_3O_2$. 0.5 $C_4H_4O_4$ [c,d]	157-160[e]	EtOH
5	-	F	-	-	$C_{23}H_{26}FN_3O_2$. 0.5 $C_4H_4O_4$. 0.3 H_2O	157-161	AcOEt-EtOH
6	-	CH_3	-	-	$C_{24}H_{29}N_3O_2$. 0.5 $C_4H_4O_4$	180-182	EtOH
7	-	OCH_3	-	-	$C_{24}H_{29}N_3O_3$. 0.5 $C_4H_4O_4$	161-163	AcOEt-EtOH
8	-	Br	-	-	$C_{23}H_{26}BrN_3O_2$. 0.5 $C_4H_4O_4$	200-204	AcOEt-EtOH
9	-	OCH_3	OCH_3	-	$C_{25}H_{31}N_3O_4$.0.5 $C_4H_4O_4$. C_2H_5OH	208-212	EtOH-H_2O
10	-	CN	H	-	$C_{24}H_{26}N_4O_2$. 0.5 $C_4H_4O_4$. 0.5 C_2H_5OH	202-204	EtOH
11	CO_2Et	H	H	B	$C_{25}H_{28}N_2O_4$. 0.5 $C_4H_4O_4$ [f]	219-226	EtOH
12	CO_2Et	-	-	A	$C_{26}H_{31}N_3O_4$	137-142	CH_3CN
13	-	F	-	-	$C_{26}H_{30}FN_3O_4$. 0.5 $C_4H_4O_4$	236-238	AcOEt-EtOH
14	-	CH_3	-	-	$C_{27}H_{33}N_3O_3$. 0.5 $C_4H_4O_4$	252-256	AcOEt-EtOH
15	-	OCH_3	-	-	$C_{27}H_{33}N_3O_3$ [g]	122-124	$CHCl_3$- i-Pr_2O
16	-	CN	-	-	$C_{27}H_{30}N_4O_4$. 0.5 $C_4H_4O_4$	246-248	AcOEt-EtOH
17	-	Cl	-	-	$C_{26}H_{30}ClN_3O_4$. 0.5 $C_4H_4O_4$	249-251	AcOEt-EtOH
18	CO_2-CH $(CH_3)_2$	H	-	-	$C_{27}H_{33}N_3O_4$. HCl. H_2O	144-148	AcOEt- i-Pr_2O
19	CN	-	-	-	$C_{24}H_{26}N_4O_2$. 0.5 $C_4H_4O_4$. C_2H_5OH [h]	140-145	EtOH
20	$CONH_2$	-	-	-	$C_{24}H_{28}N_4O_3$. 0.5 H_2O	195-199	EtOH
21	CON $(CH_3)_2$	-	-	-	$C_{26}H_{32}N_4O_3$. 0.5 $C_4H_4O_4$. 0.4 H_2O	223-226	EtOH
22	CON $(Bu)_2$	-	-	-	$C_{32}H_{44}N_4O_3$. 0.5 $C_4H_4O_4$. 0.5 CH_3CO_2Et	147-149	AcOEt-EtOH
23	CH_2OH	-	-	-	$C_{24}H_{28}N_2O_3$. 0.5 C_2H_5OH	103-145	EtOH
24	CH_3	-	-	-	$C_{24}H_{28}N_2O_2$. CH_3COOH	163-166	EtOH
25	CH_3	CH_3	H		$C_{25}H_{31}N_3O_2$. $C_4H_4O_4$. 0.25 CH_3CO_2Et	125-129	AcOEt-EtOH
26	CH_3	OCH_3	H		$C_{26}H_{33}N_3O_3$. HCl. 0.3 H_2O [i]	125-145	i-PrOH
27	CH_3	CN	H		$C_{26}H_{28}N_4O_2$. 0.5 $C_4H_4O_4$. 0.5 H_2O	163-166	EtOH
31	H	H	H	B	$C_{25}H_{28}N_2O_2$. 0.5 $C_4H_4O_4$	183-184	EtOH

[a] A = $C(CH_3)_2$; B = —CH (CH_3)—.
[b] Uncorrected melting point.
[c] $C_4H_4O_4$ = fumaric acid.
[d] Calc for C: 68.94; found: 68.20.
[e] In some instances, a salt (mp = 210—212°C) was obtained in ethanol—water mixtures (polymorphism).
[f] Calc for C: 65.70; found: 65.05.
[g] Calc for C: 67.62; found: 66.32.
[h] Calc for C: 66.38; found: 65.46.
[i] C, H, N, Cl. Calc for N: 9.8; found: 9.10.



1a-c 2a-i 3a-h

4-27 ; 31

1 a R = Bzl, X = H
1 b R = H, X = H
1 c R = Bzl, X = $CO_2CH(CH_3)_2$

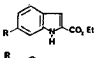
2 a	X = CO_2Et	3 a	W = $CHCH_3$,	Y = H,	Z= H
b	X = CH_3	b	W = $C(CH_3)_2$,	Y = H,	Z= H
c	X = CO_2-$CH(CH_3)_2$	c	W = $C(CH_3)_2$,	Y = F,	Z= H
d	X = CN	d	W = $C(CH_3)_2$,	Y = Cl,	Z= H
e	X = $CONH_2$	e	W = $C(CH_3)_2$,	Y = Br,	Z= H
f	X = $CON(CH_3)_2$	f	W = $C(CH_3)_2$,	Y = OCH_3,	Z= H
g	X = $CON(Bu)_2$	g	W = $C(CH_3)_2$,	Y = CN,	Z= H
h	X = CH_2OH	h	W = $C(CH_3)_2$,	Y = OCH_3,	Z= OCH_3
i	X = H				

Scheme 1.

philic groups (F, OCH_3, CN) were preferred to larger lipophilic substituents (CH_3, Br) in order to retain a high level of binding affinity **(5, 7, 10)**.

As a result of increased β_2 **(19)** or decreased β_1-binding affinity **(18)**, a tendency to β_2-selectivity ($K_i(\beta_1)/K_i(\beta_2) \geqslant 100$) was evidenced in the series of compounds substituted in the oxyindole ring **(18, 19, 27)**.

Table II. Miscellaneous and reference compounds.

Cp	FORMULA	Recrystal. solvent	Mp, °C	β_1 receptor IC_{50}nM ± SEM	Ki (nM)	β_2 receptor IC_{50}nM ± SEM	Ki (nM)	α_1 receptor IC_{50}nM ± SEM	Ki (nM)
28 	$C_{23}H_{27}N_3O_2$.0.5$C_4H_4O_4$[b]. 0.5CH_3CO_2Et	AcOEt-EtOH	140-144	75 (60-90)	40	2 (1-3)	0,4	1300 (930-1850)	530
29 	$C_{26}H_{31}N_3O_4$.0.5$C_4H_4O_4$	EtOH	164-166	7700 (6000-10000)	3800	1500 (1100-2000)	300	5900 (4100-8500)	4200
30 	$C_{26}H_{31}N_3O_4$. HCl	EtOH	196-198	8200 (5700-12000)	4000	500 (370-750)	100	1200 (750-2000)	870
Labetalol				500 (320-770)	250	410 (310-550)	77	140 (110-170)	100
Pindolol				68 (49-93)	34	16 (11-24)	3,0	2300 (1400-3600)	1600
Propranolol				39 (28-55)	20	35 (27-45)	6,5	15000 (10000-22000)	10000

[a] R = -OCH_2-$CH(OH)CH_2NH$-$C(CH_3)_2$-CH_2 

[b] $C_4H_4O_4$ = fumaric acid

Table III. Inhibition of [^{3}H]DHA and [^{3}H]WB.4101 binding to mammalian membranes.

