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Original article

Synthesis and structure–activity of 4(5)-(2,2-diphenylethyl)imidazoles as new α_2-adrenoreceptor antagonists

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Summary — 4(5)-(2,2-diphenylethyl)imidazole **6a** is described as being a potent (pK_i = 8.16 for displacement of [^{3}H] *p*-aminoclonidine) and selective (selectivity = –log (pK_i α_2 – pK_i α_1) = 81) α_2-adrenoreceptor antagonist (pA_2 = 8.73 *versus* clonidine in the electrically stimulated guinea pig ileum) with additional activity as a norepinephrine uptake inhibitor (IC_{50} = 0.3 μM). An investigation of the structure–activity relationships of compounds closely related to **6a** has been carried out. This included substitution of the phenyl and imidazole rings and modification of the link between these two ring systems. The various synthetic routes used are described. The affinity and the selectivity of the compounds for the α_2 site were determined by studying the displacement of [^{3}H] *p*-aminoclonidine and [^{3}H] WB-4101 from rat forebrain membranes. The efficacy of the compounds was defined by measuring the antagonism of the clonidine effect on the electrically stimulated guinea pig ileum.

Résumé — Synthèse et relation structure–activité de 4(5)-(2,2-diphényléthyl)imidazoles comme nouveaux antagonistes du récepteur α_2-adrénergique. *Le 4(5)-(2,2-diphényléthyl)imidazole **6a** est décrit comme étant un antagoniste α_2-adrénergique puissant et sélectif (pA_2 = 8,73 vis-à-vis de la clonidine dans l'iléon de cobaye stimulé électriquement, pK_i = 8,16 pour le déplacement de la [^{3}H] p-aminoclonidine. La sélectivité est définie comme suit: –log (pK_i α_2 – pK_i α_1) = 81). De plus, le produit est un inhibiteur de la recapture de la noradrénaline (IC_{50} = 0,3 μM). Une recherche de relation structure–activité dans une série de produits proches de **6a** a été entreprise. Ceci inclut la substitution des noyaux phényle et imidazole, ainsi que la modification du lien unissant ces deux parties de la molécule. Les différentes voies de synthèse qui ont été utilisées, sont décrites. L'affinité et la sélectivité des produits pour le site α_2-adrénergique sont déterminées en étudiant le déplacement de la [^{3}H] p-aminoclonidine ainsi que du [^{3}H] WB-4101 de membranes de cerveau de rat. L'efficacité des composés est définie en mesurant l'antagonisme des effets de la clonidine sur l'iléon de cobaye stimulé électriquement.*

α_2-adrenoreceptor antagonist / norepinephrine uptake inhibitor / imidazole derivatives

Introduction

Existing antidepressants appear to act by potentiating monoaminergic neurotransmission, predominantly by inhibiting neuronal uptake sites [1, 2, 3] or inhibiting monoamine oxidase (MAO-I) [4]. The recent identification of multiple α-adrenoreceptor sites in the central nervous system (CNS) [5] has led to the concept that the facilitation of noradrenergic transmission can also be achieved by the blockade of the presynaptic α_2-autoreceptors [6, 7]. There is presently no drug on the market with this mode of action. The prototypes for α_2-antagonists are the indole alkaloids extracted from the *Rauwolfia* root, yohimbine **1** [8] and rauwolscine [9]. These compounds have been of only limited therapeutic utility due to their lack of specificity. More recently a number of compounds have been reported to possess potent and selective α_2-antagonists properties [10]. Of these, idazoxan **2** [11] has been extensively studied.

In the course of our investigation of imidazole derivatives, we have discovered that 4(5)-diarylethylimidazoles are potent and selective α_2-antagonists. We report here the synthesis, pharmacological eva-

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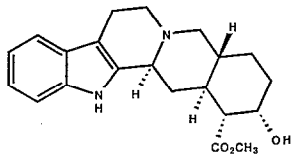
luation and structure–activity relationships of 4(5)-(2,2-diphenylethyl)imidazole **6a** and some of its derivatives.



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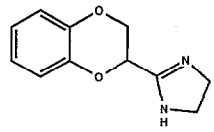

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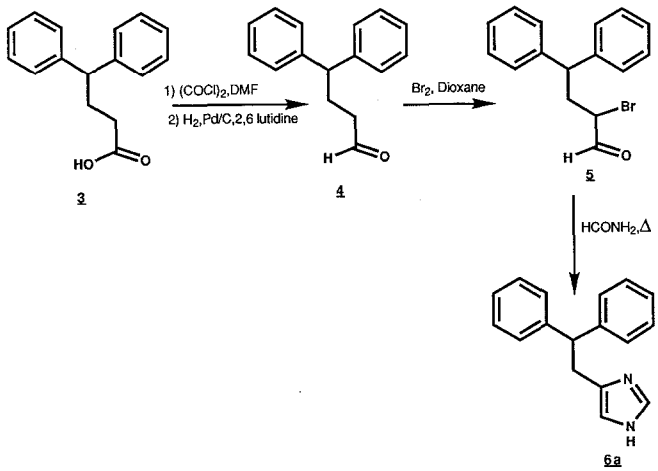
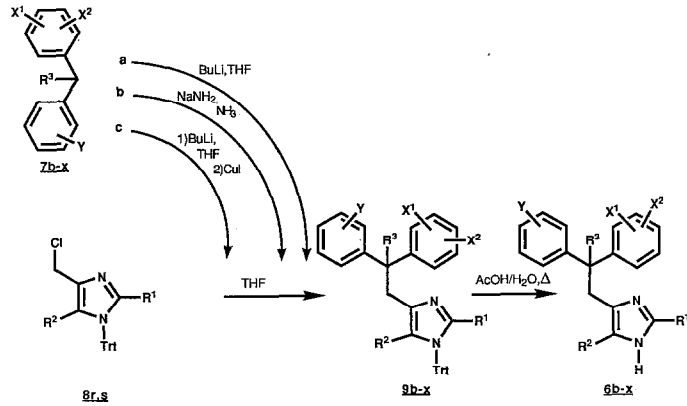
Chemistry

Synthesis of **6a** was achieved following a classical imidazole synthesis (scheme 1). Starting from 4,4-diphenylbutanoic acid [12] **3**, the acid function was reduced to the aldehyde through a modification of the Rosenmund reduction [13]. The aldehyde **4** was brominated in ether in the presence of a catalytic amount of dioxane. The bromo aldehyde **5** was reacted with formamide at high temperature according to Bredereck's method [14]. This synthetic scheme was



Scheme 1.

amenable to aldehydes and acids that were commercially or readily available. However, as the objective was to explore the structure–activity relationships of **6a** a more flexible synthetic scheme was required that would allow the synthesis of a range of compounds from a common intermediate. A retrosynthetic analysis showed that such an intermediate could be a nitrogen protected form of 4(5)-chloromethylimidazole [15]. The triphenylmethyl protecting group (trityl, Trt) was selected mainly because its bulk prevents the formation of mixtures of N^{τ} and N^{π} protected imidazoles [16]. These mixtures, if carried along the synthetic path, would make purification steps more difficult and analysis of the reaction mixtures ambiguous. Our main synthon was 4-chloromethyl-1-tritylimidazole **8a** [17], as well as analogs **8r, s** (scheme 2), derivatives **10a, b, c** (scheme 3) and **19** (scheme 4). Metalated forms of the various diphenylmethane derivatives were alkylated with the chloromethyl imidazol synthons. The first syntheses were carried out using the lithium salts obtained by reacting diphenylmethane derivatives with butyllithium in THF. When diphenylmethanes were bearing substituants other than alkyl, the use of sodium amide in ammonia was found to be necessary. In addition, when the specific chloromethylimidazole derivative was of limited availability, we tried to maximize the coupling yields by use of the corresponding diphenylmethyl cuprate reagent [18, 19]. The use of a cuprate nucleophile was critical in the synthesis of **21** in order to favor the coupling rather than the ß elimination leading to vinylimidazole (scheme 4). These different synthons (schemes 2, 3 and 4) allowed us to realize all of our target molecules

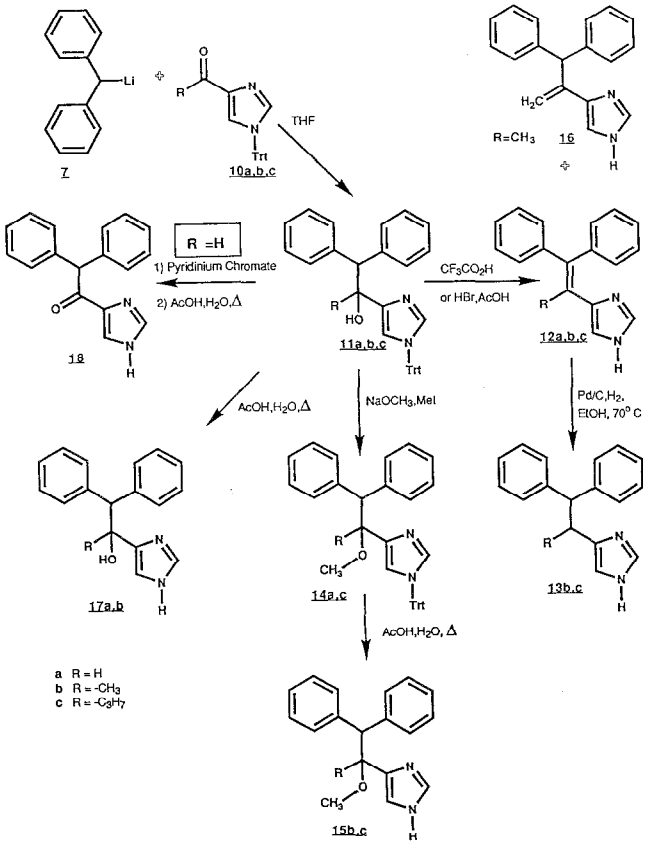


b X^1 = 2-CH_3; X^2, Y, R^1, R^2, R^3 = H
c X^1 = 3-CH_3; X^2,Y, R^1, R^2, R^3 = H
d X^1 = 4-CH_3; X^2, Y, R^1, R^2, R^3 = H
e X^1 = 2-CH_3, X^2 = 4-CH_3; Y, R^1, R^2, R^3 = H
f X^1 = 2-CH_3, X^2 = 5-CH_3; Y, R^1, R^2, R^3 = H
g X^1 = 3-CH_3, X^2 = 4-CH_3; Y, R^1, R^2, R^3 = H
h X^1 = 4-OCH_3; X^2, Y, R^1, R^2, R^3 = H
i X^1 = 4-OCH_3, Y = 4-OCH_3; X^2, R^1, R^2, R^3 = H
j X^1 = 2-F; X^2, Y, R^1, R^2, R^3 = H
k X^1 = 4-F; X^2, Y, R^1, R^2, R^3 = H
l X^1 = 2-F, X^2 = 6-F; Y, R^1, R^2, R^3 = H
m X^1 = 2-F, Y = 4-F; X^2, R^1, R^2, R^3 = H
n X^1 = 2-Cl; X^2,Y, R^1, R^2, R^3 = H
o X^1 = 4-C_6H_5; X^2,Y, R^1, R^2, R^3 = H
p X^1 = 2-CO_2H; X^2,Y, R^1, R^2, R^3 = H
q X^1, Y = 2-CH_2-CH_2-2; X^2, R^1, R^2, R^3 = H
r R^1 = -CH_3; X^1, X^2,Y, R^2, R^3 = H
s R^2 = -CH_3; X^1, X^2,Y, R^1, R^3 = H
t R^3 = -CH_3; X^1, X^2,Y, R^1, R^2 = H
u R^3 = -C_6H_5; X^1, X^2,Y, R^1, R^2 = H
v Aryl-Y = 2-pyridyl; X^1, X^2, R^1, R^2, R^3 = H
w Aryl-Y = 3-pyridyl; X^1, X^2, R^1, R^2, R^3 = H
x Aryl-Y = 4-pyridyl; X^1, X^2, R^1, R^2, R^3 = H