	β_1-receptor IC_{50}[b], nM, [^{3}H]DHA[c]-Dog hearts membranes	Ki[a](nM)	β_2-receptor IC_{50}[b], nM, [^{3}H]DHA-Rat lungs membranes	Ki[a](nM)	α_1-receptor IC_{50}[b], nM, [^{3}H]WB.4101 rat brain membranes	Ki[a](nM)
4	1.3 (1-2)	0.7	2.5 (2-3)	0.5	180 (140-240)	130
5	0.3 (0.2-0.5)	0.15	0.4 (0.2-0.5)	0.07	290 (210-390)	200
6	68 (45-100)	20	570 (360-900)	107	700 (550-950)	500
7	3 (2-4)	1.5	2.5 (2-4)	0.5	200 (140-280)	140
8	400 (300-500)	200	1600 (1100-2400)	310	600 (450-800)	420
9	100 (70-150)	50	0.5 (0.3-0.7)	0.1	200 (140-280)	140
10	8 (5-13)	4	1.5 (1-3)	0.3	165 (110-240)	110
11	6 (5-8)	3	3 (2-4)	0.6	400 (300-550)	290
12	20 (14-25)	10	5 (4-6)	1	500 (400-650)	360
13	10 (6-14)	5	0.5 (0.3-0.7)	0.1	630 (450-870)	440
14	240 (170-330)	120	3 (2-4)	0.6	900 (700-1200)	630
15	110 (80-140)	55	2 (1-5)	0.4	580 (440-750)	410
16	6 (4-9)	3	0.5 (0.4-1)	0.1	300 (230-400)	210
17	40 (30-70)	20	8 (5-11)	4	1500 (1100-2000)	1000
18	350 (250-450)	170	1 (0.6-2)	0.2	660 (520-840)	460
19	2 (1-3)	1	0.07 (0.03-0.1)	0.01	530 (410-690)	380
20	12 (8-16)	6	30 (20-35)	5	680 (540-860)	480
21	4 (3-5)	2	1 (0.7-2)	0.2	240 (190-300)	170
22	900 (600-1200)	450	500 (300-800)	100	570 (380-850)	400
23	13 (10-16)	6	7 (4-12)	1.3	350 (260-460)	250
24	4 (3-5)	2	14 (11-18)	3	210 (170-270)	150
25	13 (9-18)	6	5 (3-12)	1	550 (420-730)	390
26	15 (12-20)	8	1 (0.9-2)	0.2	230 (150-350)	160
27	15 (10-20)	8	0.1 (0.06-0.2)	0.02	230 (180-290)	160
31	10 (7.5-14)	5	3 (2-4)	0.6	105 (80-140)	75

[a]All K_i were calculated, assuming pure competitive behavior.
[b]95% confidence limits.
[c][^{3}H]DHA = [^{3}H]dihydroalprenolol.

Table IV. Comparison of vasodilator potencies *(IC_{50})* of tryptamine derivatives in rat tail artery contracted by a hyperpotassic solution (90 mM) and phenylephrine (5×10^{-6} M).

Compound	IC_{50} (M)	
	Hyper K[a]	Phenylephrine[b]
4	1.4×10^{-5}	1.1×10^{-6}
8	3×10^{-6}	6×10^{-6}
9	2.7×10^{-5}	5.2×10^{-7}
12	1×10^{-5}	7.5×10^{-6}
19	8×10^{6}	3×10^{-7}
25	3×10^{-6}	2.5×10^{-6}
31	1.2×10^{-5}	1.6×10^{-5}
Pindolol	$> 1 \times 10^{-4}$	5×10^{-5}
Labetalol	$> 1 \times 10^{-4}$	1.5×10^{-7}
Verapamil	1.4×10^{-7}	4.6×10^{-7}
Phenoxybenzamine	$> 1 \times 10^{-4}$	2.0×10^{-9}

No. of experiments ≥ 4.
[a]Ca entry blocker properties.
[b]Sum of alpha^{-1} and Ca entry blocker properties (see Experimental protocols).

Since no clearcut structure—activity relationship was found, the reasons for which substitution might alter selectivity will be analyzed as trends in comparison to **4**.

In the sp^3-substituted series, a theory of lipophilicity or H-donor/acceptor properties was ruled out because analogs **23, 24** (X = CH_2OH, CH_3) bind with high affinity, with no selectivity in spite of the large shifts in substituent lipophilicity parameters [21]. However, the theory of lipophilicity must be considered in the sp^2-substituted series. More lipophilic substituents were preferred to hydrophilic ones in order to retain a high level of binding affinity for the β_2-adrenoceptor (**20** *vs* **12; 12** *vs* **18; 20** *vs* **21**). But increased lipophilicity does not always lead to an increase in selectivity **(22)**. Consequently, a bulk hypothesis must be considered. When one of the butyl groups of the amide **22** was removed by insertion of an oxygen atom, this change proved to be detrimental for binding at the β_1-adrenoceptor, but led to a selective analog **(18)** with high affinity for the β_2-adrenoceptor.

However, none of these observations can account for the 50-fold increase in binding affinity displayed by **19** at the β_2-adrenoceptor site. We are left with the conclusion that electronic parameters are also operating here.

The CN group is hydrophilic and, from the previous discussion, should not be a preferred one, but it manifests strong resonance and field effects [21]. A consequence might be a weakening of the indole—NH bond and a strengthening of the NH—receptor interaction at the β_2-adrenoceptor, leading to an increased binding affinity.

A 2-alkoxycarbonyl group also displays strong field and resonance effects. Taken in conjunction with the bulk and lipophilic effects mentioned above, these might account for the large shift towards β_2-selectivity elicited by **18** leading to an *in vitro* ratio $K_i(\beta_1)/K_i(\beta_2) = 850$.

Table V. *In vivo* experiments.

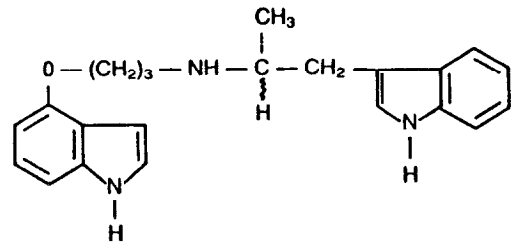
Compound	Comparative effects on MAP[a] of SHR[b] (1 mg/kg) iv			Alpha-adrenergic blockade and cardiovascular effects (heart rate and arterial pressure) of selected compounds studied at beta-blocking dose (iv) in anaesthetized dogs							
				Beta-adrenergic blockade					α-adrenergic blockade	Maximum variation	Maximum fall in
	No of SHR	Maximum fall in MAP mmHg ± SEM[d]	Duration of effect in h	No of dogs	ß-blocking dose (mg/kg) iv	% blockade of isoproterenol induced Tachycardia	% blockade of isoproterenol induced Hyputension	Duration of ß-blocking effect in h	% blockade of NE[c] induced hypertension	in heart rate beat/min ± SEM	diastolic arterial pressure mmHg ± SEM
4	5	28 ± 5,2**	> 2.30	4	0,04	89	85	> 1	59	-25 ± 4	-10 ± 2
5				4	0,10	92	86	> 3	60	-4 ± 12	-11 ± 3
6	4	41 ± 3,8**	> 2.30	4	0,40	93	92	> 3	56	-7 ± 18	-18 ± 5
7	4	46 ± 4,4**	> 2.30	4	0,20	85	87	> 2.30	55	-5 ± 19	-16 ± 4
8	3	40 ± 7**	> 2.30	3	0,30	95	88	3.30	39	+32 ± 13	-26 ± 11
9	4	21 ± 4*	> 2.30								
10	6	38 ± 4,5**	> 2.30	4	0,10	87	79	> 3	42	-25 ± 1	-11 ± 2
12	4	19 ± 6,0*	> 2.30	4	0,10	89	65	> 3	45	-14 ± 10	-15 ± 6
13				3	0,10	96	93		43	-26 ± 16	-8 ± 3
16				4	0,10	89	86	> 3	47	-29 ± 10	-11 ± 6
19	3	28 ± 1,8*	> 2.30	3	0,07	96	97	> 4	31	+21 ± 7	-14 ± 1
20				4	0,03	94	96	> 3	49	+6 ± 11	-20 ± 7
21				4	0,15	88	95	> 3	21	+1+15	-11 ± 5
23	4	15 ± 4,2*	< 1	3	0,05	86	82	> 3.30	36	-31 ± 6	-22 ± 6
24	4	25 ± 9,4	> 2.30	4	0,03	91	86	> 2.30	55	-28 ± 8	-11 ± 4
25	3	38 ± 2,6*	> 2.30	3	0,10	92	82	> 3	57	-32 ± 8	-16 ± 5
27	3	30 ± 2,4**	> 2.30	3	0,08	97	98	> 3	73	-40 ± 11	-19 ± 4
31	4	22 ± 4,9*	> 2.30	2	0,05	68	64	2	40	-20 ± 5	-15 ± 0
Labetalol	4	25 ± 3**	1	4	1,4	89	88	> 1	73	-18 ± 6	-14 ± 4
Pindolol	3	40 ± 2**	> 2	4	0,025	84	82	> 4		+17 ± 5	-10 ± 2
Propranolol	4	0		4	0,75	84	80	2.30		-33 ± 5	-13 ± 5
Hydralazine	3	52 ± 15*	> 2.30								

[a] *MAP* = mean arterial pressure.
[b] SHR = spontaneously hypertensive rats.
[c] NE = norepinephrine.
[d] $*p \leq 0.05$; $**p \leq 0.01$.

Table VI. Intrinsic sympathomimetic activity *(ISA)*.

Compound	No. of rats	Δ HR[a] (bpm)
4	6	54 ± 5
19	4	100 ± 7[b]
23	4	48 ± 4
24	4	63 ± 7
25	4	43 ± 9
27	3	54 ± 2
Pindolol	5	58 ± 6
Isoproterenol	5	150 ± 7

[a] Values represent mean ± SEM increase in heart rate (beat/min) of reserpinized rats (n = 3—6) given 0.3 mg/kg i.v.
[b] Student's t test: **19** significantly different from **4** ($p \leq 0.01$); **23**, **24** and **25** non-significantly different from **4**.



Steric hindrance might be determinant in increasing K_i values (10—450 nM) at the β_1 level **(12, 18, 22)**. Otherwise, affinity for the β_1-adrenoceptor was mostly insensitive (0.7—6 nM) to the structural character of substituents **(4, 19, 20, 21, 23, 24)**. In the disubstituted series, other theories can be put forward to account for the following data. In the group of compounds **(12—17)** substituted by the 2-CO_2Et group, affinity for the β_1-adrenoceptor stretched from 3 **(16)** to 120 nM **(14)**. In the case of the β_2-adrenoceptor (with the exception of **17**), changes in affinity were less pronounced from 0.1 **(16)** to 1 nM **(12)**. Consequently, β_2-selectivity was gained by a loss of affinity **(14, 15)** for the β_1-adrenoceptor. Changing the 2-CO_2Et group by the isolipophilic [21] 2-CH_3 group **(24—27)** gave the following results. Affinity for the β_1-adrenoceptor was kept constant from 2 **(24)** to 8 nM **(27)**. A unique increase in affinity for the β_2-adrenoceptor from 3 **(24)** to 0.02 nM **(27)** rendered **27** one of the most selective analogs found in this study ($K_i(\beta_1)/K_i(\beta_2) = 400$). The influence of the 5'-CN group was unprecedented. In fact, substitution by a CN group (**10** *vs* **4**, X = H; **16** *vs* **12**, X = CO_2Et) did not alter dramatically the binding affinity and selectivity in the other series. It has been shown from PCILO calculations [22] that β-adrenergic drugs of the isopropyl or tertiobutyl-type in the aryloxy propanolamine family only exist in three stable conformations.

The constraints imposed by the field/resonance effects

(*i.e.*, induced dipole, restricted rotation of the C_2—CO bond) in the 2-CO_2Et substituted family might strongly favor one conformer over the others. These would force the tryptamine indole ring to be translocated respectively: 1) in a non-interacting domain (β_2) and 2) in a domain (β_1) where repulsive interactions exist in the series **12—17**. Conversely, with a substituent lacking resonance and field effects (2-CH_3) the population of the other conformers might be increased. The tryptamine indole ring **(24—27)** would have freedom either to choose a 'better fit' or be forced 1) into a non-interacting domain (β_1) and 2) into a domain (β_2) where attractive forces are operating. Turning to the monosubstituted series (X = H), whenever comparison is made possible (Y = H, F, CH_3, OCH_3, CN) and with the exception of **6** at the β_2-adrenoceptor, the binding modes more closely resemble that of the 2-CO_2Et than that of the 2-CH_3 substituted families. The origin of the repulsive forces in the β_1-domain might be steric bulk. In a series where hydrophilicity [21] varied little **(4, 5, 7, 9)** binding affinity varied widely (over 300-fold), with a preference for the small F atom (molecular refractivity *(MR)* value) over the H atom (compare **4, 5** and **12, 13**). The attractive forces in the β_2-domain (X = CH_3) would favor hydrophilic groups and a group (CN) able to increase a tryptamine indole—NH—protein interaction, undemonstrated for the moment, due to the resonance effect of the 5′-CN group (more analogs are needed to test this hypothesis).