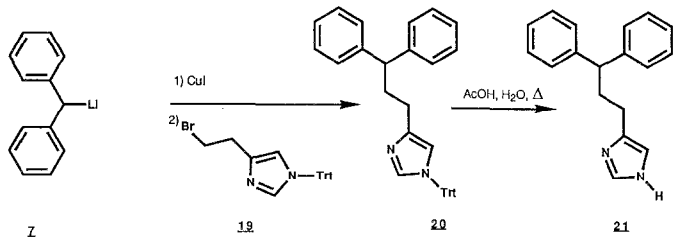
Trt = trityl, -$C(C_6H_5)_3$

Scheme 2.

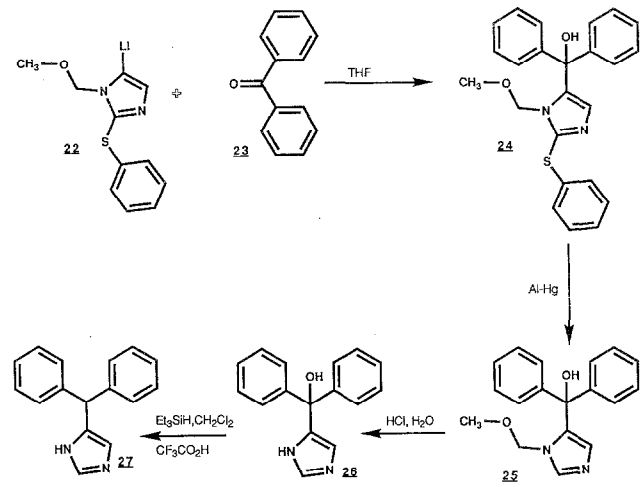
except **27**. To synthesize **27**, we relied on the use of a synthon developed by R Breslow [20] (scheme 5). In this synthesis, the reagents' reactivity was reversed, *ie* the imidazole fragment serves now as a nucleophile, while the diphenylalkyl partner reacts as the electrophile. Attempts to reverse the reactivity of an imidazole synthon by preparing a Grignard reagent from 4-chloromethyl-1-tritylimidazole **8a** were unsuccessful.



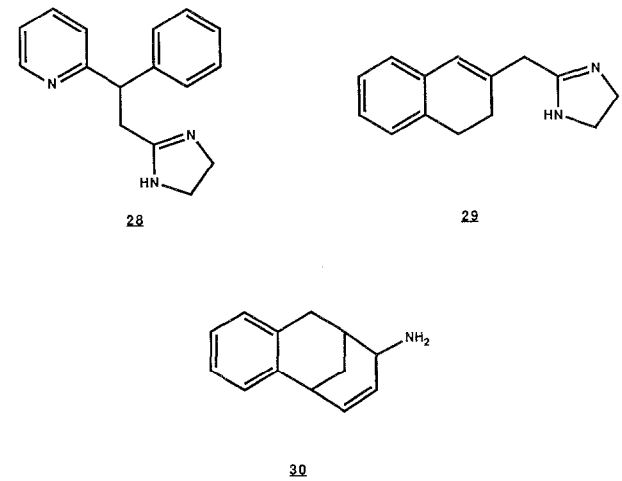
Scheme 3.



Scheme 4.



Scheme 5.



Pharmacology

α_1- and α_2-adrenoceptor binding constants were obtained by studying the displacement of [^{3}H]-WB-4101 (α_1) [21, 22] and [^{3}H]-*p*-aminoclonidine (α_2) [23] from rat forebrain membranes. New compounds were tested at 1 and 0.1 μM concentrations, and only compounds showing sufficient affinity were investigated further to define the pK_i (–log K_i). Compounds with an IC_{50} lower than 0.1 μM were tested in the electrically stimulated guinea pig ileum [24]. In this system, contractions of the longitudinal muscles were produced by acetylcholine released from the stimulated cholinergic nerves. Adrenergic stimulation by clonidine, an α_2-agonist, reduced the contraction induced by electrical stimulation. The adrenergic inhibition was selectively antagonized by α_2-

antagonists. This assay was therefore used to define the antagonistic or agonistic potency of the compounds tested. As the objective was to discover a new potential antidepressant, it was important to assess the bioavailability of the compounds and in particular their ability to cross the blood–brain barrier. To do so, we chose to investigate the effect of selected compounds on clonidine-induced hypoactivity [25]. Clonidine is an α_2-agonist which has various behavioral side-effects of central origin. In addition, to assess our compounds antidepressant potential, we studied the effect some of them had on head twitches induced by administration of 5-hydroxytryptamine (5-HTP) [26, 27], a precursor of serotonin. Finally, we investigated the effect of the compounds on the monoamine uptake in brain synaptosomes because this is thought to be the mode of action responsible for the efficacy of the tricyclic antidepressants. The brain synaptosomes were prepared according to the method of Wong [28] and the uptake inhibition was assessed according to the method of Kuhar [29].

Results and Discussion

The results of the biological evaluation are given in table I. In our search for structure–activity relationships, we investigated different ways of modifying the structure of **6a**: 1) modifications of the diphenylmethyl moiety (**6b** to **6g** and **6t** to **6x**); 2) modifications of the imidazole residue (**6r** and **6s**); 3) modifications of the link between the imidazole ring and the diphenylmethyl moiety (**12**, **13**, **15–18**, **21** and **27**).

Substitution of the imidazole ring imparted an important loss of activity, with methyl substitution in the 2 position (**6r**) being substantially more detrimental than in the 5(4) position (**6s**). Replacement of the imidazole ring by other basic heteroaromatic rings produced totally inactive compounds (results not shown). Any modification in the link between the diphenylmethyl residue and the imidazole ring led to a reduction in activity. When the link between the diphenylmethyl moiety and the imidazole was extended by one carbon (**21**), agonist properties became evident. By contrast, when the link was one carbon shorter (**27**), partial agonist character was manifested. This last discovery was not as puzzling as the first one, given that different agonists (clonidine [30], detomidine [31]) shared the same 1 atom connectivity between the basic residue (imidazoline or imidazole) and an aromatic ring. Substitution in the α position of the imidazole was detrimental whatever the substituent; ketone (**18**), hydroxyl (**17**), alkyl (**13**) or methoxy (**15a**). The design of these compounds was inspired by a recent publication which revealed increased activity for α-alkyl [32] and α-alkoxy [33] substituted idazoxan derivatives. In each of these cases, the idazoxan analogs has a quaternary carbon next to the imidazoline ring. In our case, the carbon was only trisubstituted. We therefore decided to synthesize a tetrasubstituted derivative (**15c**) which also proved inactive.

When a double bond was introduced in the α–ß position, the effect on the compound's activity varied according to the α substitution. The unsubstituted compound (**12a**) was less active than the methyl substituted one (**12b**), in contrast with data obtained with the saturated series where the unsubstituted derivative (**6a**) was much more potent than the methyl

Table I. Pharmacological activity *in vitro*. [a]Selectivity : –log ($pK_i\alpha_2 - pK_i\alpha_1$). [b]Agonistic effect: pD_2.

No	$pK_i\alpha_1$	$pK_i\alpha_2$	*Selectivity* [a]	pA_2 *Guinea pig ileum*	LD_{50} *(mg/kg)*
6a	6.25	8.16	81	8.73	155
6b	6.68	7.65	9	7.9	155
6c	6.53	8.08	35	7.8	155
6d	6.49	7.65	14	7.6	350
6e	6> <7	6.47	–	–	–
6f	<6	6.94	>9	7.00	>300
6g	6.54	7.71	15	7.80	155
6h	6.15	7.94	62	7.90	350
6i	<6	6.28	–	–	–
6j	6.36	8.37	102	–	94
6k	6.56	7.87	20	7.8	155
6l	6.23	7.98	56	–	173
6m	6.70	7.89	15	7.8	–
6n	6.38	8.02	44	8.0	172
6o	<6	6.37	–	–	>300
6p	<6	<6	–	–	>300
6q	6> <7	6.83	–	7.3	–
6r	<6	5.70	–	–	–
6s	<6	6.81	–	7.3	–
6t	<6	<6	–	–	–
6u	<6	<6	–	–	1750
6v	<6	7.14	>14	7.4	130
6w	5.57	8.32	562	–	175
6x	4.99	8.34	2238	–	–
12a	<6	6.60	–	–	–
12b	<6	7.38	>24	7.3	–
12c	<6	<6	–	–	270
13b	<6	6.37	–	6.5	–
13c	<6	6.19	–	–	350
15a	<6	<6	–	–	–
15c	<6	<6	–	–	>300
16	<6	6.70	–	6.8	–
17a	<6	5.09	–	–	>300
17b	<6	<6	–	–	–
18	<6	5.64	–	–	–
21	<6	6> <7	–	5.2[b]	–
27	<6	6> <7	–	6.0	>300
2	6.60	7.82	17	8.24	85

substituted example (**13b**). The reason for such behavior is not clear. However, given that the steric hindrance around the double bond is sufficient to prevent the 3 aromatic rings being coplanar and fully conjugated, one can postulate that the added crowding provided by the methyl group will favor an alternate conformation which is closer to the active conformation at the receptor site.

With regard to modifications at the level of the diphenylmethyl residue, tetrasubstitution of the ß carbon (**6t** and **6u**) results in a complete loss of activity. The monosubstitution of the phenyl rings by alkyl (**6b**, **c**, **d**), alkoxy (**6h**) and halogens (**6j**, **k**, **n**) gave products roughly equivalent in activity to **6a** regardless of the position of the substituent as well as its electronic character. By contrast, monosubstitution by an aryl (**6o**) or a carboxylic function (**6p**) abolished the activity. The effect of disubstitution of the phenyl rings is more subtle. In the case of dimethyl derivatives wherein the 2 substituents are on the same phenyl ring, the substitution pattern is critical in order to maintain a high activity level (**6e**, **f** *versus* **6g**). When the 2 substituents are on different phenyl rings, the nature and the pattern of substitution appears important; *eg* the di *para* methoxy derivative (**6i**) has very low activity while the *ortho*, *para* difluoro derivatives (**6m**) have very high activity. Bridging of the 2 phenyl rings with an ethylene residue (**6g**) deserves special comment. The tricyclic structure obtained is common to various antidepressant drugs which act through inhibition of norepinephrine of serotonin uptake. In addition to a marked decrease in activity, this compound also loses its selectivity for the α_2 binding site, becoming an equipotent ligand for the α_1, H_1 and muscarinic receptors (as defined by the displacement of the binding of [^{3}H] prazosin, pyrilamine and dexitimide respectively; data not shown). This lack of selectivity is also shared by the tricyclic antidepressants and is thought to be responsible for some of the side-effects observed with this class of therapeutic agents [34]. The last change in the structure of **6a** which was investigated was the replacement of a phenyl ring by a pyridine ring. This modification was inspired by literature reports indicating that DG 5128 (**28**), a compound developed as an hypoglycemic agent, has selective α_2-antagonist properties [35]. By contrast to what was observed in the monosubstituted derivatives, the position of the nitrogen not only affects the potency of the compound (**6v** *versus* **6w** and **6x**) but also its selectivity for the 2 α-receptors subtypes. It is worth mentioning that **6v**, which is the closest analog of DG 5128 with its pyridyl nitrogen in *ortho* position, has the weakest activity of the 3 isomers.