The 5′,6′-dimethoxy analog **9** was synthesized with the aim of shifting the biological response towards β_1-selectivity, a transformation which proved to be advantageous in a series in which the side chain N-substituent was the 3,4-dimethoxyphenethyl moiety [23]. The opposite was true and **9** was found to bind selectively ($K_i(\beta_1)/K_i(\beta_2)$ = 500) at the β_2-adrenoceptor, for the reasons discussed above.

The results of biochemical assays were confirmed *in vivo* in the anesthetized dog, by i.v. injection of low doses of the most β_2-selective analogs.

At 0.003 mg/kg i.v. β_2-blockade by **9, 18** and **27** was, respectively, 16, 36, 51% with no effect at the β_1-adrenoceptor. At 0.01 mg/kg i.v., results for **9** and **18** were 65, 60% (β_2) and 19, 17% (β_1).

Finally, shifting the propanoloxy side chain from the 4 to the 5 or 6 position in the indole nucleus **(29, 30)** led to inactive compounds.

In summary, with the exception of a minor structural change exemplified by the 5′-F atom, none of the substituent patterns appeared to be highly advantageous for non-selective binding with high affinity in this family. It can be seen that the β_2-adrenoceptor was quite tolerant towards a large number of chemical variations with a consequence that the underlying property in this family was β_2-adrenoceptor selectivity. A new series of pindolol analogs in which the N-atom was substituted by various phenethyl groups has recently been reported. Some compounds listed in this paper showed a low degree of β_1-selectivity [24].

α-Adrenergic receptor affinity

Most of the tested compounds in this study showed moderate and similar affinities for the α_1-receptor (IC_{50} = 10^{-6}—10^{-7} M). In our binding assays, some of them **(6, 8, 22)** with reduced β-affinity behaved like labetalol. None of them was able to displace [^{3}H]clonidine from its α_2-binding site at the 10^{-5} M level (data not showned).

Factors responsible for the binding at the α_1- and β-receptors were analyzed in the phenoxypropanol series **(32—36)**.

Results in Table VII show that **32** binds non-selectively at the β-adrenoceptors. The highest affinity resides in the two enantiomers **33** and **34** with the *S*-configuration at the carbon bearing the hydroxyl group. However, the 30—100-fold decrease in binding affinity observed for analogs **35** and **36** with the opposite chirality at the secondary hydroxy center is larger than the 10-fold variation recently reported for an oxymethylene class of *N*-aralkylamino-substituted compounds [11]. A reason for this discrepancy might stem from a loss of conformational flexibility of the (1H-indol-3 yl)-1-methylethyl group, as compared to the 4-phenyl-2-butyl group used in the reference article.

Affinity for the α_1-adrenoceptor is weakly influenced (4 times) by the configuration of the secondary hydroxy center, a conclusion strengthened by the fact that the deoxy analog rac. **37** binds slightly better than the less well bound enantiomers **35** and **36** in agreement with [11]. The two distinct enantiomers of labetalol [9] that respectively and selectively block α_1- and β-adrenoceptors share the same *R*(—)1-methyl-3-phenylpropylamine. The *R*(—)α-methyl-(1H)-indole-3-ethanamine used in this study does not manifest discriminative properties except for the β-adrenoceptor site in the *R*-propanol series (compare **35** *vs* **36**).

In vitro *vasodilating properties*

Some analogs were selected for a study of the phenylephrine-induced vasoconstriction in the rat tail artery (Tables IV and VII). Results show that the IC_{50} values (3×10^{-7}—1.6×10^{-5} M) do not closely parallel the K_i values obtained from the binding experiments using [^{3}H]WB.4101. For example, compound **4** with an α,α-dimethyl group at the side chain N-atom was more active than its congener **31** with a monomethyl group. Preference for analogs with a dimethyl group is in opposition to results observed in a set of salicylamide derivatives related to labetalol, using the same agonist [9].

Since Ca^{2+} entry blockers also antagonize phenylephrine-induced vasoconstriction, a selective analysis of Ca^{2+} blockade was performed in a test in which vasoconstriction was induced by a hyper K^+ solution [20]. Indeed, a moderate, unexpected Ca^{2+} entry blocker property was observed (IC_{50} = 3×10^{-6}—1×10^{-5} M), and was attributed to the (1H-indol-3-yl)-1,1-dimethylethyl group for labetalol and pindolol proved to be roughly 10—100 times less active in this test.

Table VII. Stereoisomers of 1-((2-(1H-indol-3-yl)-1 methylethyl)amino)-3-(2-methylphenoxy)-2-propanol **32.**

Cpd	Abs. config.	mp°C[a]	$[\alpha]^{25}$ deg.[b]	Adrenoceptors IC_{50} nM (± SEM); K_i nM						Vasodilator potency (IC_{50} M)		Comparative effects on *MAP* of SHR[h]		
				β_1		β_2		α_1		hyper K	phenyl-ephine	No. of rats	max fall in *MAP* mm Hg ± SEM[j]	duration of effects (min)
32		185—189[c]		4(3—5)	2	12(8—17)	1	190(150—230)	160	5×10^{-6}	5×10^{-7}	4	32±9*	>90
33	*SS*	163—164[d]	0	2(1—3)	1	13(10—16)	1	50(30—85)	43	4×10^{-6}	2×10^{-7}	4	30±5**	>90
34	*SR*	166—167[d]	−29.2	1(0.5—2)	0.5	12(9—15)	1	70(50—100)	60	5×10^{-6}	2×10^{-7}	3	40±2**	>90
35	*RS*	169—170[e]	+31.4	240(170—300)	120	1100(900—1400)	100	300(250—400)	250	3×10^{-6}	3×10^{-6}	3	10±2	30
												3[i]	35±8*	>60
36	*RR*	162—163[f]	0	60(40—100)	30	370(300—400)	34	270(210—350)	225	3×10^{-6}	1.5×10^{-6}	3	20±2**	>90
37		177—179[g]				6500(5400—800)	600	220(170—280)	180	2×10^{-6}	5×10^{-7}	3	13±4	60
(rac. deoxy)												3[i]	19±1**	>90
Hydralazine												3	19±1**	>90

[a]Hemifumarates.
[b]$C = 1.0$, EtOH—H_2O (6:4).
[c]From EtOH—H_2O (3:1).
[d]From EtOH—H_2O (3:1).
[e]From EtOH.
[f]From AcOEt.
[g]From AcOEt—EtOH.
[h]Cumulative dose 0.1 + 0.3 mg/kg i.v.
[i]Cumulative dose 1 + 3 mg/kg i.v.
[j]*$p \leqslant 0.05$; **$p \leqslant 0.01$.

Pharmacological studies

Intrinsic sympathomimetic activity (ISA)

ISA of pindolol has been explained by an interaction of the indole—NH with the β-adrenoceptor binding site [17]. Bucindolol also displays an *ISA* [25]. Effects of ring substitution were analyzed by introducing an electronegative CN group, whose bulk is similar to that of a CH_3 group (*MR* values [21]).

To rule out the possibility that agonism could be varied by loss of cardiac affinity, a set of analogs **(4, 19, 23, 24, 25, 27)** was selected whose *in vitro* affinities for β_1-receptor were kept constant within 1 log unit.