In a further investigation of the pharmacology of **6a**, the compound was tested for inhibition of centrally mediated behavioral effects of clonidine [17]. The results given in table II show that **6a** was able, at very low doses, to inhibit both the hypomotility and decrease in rearings induced by clonidine administration, thereby demonstrating its brain bioavailability. Morcover, in a test predictive of antidepressant efficacy (5-HTP induced head twitches), **6a** was more active than idazoxan.

The effect of different compounds on norepinephrine and serotonin uptake in brain synaptosomes is summarized in table III. Some of these molecules are both very potent α_2-antagonists and very good selective noradrenaline uptake blockers. As a result of their unique pharmacology, we expect the 2 pharmacological effects to work in synergy to increase the concentration of noradrenaline in the synaptic cleft in a functional way. This hypothesis is strengthened by the observation that in the rat, the coadministration of an uptake blocker and an α_2-antagonist, accelerates ß receptor desensitization [36, 37] a phenomenon potentially linked to the therapeutic efficacy of antidepressants.

Recently napemazole **29** [38] was shown to have a pharmacological profile close to that of **6a**, being both an α_2-antagonist and a serotonin uptake blocker.

Table II. *In vivo* pharmacology.

No	*Clonidine effect on:*		*100% increase in*
	hypomotility 100% abolished	*rearing at (mg/kg)*	*5-HTP twitches at (mg/kg)*
6a	3	3	1.9
2	3	3	7.5

Table III. Bioamine uptake inhibition *in vitro*.

No	*IC_{50} (µM)*	
	NA	*5HT*
6a	0.3	>10
6b	0.5	>10
6c	0.25	>10
6d	0.3	>10
6f	>10	>10
6g	0.35	0.28
6h	0.4	>10
6i	1.3	>10
6k	0.65	>10
6l	>10	>10
6m	0.4	>10
6n	0.17	>10
6v	>10	>10
6w	1.4	>10
6x	0.6	>10
12b	>10	>10
2	>10	>10

Organon has reported compounds with an ever closer profile: mianserin [39, 40] and Org-6906 **30** [41]. The latter compound is a specific α_2-antagonist and a noradrenaline uptake blocker devoid of any interaction with other receptors. Nevertheless, its level of activity as reported is an order of magnitude lower than that of **6a**.

Conclusions

Our search for an α_2-adrenoreceptor antagonist has led us to investigate a large series of diphenylethyl derivatives which are characterized not only by a high affinity and a high specificity to the α_2-receptor but also by a potent inhibition of the NA uptake process. Amongst the compounds tested, **6a** has been selected for further development.

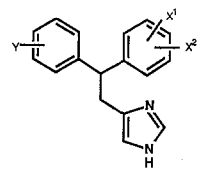
Experimental protocols

Chemistry

Reagents were commercially available and of synthetic grade unless otherwise stated.

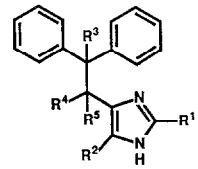
Proton NMR spectra were recorded on a Varian T60. Infrared spectra were obtained as KBr pellets on a Perkin–Elmer 457 spectrometer. Mass spectra were obtained on a Varian CH-7 spectrometer. All new substances exhibited spectroscopic data consistent with the assigned structures. Elemental analyses were performed on a Perkin–Elmer 240B Elemental Analyzer and, unless otherwise stated, agree with calculated values within ± 0.4%. When necessary, water content was determined by Karl–Fisher analysis. Melting point (mp) were obtained on a Mettler FPS and are uncorrected. Structures and chemical data of compounds **6**, **12**, **13**, **15**–**18**, are given in tables IV–VI.

Table IV. Chemical data of **6a**–**q**.



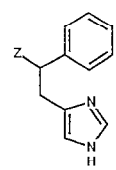
No	X_1	X_2	*Y*	*Synthetic route*	*Melting point*	*Crystallization solvent*	*Formula*
6a	H	H	H	1, 2a	157–158	toluene	$C_{17}H_{16}N_2$
6b	2-CH_3	H	H	2a	182	CH_3CN-Ether	$C_{18}H_{18}N_2$·HCl·0.7 H_2O
6c	3-CH_3	H	H	2a	140	isopropOH-Ether	$C_{18}H_{18}N_2$·HCl·0.8 H_2O2
6d	4-CH_3	H	H	2a	219	CH_3CN-Ether	$C_{18}H_{18}N_2$·HCl·0.7 H_2O
6e	2-CH_3	4-CH_3	H	2a	245–246	isopropOH	$C_{19}H_{20}N_2$·HCl
6f	2-CH_3	5-CH_3	H	2a	171	CH_3CN	$C_{19}H_{20}N_2$·HCl·0.5 H_2O
6g	3-CH_3	4-CH_3	H	2a	187	CH_3CN	$C_{19}H_{20}N_2$·HCl·0.2 H_2O
6h	4-OCH_3	H	H	2b	142	CH_3CN	$C_{18}H_{18}N_2O$
6i	4-OCH_3	H	4-OCH_3	2b	178–179	CH_3CN	$C_{19}H_{20}N_2O_2$·HCl·0.4 H_2O
6j	2-F	H	H	2b	161	CH_3CN	$C_{17}H_{15}FN_2$·HCl
6k	4-F	H	H	2b	157	CH_3CN	$C_{17}H_{15}FN_2$
6l	2-F	6-F	H	2b	193	CH_3CN	$C_{17}H_{14}F_2N_2$·HCl
6m	2-F	H	4-F	2b	141	CH_3CN-Ether	$C_{17}H_{14}F_2N_2$·HCl
6n	2-Cl	H	H	2b	159	CH_3CN-Ether	$C_{17}H_{15}ClN_2$·HCl·0.7 H_2O2
6o	4-C_6H_5	H	H	2b	192	CH_3CN	$C_{23}H_{20}N_2$
6p	2-COOH	H	H	2b	258	DMF	$C_{18}H_{16}N_2O_2$·HCl·0.2 H_2O
6q	H	2-CH_2-CH_2-2		2b	242	Toluene	$C_{19}H_{18}N_2$

Table V. Chemical data of **6r–u**, **12a–c**, **13b–c**, **15a**, **15c**, **16**, **17a**, **17c**, **18**.



No	*R1*	*R2*	*R3*	*R4*	*R5*	*Synthetic route*	*Melting point*	*Crystallization solvent*	*Formula*
6r	CH_3	H	H	H	H	2c	168–170	CH_3CN	$C_{18}H_{18}N_2$
6s	H	CH_3	H	H	H	2c	217–218	Ether	$C_{18}H_{18}N_2$
6t	H	H	CH_3	H	H	2a	153	CH_3CN	$C_{18}H_{18}N_2 \cdot 0.35\ H_2O$
6u	H	H	C_6H_5	H	H	2a	290–300	EtOH	$C_{23}H_{20}N_2 \cdot HCl$
12a	H	H	C=C		H	3	170–171	MeOH-H_2O	$C_{17}H_{14}N_2$
12b	H	H	C=C		CH_3	3	166	Toluene	$C_{18}H_{16}N_2$
12c	H	H	C=C		C_3H_7	3	187–188	EtOAc	$C_{20}H_{20}N_2$
13b	H	H	H	H	CH_3	3	190	Toluene	$C_{18}H_{18}N_2$
13c	H	H	H	H	C_3H_7	3	116–119	Heptane	$C_{20}H_{22}N_2 \cdot 0.25\ H_2O$
15a	H	H	H	OCH_3	H	3	177–179	CH_3CN	$C_{18}H_{18}N_2O$
15c	H	H	H	OCH_3	C_3H_7	3	161–163	CH_3CN	$C_{21}H_{24}N_2O$
16	H	H	H	$C=CH_2$		3	218	IsoPrOH	$C_{18}H_{16}N_2 \cdot HCl$
17a	H	H	H	OH	H	3	177–178	CH_3CN	$C_{17}H_{16}N_2O$
17b	H	H	H	OH	CH_3	3	183–184	Toluene	$C_{18}H_{18}N_2O$
18	H	H	H	=O		3	245	IsoPrOH–H_2O	$C_{17}H_{14}N_2O \cdot HCl \cdot H_2O$

Table VI. Structure and chemical data of **6v–w**.



No	*Z*	*Synthetic route*	*Melting point*	*Crystallization solvent*	*Formula*
6v	2-pyridyl	2a	108	toluene	$C_{16}H_{15}N_3$
6w	3-pyridyl	2b	138	AcOEt	$C_{16}H_{15}N_3$
6x	4-pyridyl	2b	152	AcOEt	$C_{16}H_{15}N_3 \cdot 0.15\ H_2O$

Route 1

2-Bromo-4,4-diphenylbutanal ***5***
4,4-Diphenylbutanal(45.6 g, 0.2 mol) was dissolved, under nitrogen, in anhydrous ether (200 ml) containing dioxane (0.7 ml). A few drops of bromine were added to the solution and when the color disappeared, bromine (10.42 ml, 0.2 mol) was added dropwise over 90 min at such a rate that the solution remained colorless. The reaction mixture was then neutralized using a saturated solution of sodium carbonate. The ethereal phase was separated, washed 3 times with water (50 ml), dried over magnesium sulfate and evaporated in the dark, under reduced pressure. The colorless oil was used immediately without further purification in the preparation of **6a**.

4(5)-(2,2-Diphenylethyl)imidazole ***6a***
Formamide (470 ml) was heated to 160°C under nitrogen. The bromo aldehyde **5** was then added. The mixture was heated for 4 h at this temperature, then cooled and poured into a mixture of ice and water (1 l). The solution was adjusted to pH 2 by addition of concentrated HCl. The aqueous phase was extracted twice with portions of dichloromethane (100 ml) and made alkaline (pH 10) by addition of 4 N NaOH. The white solid which formed was filtered and recrystallized from toluene. Yield: 15 g (30.5% from the aldehyde). Mp: 158°C. Anal ($C_{17}H_{16}N_2$) C, H, N.

Route 2b

2,4'-Difluorodiphenylmethane ***7m***
To a suspension of 10% palladium on carbon (1.1 g) in ethanol (100 ml) was added a solution of 2,4'-difluorobenzophenone (10.9 g, 50 mmol) in ethanol (100 ml) containing methanol saturated with HCl (1 ml). The mixture was hydrogenated under a pressure of 2.75 bars for 2 h at 20°C. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. **7m** was purified by distillation under reduced pressure; 66–70°C/0.4 mbar.

4-{[2-(2-Fluorophenyl)-2-(4'-fluorophenyl)]-ethyl}-1-trityl-imidazole ***9m***
Small amounts of sodium were added to a reactor containing liquid ammonia (50 ml) under a slight flow of nitrogen, until a persistent blue color was obtained. A few crystals of Fe III nitrate were added to the solution, followed by a further amount of metallic sodium (253 mg, 11 mmol). The suspension was stirred at –75°C for 30 min. A solution of **7m** (2.04 g, 10 mmol) in anhydrous ether (5 ml), was then added. After an additional half hour, a solution of 4-chloromethyl-1-tritylimidazole **8a** [17] (3.21 g, 9 mmol) in THF (20 ml) was added. The ammonia was allowed to evaporate and water (30 ml) was added to the residue. The aqueous phase was then extracted 3 times with dichloromethane (10 ml). The combined extracts were dried over magnesium sulfate and evaporated to dryness under reduced pressure. **9m** was used in the following step without further purification.