Results (Table VI) show that, compared to isoproterenol, the degree of agonism achieved by **27** (Y = CN, 36% *ISA*) was not significantly different from that of **24** (Y = H, 42% *ISA*), **25** (Y = CH_3, 29% *ISA*) and **4**. This suggests that a tryptamine indole—NH—protein interaction is not critical for agonism. In the oxyindole ring, the trend **19** (X = CN, 66% *ISA*) > **4** (X = H, 36% *ISA*) = **23** and **24** would lend support to the phenolic equivalent concept [26], although other theories [27] cannot be excluded from the present study.

Anti-hypertensive activity

In the SHR, except for the hydroxymethyl derivative **23,** all compounds studied at 1 mg/kg i.v. were shown to elicit a long-lasting fall in *MAP* ($\geqslant$ 19 to 46 mm Hg) a value quite similar to that found for hydralazine (52 ± 15 mm Hg) for **6, 7** and **8**. A true β-antagonist, like propanolol, is inactive in this test. As likely components of the observed vasodilating effect, the α_1 and Ca^{2+} blocker properties might contribute to the overall anti-hypertensive effect. Study of Table IV demonstrates that the fall in *MAP* is not correlated to the sole antagonism of phenylephrine vasoconstriction **(9, 19** and labetalol; $IC_{50} = 1.5 \times 10^{-7}$—$5.2 \times 10^{-7}$ M, maximum fall in *MAP*: 21—28 mm Hg), but also to an increase in Ca^{2+} blockade (**8** and **25**; $IC_{50} = 3 \times 10^{-6}$ M, maximum fall in *MAP*: 38—40 mm Hg).

In the phenoxypropanol series **(33—36)** data (Table VII) show that the maximal blood lowering effect (0.3 mg/kg, i.v.) roughly parallels the affinity of compounds for the β- and α_1-adrenoceptors and their vasodilator potency against phenylephrine in the rat tail artery. But there is evidence that the maximal fall in *MAP* is not related to these vasodilating properties only. For example, the deoxy analog **37,** the reference vasodilator from the *in vitro* experiments (Ca^{2+} blockade: 2×10^{-6} M, α_1 blockade: 5×10^{-7} M) (compare with **32**) does not lower blood pressure significantly at 0.3 mg/kg i.v. Moreover, pindolol which lacks the α_1 and Ca^{2+} components, but stimulates the β_2-adrenoceptors *(ISA)* is active in this test. The extent by which α_1 and Ca^{2+} blockade, *ISA* or direct vasodilation as in bucindolol [25], (compound **4** does not display β_2-agonist activity, results from Panlabs, Inc.) influence arterial pressure is currently under investigation for selected compounds.

Anti-adrenergic effects

Since these compounds possess a variety of pharmacological effects and to varying degrees, their effect on heart rate is unpredictable. Examination of Table V shows that **8,** with a labetalol-like profile (see above) was found to increase heart rate when labetalol did not. A more potent analog **19** also elicits a tachycardic effect. For analogs **5, 6, 7, 12, 13, 20** and **21,** a decrease in heart rate was not evenly observed in all the animals. However, a group of analogs (**4, 10, 13, 16, 23, 24, 25** and **27**) was found to be bradycardic agents (> 25 beats/min); some of them (**23, 25** and **27**) eliciting a consistent decrease in diastolic blood pressure. At the threshold dose of 0.1 mg/kg i.v., or lower (a minimum 14-fold increase in potency as compared to labetalol) 5 analogs (**4, 5, 24, 25** and **27**) inhibited more than 50% of the NE-induced vasoconstriction. Two out of these compounds (**4** and **25**) were selected for further pharmacological studies. Compound **4** had also a noticeable capacity to block β-adrenoceptors by 80% in the conscious dog at 1 mg/kg *p.o.* for 24 h.

Conclusion

We have synthesized a series of indolyloxypropanolamines in which the substituent at the side chain nitrogen atom was the 2-(1H-indol-3-yl)-1,1-dimethylethyl group. They blocked the α_1-adrenoceptors *in vivo* and possessed a moderate additional vasodilating property (Ca^{2+} entry blockade) which, however, might not account for the overall anti-hypertensive effect in SHR. Selective β_2-antagonism (**9, 18** and **27**) reduced the number of candidates for anti-hypertensive therapy. In the anesthetized dog, 5 of them (**4, 5, 24, 25** and **27**) were able to decrease the stimulating effect of isoproterenol and norepinephrine by 50% at doses equal to or lower than 0.1 mg/kg i.v. Two non-selective analogs (**4** and **25**) with partial *ISA* not exceeding that of pindolol underwent further pharmacological evaluation.

Experimental protocols

Chemistry

Melting points were taken on a Tottoli melting apparatus (Büchi) in unsealed capillaries and were uncorrected. IR spectra in KBr pellets were obtained with a Perkin—Elmer 397 spectrometer. NMR spectra were obtained with a Perkin—Elmer R12B spectrometer; hexamethyldisiloxane (HMDSO) was used as an internal standard. Unless otherwise specified, analytical data from Perkin—Elmer 240A and 240C analyzers were within 0.5% of the theoretical values for C and 0.4% for N and H. Corrections for contaminating solvents were deduced from the NMR spectra and/or estimated by gas chromatography on a Perkin—Elmer sigma 3B.G.C. chromatograph with a 100—120 mesh porapak Q chromatography column.

The following compounds: **1a—c, 2a—i** and **3a—h,** used in this study, were known and prepared according to methods described in the literature: 4-benzyloxy [28]; 5-benzyloxy [29]; 6-benzyloxy-2-ethoxycarbonyl-1H-indoles [30]; 4-hydroxy-2-methyl-1H-indole [31]; 4-benzyloxy-2-carboxamido-1H-indole [32]; 4-(2,3-epoxypropoxy)-2-carboxamido-1H-indole [32]; 4-(2,3-epoxypropoxy)-2-cyano-1H-indole [33]; 4-benzyloxy-2-hydroxymethyl-1H-indole [28]; 4-benzyloxy-2-dimethylaminocarbonyl-1H-indole [34]; 4-(2,3-epoxypropoxy)-2-dimethylaminocarbonyl-1H-indole: mp: 173—176°C. Anal. ($C_{14}H_{15}N_2O_3$) C, H, N; 4-benzyloxy-2-dibutylaminocarbonyl-1H-indole according to [34]: mp: 121—123°C. ($CHCl_3$); 4-(2,3-epoxypropoxy)-2-dibutylaminocarbonyl-1H-indole: mp: 128—130°C. Anal. ($C_{20}H_{28}N_2O_3$) C, H, N; 8-acetyl-indolizinol [35].

5-Cyano-α,α-dimethyl-1H-indole-3-ethanamine 3g
5-Bromo-α,α-dimethyl-1H-indole-3-ethanamine (**3e**) (6.45 g, 0.024 mol), $(CuCN)_2$ (3.25 g; 0.036 mol) in *N*-methylpyrrolidone [19] (40 ml), were heated under reflux for 5 h. The solvent was removed at 80°C under reduced pressure (1 mm Hg), and the black residue was stirred for 30 min with 40% aqueous ammonia (50 ml) with added $CHCl_3$. An insoluble material was filtered off, washed 5 times with boiling $CHCl_3$ (total amount 150 ml). The organic phases were combined, washed with water and dried (Na_2SO_4). Chromatography of the residue (4.0 g) on silica gel and elution with AcOET, MeOH (8:2) afforded amine **3g** as a black solid, which was further treated with charcoal and crystallized from Et_2O. (1.4 g; 27%, mp: 145°C) used as such in the next step. Anal. ($C_{13}H_{15}N_3$) C, H, N.

5,6-Dimethoxy-3-(2,2-dimethyl-2-nitroethyl)-1H-indole 38
5,6-Dimethoxygramine [36] (13 g, 0.055 mol), excess NaOH pellets (4.8 g, 0.12 mol) in 2-nitropropane (100 ml) were refluxed for 18 h. The solvent was removed under reduced pressure. The residue was acidified with 10% acetic acid in water (150 ml). Extraction with CH_2Cl_2, washing with water, and drying (Na_2SO_4) afforded a crude compound, **38,** which was purified by silica gel chromatography (CH_2Cl_2): (6.9 g, 45%, mp: 179—180°C) used as such in the next step.

5,6-Dimethoxy-α,α-dimethyl-1H-indole-3-ethanamine 3h
W2. Raney's nickel (3 tsp) and **38** (6.9 g, 0.025 mol) were refluxed in ethanol (100 ml). The oil bath was removed and hydrazine hydrate (80% in water, 13.5 ml, 0.35 mol) was slowly added so as to maintain a gentle reflux of the solution. At the end of the addition, reflux was maintained for 2 h. After cooling, the catalyst was filtered off, washed 3 times with boiling ethanol, and the solvent was removed under reduced pressure. Chromatography on Al_2O_3 and elution with AcOEt—MeOH (8:2) yielded **3h** which crystallized from CH_2Cl_2—*i*-Pr_2O as a pale yellow solid (3.0 g, 49%) mp: 87—88°C. Anal. ($C_{14}H_{20}N_2O_2$) C, H, N.

Synthesis of amino alcohols: general procedure

1-((2-(1H-Indol-3-yl)-1,1-dimethylethyl)amino)-3-(1H-indol-4-yloxy)-2 propanol hemifumarate 4
Epoxide **2i** (48 g, 0.254 mol, contaminated by a trace amount of chloroalcohol), amine **3b** (48 g, 0.254 mol) were boiled in ethanol (190 ml) for 5 h. The solvent was evaporated under reduced pressure, the residue was taken up in boiling AcOEt and, after cooling (at this stage a basic wash with dilute sodium hydroxide or sodium carbonate solution was occasionally performed), was passed through a 1 kg silica gel column preequilibrated with AcOEt. The aminoalcohol was eluted with an 8:2 mixture of AcOEt and MeOH as an oil (yield: 65%). *Salt formation.* Fumaric acid (1 M eq.) in boiling ethanol (250 ml) was added to a solution of the aminoalcohol (60.9 g, 0.161 mol) in boiling ethanol (500 ml). The mixture was left to boil over a water bath until crystals appeared (10 min). After cooling, the solid was filtered off, thinly ground in a mortar and dried under vacuum (10 mm Hg) at 90°C for 8 h (yield: 82%), mp: 157—160°C. NMR (DMSO-d_6): δ 11.03 and 10.93 (s, 2H, ind NH); 7.52 (d, J = 7 Hz, 1H, H'-4); 7.29 (d, J = 8 Hz, 1H, H'-7); 7.16 (broad s, 2H, H-2, H'2); 7.05—6.88 (m, 4H, H'-5, H'-6, H-6, H-7); 6.50—6.38 (m, 3H, H-3, H-5; CH=CH—); 4.16—3.95 (m, 3H, O—CH_2, CHOH); 3.13—2.81 (m, 4H, CH_2N, CH_2 ind); 1.09 (s, 6H, CH_3).

1-((2-(1H-Indol-3-yl)-1-methylethyl)amino-3-(1H-indol-4-yloxy)-2 propanol hemifumarate 31
By exchanging amine **3b** with amine **3a,** compound **31** was isolated in two yields as a mixture of stereoisomers. mp: 183—184°C.

4-Phenylmethoxy-2-methylethyloxycarbonyl-1H-indole 1c
4-Phenylmethoxy-2-carboxy-1H-indole (5.9 g, 0.022 mol) was suspended in benzene and treated at 45°C under N_2 with $SOCl_2$ (5.9 g,

0.05 mol). The bath temperature was raised to 65°C within 4 h, and finally the reaction mixture was left overnight at room temperature. The clear solution was distilled in a rotavapor, toluene was added twice to the slurry, and the solvent was evaporated again. 2-Propanol (20 ml) in dry pyridine (15 ml) was added to the resulting mass at room temperature. After 3 h, excess reagents were evaporated under reduced pressure, water and AcOEt were added to the residue, and the organic phase was washed successively with a dilute hydrochloric acid solution, water, a sodium hydrogenocarbonate solution, and brine. After drying and distillation of the solvent, the solid thus obtained was purified by silica gel chromatography and was eluted with pentane—AcOEt (7:3). Yield 73%, mp: 167—169°C (AcOEt).

2-Methylethyloxycarbonyl-4-(2,3-epoxypropoxy)-1H-indole **2c**
This ester (4.6 g) in 2-propanol (100 ml) was hydrogenated at ordinary pressure with 10% Pd on charcoal (1.1 g), with intermittent heating at 70°C to ensure dissolution of the barely soluble starting material. After 18 h, the catalyst was filtered off and the filtrate was washed several times with boiling 2-propanol.

The filtrate was concentrated under vacuum and the crude phenol (3.15 g) was used for the next step without purification. This phenol (3.15 g, 0.014 mol), 2-propanol (30 ml), and glycerol epichlorhydrin (30 ml) were heated with stirring at 80°C under N_2, then NaOH pellets were added (680 mg, 0.017 mol). Stirring and heating were continued for *ca.* 140 min, and the solvents were removed by distillation under vacuum. Water and AcOEt were added, the organic phase was washed with water and brine, then dried (Na_2SO_4). Evaporation left a brown residue. Chromatography on silica gel and elution with $CHCl_3$ afforded a mixture of 2 compounds (1.9 g, yield *ca.* 50%) from which pure oxiranne was obtained as needles after crystallization from CH_2Cl_2 (*i*-Pr_2O). mp: 149—150°C Anal. ($C_{15}H_{17}NO_4$) C, H, N.