4(5)-{[2-(2-Fluorophenyl)-2-(4'-fluorophenyl)]-ethyl}-imidazole ***6m***
The tritylated derivative **9m** was mixed with 90% aqueous acetic acid (20 ml), heated to reflux for 5 min and then evaporated to dryness under reduced pressure. The residue was dissolved in 5% aqueous sodium bicarbonate (10 ml) and extracted 3 times with dichloromethane (10 ml). The organic extracts were combined, dried over potassium carbonate and evaporated. The residue was dissolved in ether and the solution was saturated with anhydrous gaseous hydrochloric acid. The precipitated hydrochloride salt was filtered and precipitated from acetonitrile by the addition of ether. Yield: 1.50 g (62%). Mp: 141°C. Anal ($C_{17}H_{14}N_2F_2$·HCl) C, H, N.

Route 2c

4-Hydroxymethyl-5-methyl-1-tritylimidazole
Chlorotriphenylmethane (71.6 g, 0.26 mol) was added portionwise under nitrogen to a solution of 4(5)-hydroxymethyl-5(4)-methylimidazole [42] (38.2 g, 0.26 mol) and triethylamine (75 ml) in anhydrous DMF (150 ml), cooled at 0°C. The reaction mixture was stirred for 16 h at room temperature, and then poured into water (1.2 l). The water phase was extracted 3 times with chloroform (150 ml). The organic phase was washed with water (100 ml) and dried over anhydrous magnesium sulfate. The chloroform was evaporated under reduced pressure and the residue was dissolved in ether (1 l). The white solid which crystallized upon cooling (0°C) was filtered and washed with hot isopropanol and ether. Yield: 9.3 g (10%). Mp: 231–232°C.

4-Chloromethyl-5-methyl-1-tritylimidazole ***8s***
Thionyl chloride (0.41 ml, 5.6 mmol) was added dropwise to a solution of 4-hydroxymethyl-5-methyl-1-tritylimidazole (2 g, 5.6 mmol) and triethylamine (0.83 ml, 6.5 mmol) in anhydrous benzene (28 ml). After 45 min of stirring at room temperature, the solution was filtered and the solid washed with benzene. The combined organic phases were dried over calcium chloride and evaporated under reduced pressure. **8s** was obtained in the form of a yellow solid which was used in the following step without further purification.

4-(2,2-Diphenylethyl)-5-methyl-1-tritylimidazole ***9s***
A 0.5 M solution (22.5 ml, 11.25 mmol) of the lithium salt of diphenylmethane (prepared from diphenylmethane and butyllithium) in THF was added dropwise to a suspension of cuprous cyanide (0.5 g) in anhydrous THF (10 ml) cooled to –78°C. The reaction mixture was then allowed to warm to room temperature for one min. It was then recooled to –78°C; and a solution of **8s** (2 g, 5.6 mmol) in anhydrous THF (10 ml) was added. After stirring for 1 h at –78°C, the reaction mixture was kept at –20°C for 48 h. A 10% aqueous ammonia solution (30 ml) saturated with ammonium chloride was then added and the mixture was extracted with ether. The organic phase was washed with water, dried over potassium carbonate and evaporated under reduced pressure. The residual oil was dissolved in heptane and cooled with an ice bath. The yellow solid which precipitated was collected and crystallized in isopropanol. Yield: 0.89 g (31%). Mp: 205–206°C.

4(5)-(2,2-Diphenylethyl)-5(4)-methylimidazole ***6s***
A solution of 4-(2,2-diphenylethyl)-5-methyl-1-tritylimidazole **9s** (0.83 g, 1.65 mmol) in 90% acetic acid (20 ml) was heated to reflux for 15 min. The resulting clear solution was then poured into a mixture of ice and water (50 ml) and extracted with dichloromethane. The water phase was made alkaline by portionwise addition of a 10 N sodium hydroxide solution and extracted with chloroform. The chloroform was evaporated, the residue dried by azeotropic distillation of toluene and the residual oil dispersed in ether where it crystallized as a white solid. Yield: 0.23 g (53%). Mp: 217–218°C. Anal ($C_{18}H_{18}N_2$) C, H, N.

Route 2a

4-(2,2-Diphenylethyl)-2-methyl-1-tritylimidazole ***9r***
Under a nitrogen atmosphere, butyllithium (16.8 ml, 1.7 M in hexane) was added to diphenylmethane (4.3 g, 26 mmol) dissolved in THF (50 ml). The cooled red solution (ice bath) was stirred for 15 min and 4-chloromethyl-2-methyl-1-trityl-imidazole **8r** [43] (5 g, 12.9 mmol) in THF (30 ml) was added. After stirring overnight at room temperature, a saturated aqueous solution of sodium chloride (40 ml) and water (100 ml) were successively added to the reaction mixture. The aqueous phase was extracted 3 times with dichloromethane (50 ml), dried over magnesium sulfate and evaporated. The residue was used without further purification in the following step.

4-(2,2-Diphenylethyl)-2-methylimidazole ***6r***
The tritylated derivative **9r** was heated to reflux for 5 min in 90% acetic acid (100 ml). The solution was then evaporated to dryness under reduced pressure. Water (200 ml) was added to the residue and the resulting suspension was filtered. The filtrate was neutralized with a 5% aqueous sodium bicarbonate solution, then extracted 3 times with dichloromethane (50 ml). The combined organic phases were dried over potassium carbonate, evaporated under reduced pressure and the solid crystallized from acetonitrile. Yield: 1.9 g (56%). Mp : 168–170°C. Anal ($C_{18}H_{18}N_2$) C, H, N.