(4-(2-Hydroxy-3-(2-(1H-indol-3-yl)-1,1-dimethylethyl)amino)propoxy)-1H-indole-2-carboxylic acid, methylethylester hydrochloride **18**
The mixture of the above ethers (1.25 g, *ca.* 0.0045 mol) and **3b** (1.03 g, 1.2 eq) was refluxed in 2-propanol for 5 h. After removal of the solvent and chromatography on silica gel, crude aminoalcohol **18** was eluted with a 9:1 AcOEt—2-propanol mixture (yield: *ca.* 66%). The hydrochloride was formed in AcOEt by the addition of the minimal amount of an ethereal hydrochloric acid solution. The salt was recrystallized from 2-propanol—AcOEt and then from AcOEt—*i*-Pr_2O mixtures. Crystals were ground in a mortar and dried under vacuum (0.1 mm Hg) at 70°C for 16 h. mp: 144—148°C Anal. ($C_{27}H_{33}N_3O_4$, HCl, H_2O) C, H, N.

α-Methyl-N-((2-methylphenoxy)-3-propyl)-1H-indole-3-ethanamine hemifumarate **37**
The sodium salt of *o*-cresol (10.8 g, 0.1 mol) was prepared from 2.3 g of Na in methoxyethanol (50 ml) at 150°C for 30 min. Then, 1-bromo 3-chloropropane (20 ml) was added at once at 120°C. The mixture was left at this temperature for 72 h. After cooling, water was added and the residue was extracted with CH_2Cl_2 (2 ×). The organic phase was dried ($MgSO_4$) and the residue was distilled twice to give a mixture of 3-(2-methylphenoxy)-1-chloro(bromo)propane **39**. Yield: 6.5 g (*ca.* 35%), bp: 105—112°C (3.5 mm).

Amine **3a** (10.5 g, 0.06 mol) and **39** (5.75 g, *ca.* 0.03 mol) were refluxed in EtOH (100 ml) for 36 h. After cooling the reaction mixture was made basic with 1 N NaOH (50 ml) and ethanol was distilled off. Amine **37** and excess **3a** were extracted with AcOEt (2 ×). Purification was achieved by gel chromatography (AcOEt—MeOH, 8:2) followed by salt formation with fumaric acid in AcOEt—EtOH. Yield 2.3 g, 20%).

Biological and Pharmacological Methods

β-Adrenoceptor binding assays

Membrane preparations
Heart and lung microsomal preparations were obtained by the method of Wayne Alexander *et al.* [37]. Hearts were removed from anesthetized mongrel dogs. Ventricles were minced with scissors and homogenized (polytron) in 4 vol. of cold buffer (0.25 M sucrose, 5 mM Tris-HCl, pH 7.4, 1 mM $MgCl_2$). Lungs were removed from freshly killed rats and homogenized in 8 vol of cold buffer. Heart and lung homogenates were centrifuged at 700 × *g* for 10 min at 4°C and the pellet was discarded. The supernatant was centrifuged at 10 000 × *g* for 10 min and, after the pellet had been discarded, centrifuged at 29 000 × *g* for 15 min at 4°C. The pellet from the final centrifugation was resuspended in 1 ml of 'incubator buffer' (75 mM Tris-HCl, pH 7.4, 25 mM $MgCl_2$) for 10 g of heart or 2 g of lung tissue.

Binding assays
Membrane suspensions from dog heart or rat lung (100 μl) were incubated with [^{3}H]dihydroalprenolol (10 nM) and with the test compounds for 10 min at 37°C in a total volume of 300 μl of incubation buffer. The incubations performed in duplicate were terminated by the addition of 5 ml of ice cold incubation buffer, followed by rapid filtration through Whatman GF/B glass filter disks. The filter disks were washed twice with 5 ml of ice cold incubation buffer and placed in 10 ml of scintillation fluid (Biofluor). Radioactivity was measured in a liquid scintillation counter. Non-specific binding was defined as non-displaceable binding in the presence of 100 μM propranolol, and specific binding as the difference between total and non-specific binding.

α-Adrenoceptor binding assays
Rat brain membrane preparations were obtained according to O'Prichard *et al.* [38]. Binding assays: 1 ml of membrane suspensions were incubated with [^{3}H]WB.4101 (0.2 nM) or [^{3}H]clonidine (1 nM) and with various concentrations of compounds for 30 min at 37°C in a final volume of 2 ml of 50 nM Tris buffer, pH 7.7. The incubations were performed in duplicate. Filtration and measurements of β-radioactivity were performed under the same conditions as those for [^{3}H]-dihydroalprenolol binding assays. Non-specific binding was measured in the presence of 10 μM phentolamine for the [^{3}H]WB.4101 binding assays and 10 μM unlabeled clonidine for the [^{3}H]clonidine binding assays.

The equilibrium dissociation constant (K_i) for the interaction of each compound with the binding site was calculated from the equation: $K_i = IC_{50}/(1 + S/K_d)$. IC_{50} represents the concentration of each compound giving a 50% inhibition of the specific binding; it was obtained by log probit analysis. S represents the concentration of the radio ligand and K_d is the dissociation constant of the radio ligand.

Isolated artery studies
Using rat tail artery, the hypotensive action mechanism of the compounds was studied on α-receptor-mediated vasoconstriction induced by phenylephrine and on cellular depolarization induced by a hyperpotassic solution (calcium entry blocker property). A proximal 2 cm segment of the rat tail artery was perfused with constant flow of Krebs' solution (pH 7.35, 37°C); vasoconstriction was measured as an increase in the perfusion pressure. After a 1 h stabilization period, responses to hyper K (90 mM) or phenylephrine (5×10^{-6} M) were elicited. In order to avoid the release of endogenous norepinephrine, hyper K vasoconstriction was induced on the denervated artery (6-OH-dopamine method). Then, additional increasing concentrations of drugs were introduced into the organ bath and hyper K or phenylephrine responses were induced at each of these doses. Results are expressed as percentages of the control values; concentration response (mean ± SEM) curves were established and the concentration corresponding to 50% inhibition (IC_{50}) was determined.

Anti-hypertensive activity in spontaneously hypertensive rats
The anti-hypertensive activity of these compounds was studied in Wistar—Okamoto, spontaneously hypertensive conscious rats purchased from Charles River (U.S.A.). 15 week old rats were used for the experiment. Two catheters (pE50) were implanted in the rats 24 h before the experiment: one in a carotid artery for blood pressure recording and the other in a jugular vein for compound injection. On the day of the experiment, the rats were put in a cylindrical box and the arterial catheter was connected to a pressure transducer through a swivel. Blood pressure was continuously recorded. The results were expressed as means of the maximum fall in mean arterial pressure (*MAP*, mm Hg ± SEM). Student's paired *t* test was applied to these results.

Intrinsic sympathomimetic activity (ISA)

Catecholamine depletion [*39*].
Male albino rats (Sprague—Dawley, Charles River) weighing 250—300 g were pretreated with reserpine (2 mg/kg i.p.) injected 18—24 h

prior to study. On the day of the experiment, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Blood pressure was maintained by a continuous i.v. infusion of 0.01 μg/kg/min of angiotensin II and recorded, *via* a cannula, in one carotid artery connected to an arterial transducer. Heart rate (HR) was determined from the pulse pressure, using a cardiotachimeter. The test drugs were injected rapidly into the jugular vein in a volume equivalent to 1 ml/kg.

Intrinsic sympathomimetic activity; values represent mean (± SEM) increases in heart rate (bpm) of reserpinized rats (n = 3—10) given 0.3 mg/kg i.v. The maximum response elicited by isoproterenol in this model was 150 ± 17 bpm.

Anti-adrenergic effects

Mongrel dogs of either sex weighing 8—20 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Drug injections were made through a lateral saphenous vein catheter. Analysis of the standard EKG surface leads was used to evaluate changes in heart rate (beats/min). Blood pressure was recorded through a humeral artery catheter. Norepinephrine (0.5—1 μg/kg, i.v.) and isoproterenol (0.25—0.5 μg/kg, i.v.) were injected at 10 min intervals before and after the drug (1 dose per animal) every 30 min for 1—4 h.

For norepinephrine (α-adrenoceptor agonism) the change in systolic blood pressure (hypertensive effect) was recorded. For isoproterenol (β-adrenoceptor agonism) the changes in diastolic blood pressure (vasodilation) and heart rate (tachycardia) were recorded. The percentage of antagonism on the cardiovascular parameters was calculated for each of the agonists. The duration of the β-blocking effect corresponded to a 90 ± 10% blockade of the β_1-receptor. The α-adrenoceptor blockade and the effect on heart rate and blood pressure were analyzed at the β-adrenoceptor blockade dose. Heart rate and diastolic blood pressure maximal variations were expressed as means ± SEM.

Compounds **9**, **18** and **27** were injected i.v. at 0.003 mg/kg and 0.01 mg/kg 45 min after the first dose to differentiate between the β_2- and the β_1-adrenergic effects.

Acknowledgments

The authors gratefully thank Mr. T. Clim for the synthesis of compounds **4** and **31**. Special thanks are due to M. de Clavière and C. Lafille for helpful discussions. The authors are also indebted to J. P. Vergnaud and C. Deleuze for microanalytical determinations and interpretation of the NMR spectra, and M. Boitel for secretarial assistance.

References

1 Sannerstedt R., Stenberg J., Johnsson G. & Werko L. (1971) *Am. J. Cardiol.* 28, 316
2 Zacest R., Gilmore E. & Koch-Weser J. (1972) *New Engl. J. Med.* 286, 617
3 Farmer J. B., Kennedy I., Levy G. P. & Marshall R. J. (1972) *Br. J. Pharmacol.* 45, 660
4 Taylor E. M., Roe A. M. & Slater R. A. (1979) *Clin. Sci.* 57 (Suppl.), 4335
5 Sponer G. & Muller-Beckmann B. (1983) *Naunyn-Schmied Arch. Pharmacol.* 322 (Suppl.), R46
6 Kreighbaum W. E., Matier W. L., Dennis R. D., Minielli J. L., Deitchman D., Perhach J. L. & Comer W. T. (1980) *J. Med. Chem.* 23, 285
7 Dage R. C., Cheng H. C. & Woodward J. K. (1981) *J. Cardiovasc. Pharmacol.* 3, 299
8 Grisar J. M., Claxton G. P., Bare T. M., Dage R. C., Cheng H. C. & Woodward J. K. (1981) *J. Med. Chem.* 24, 327
9 Clifton J. E., Collins I., Hallett P., Hartley D., Lunts L. H. C. & Wicks P. D. (1982) *J. Med. Chem.* 25, 670
10 Gold E. H., Wei Chang, Cohen M., Baum T., Ehrreich S., Johnson G., Prioli N. & Sybertz E. J. (1982) *J. Med. Chem.* 25, 1363
11 McClure D. E., Baldwin J. J., Randall W. C., Lyon T. F., Mensler K., Lundell G. F., Raab A. W., Gross D., Risley E. A., Sweet C. S. & Williams M. (1983) *J. Med. Chem.* 26, 649
12 Van Arman C. G., Miller L. M. & O'Malley M. P. (1961) *J. Pharmacol. Exp. Ther.* 133, 90
13 Demarne H. & Wagnon J. (1979) French Patent 2463765
14 Wagnon J., Plouzane C., Tonnerre B. & Nisato D. (1986) French Patent 09680
15 Louis W. J., McNeil J. J., Jarrott B. & Drummer O. H. (1983) *Am. J. Cardiol.* 52, 104A
16 Van Brummelen P. (1983) *J. Cardiovasc. Pharmacol.* 5, 551
17 Clark B. J. (1982) *Am. Heart J.* 104, 334
18 Reale A. & Motolese M. (1981) *Eur. Heart J.* 2, 245; see however: Shanks R. G. (1981) *Eur. Heart J.* 2, 253
19 Ikan R. & Rapoport E. (1967) *Tetrahedron* 23, 3823
20 Guiraudou P., Bertrand J. P. & Gautier P. (1984) *J. Mol. Cell. Cardiol.* 16 (Suppl. 3), 17
21 Hansch C., Leo A., Ungler S. H., Kim K. H., Nikaitani D. & Lien E. J. (1973) *J. Med. Chem.* 16, 1207
22 Leger J. M., Gadret M. & Carpy A. (1980) *Mol. Pharmacol.* 17, 339
23 Hoefle M. L., Hastings S. G., Meyer R. F., Corey R. M., Holme A. & Stratton C. D. (1978) *J. Med. Chem.* 21, 678
24 Tejani-Butt S. M. & Brunswick D. J. (1986) *J. Med. Chem.* 29, 1524
25 Leff A. R., Garrity E. R., Munoz N. M., Tallet J., Cavigell M., Deitchman D. & Rajfer S. I. (1984) *J. Cardiovasc. Pharmacol.* 6, 859
26 Baldwin J. J., Engerhardt E. L., Hirschmann R., Lundell G. F., Ponticello G. S., Ludden C. T., Sweet C. S., Scriabine A., Share N. N. & Hall R. (1979) *J. Med. Chem.* 22, 687
27 Main B. J. (1982) *J. Chem. Tech. Biotechnol.* 32, 617
28 Troxler F. (1970) U.S. Patent 3705907
29 Boehme W. R. (1953) *J. Am. Chem. Soc.* 75, 2503
30 Pastor I. C., Alvarez E. F. & Lora-Tamayo M. (1972) *Bull. Soc. Chim. Fr.* 4722
31 Junghans K. & Hader J. (1979) German Patent 2906551
32 Seeman F. & Troxler P. (1973) Swiss Patent 544755
33 Berthold R. (1979) German Patent 2925448
34 Troxler F., Seeman F. & Hofmann A. (1959) *Helv. Chim. Acta* 226, 2073
35 Silva H. & Blondeau D. (1976) *Tetrahedron Lett.* 12, 933
36 Walker G. N. (1955) *J. Am. Chem. Soc.* 77, 3844
37 Wayne Alexander R., Williams L. T. & Lefkowitz R. J. (1975) *Proc. Natl. Acad. Sci. USA* 72, 1554
38 O'Prichard D. C., Greenberg D. A. & Snyder S. H. (1977) *Mol. Pharmacol.* 13, 454
39 Barret A. M. & Carter J. (1970) *Br. J. Pharmacol.* 40, 373