Route 2b

4-[2-Phenyl-2-(4-pyridinyl)ethyl]-1-tritylimidazole ***9x***
Under a nitrogen atmosphere, a 100 ml flask was charged with liquid ammonia (50 ml), a small piece of sodium and a few crystals of $Fe(NO_3)_3$. Sodium metal (0.25 g, 11 mmol) was then added and the mixture was stirred at –70°C for half an hour. At this temperature and under stirring, 4-benzylpyridine (1.69 g, 10 mmol) dissolved in anhydrous THF (5 ml) was added dropwise over 10 min. After stirring for an additional half hour, the mixture was allowed to warm to reflux (ammonia) and 4-chloromethyl-1-tritylimidazole **8a** (3.21 g, 9 mmol) dissolved in anhydrous THF (20 ml) was added dropwise. The mixture was allowed to warm to room temperature while the ammonia evaporated. Water (30 ml) was added to the residue and the aqueous phase extracted with 3 portions (30 ml) of dichloromethane. The combined organic extracts were dried over potassium carbonate and evaporated to dryness under reduced pressure. The compound was used in the following step without further purification.

4(5)-[2-Phenyl-2-(4-pyridinyl)ethylimidazole ***6x***
The crude derivative **9x** was hydrolyzed by heating to reflux for 5 min in 90% aqueous acetic acid (20 ml). After evaporation of the solvent, the solid was treated with water. The suspension was filtered and the filtrate neutralized with aqueous sodium bicarbonate and extracted with 3 portions (10 ml) of dichloromethane. The combined organic phases were dried over potassium carbonate, evaporated to dryness under reduced pressure and the solid was recrystallized from ethyl acetate. Yield: 0.6 g (27%). Mp: 152°C. Anal ($C_{16}H_{15}N_3$·O·15 H_2O) C, H, N.

Route 3

4-Butanoyl-1-tritylimidazole ***10c***
An iodine crystal was added to magnesium turnings (0.8 g, 32.6 mmol) kept under a nitrogen atmosphere. A solution of 1-bromopropane (4 g, 2.95 ml, 32.6 mmol) in anhydrous diethyl ether (25 ml) was then added at such a rate that the mixture was kept at reflux temperature. After the addition and the resulting exotherm were completed, the mixture was cooled by immersion in an ice bath. A solution of 1-trityl-4-imidazolecarboxaldehyde [17] (5.5 g, 16.3 mmol) in THF (50 ml) was then added slowly. The mixture was stirred for 2 h at room temperature and then a saturated aqueous ammonium chloride solution (100 ml) added. The aqueous phase was extracted 3 times with ether (50 ml). The organic phases were dried over magnesium sulfate and evaporated under reduced pressure. The residue which crystallized spontaneously was recrystallized from ethyl acetate. Yield: 4.75 g (76%). Mp: 154–155°C. 4-(1-Hydroxy-butyl)-1-tritylimidazole (4.75 g, 12.4 mmol) dissolved in dioxane (150 ml) was brought to reflux with manganese dioxide (11 g, 124 mmol) for one hour. The solid was filtered through a Celite layer and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized in cyclohexane. Yield: 4.4 g (93%). Mp 134–136°C.

4-[2-(1,1-Diphenyl-2-hydroxy)-pentyl]-1-tritylimidazole ***11c***
Butyllithium (16.7 ml, 1.7 M) in hexane was added to a solution of diphenylmethane (4.3 g, 25.8 mmol) in THF (50 ml), under a nitrogen atmosphere at 0°C. **10c** (4.9 g, 12.9 mmol) was added slowly to the red solution. The reaction mixture was stirred for 2 h at room temperature and then a saturated aqueous ammonium chloride solution (50 ml) was added. The aqueous phase was extracted with 3 fractions (50 ml) of ethyl acetate. The solvent was dried over magnesium sulfate and evaporated to yield a solid which was recrystallized from cyclohexane. Yield: 5.6 g (79%). Mp: 196–198°C.

4(5)-[(2,2-Diphenyl-1-n.propyl)-ethenyl]imidazole ***12c***
Compound **11c** (5 g, 9.1 mmol) was heated at reflux temperature in 90% acetic acid (20 ml) for 1 h. The solution was then evaporated to dryness under reduced pressure, diluted with water, neutralized by the addition of a 5% sodium bicarbonate solution, and extracted by 3 portions (50 ml) of dichloromethane. The organic phase was dried over potassium carbonate and evaporated under reduced pressure. The residue dissolved in dry ether was precipitated as the hydrochloride salt by addition of a HCl saturated ether solution. The solid was dissolved in HBr saturated acetic acid (110 ml) and stirred at room temperature for 16 h. The solution was then diluted with water (100 ml) and neutralized by addition of 1 N NaOH. The aqueous phase was extracted by 3 portions (100 ml) of ether. The combined ether extracts were dried over magnesium sulfate and evaporated under reduced pressure. The resulting solid was recrystallized from ethyl acetate. Yield: 1.7 g (67%). Mp: 187–188°C. Anal ($C_{20}H_{20}N_2$) C, H, N.

4(5)-[2-(1,1-Diphenyl)-pentyl]imidazole ***13c***
Compound **12c** (0.8 g, 2.8 mmol) was hydrogenated in ethanol (200 ml) for 6 h in the presence of 10% palladium on carbon (0.13 g), at 2.72 bar and 70°C. The suspension was filtered and the filtrate evaporated to dryness under reduced pressure. Ether was added to the residue and the insoluble material was discarded. The ether phase was evaporated and the solid recrystallized from heptane. Yield: 0.67 (83%). Mp: 116–119°C. Anal ($C_{20}H_{22}N_2$) C, H, N.

4-[2-(1,1-Diphenyl-2-methoxy)-pentyl]-1-tritylimidazole ***14c***
Compound **11c** (6 g, 11 mmol) was dissolved in THF (60 ml)

under a nitrogen atmosphere. Butyllithium (7 ml, 1.7 M in hexane) was added dropwise to this solution cooled in an ice bath. Iodomethane (3.12 g, 1.38 ml, 22 mmol) was then added. The mixture was stirred at room temperature for half an hour before addition of a saturated aqueous ammonium chloride solution (50 ml). The aqueous phase was extracted 3 times with portions (30 ml) of diethyl ether. The organic phase was dried over magnesium sulfate and evaporated under reduced pressure. The residue was used directly in the following step.

4(5)-[2-(1,1-Diphenyl-2-methoxy)-pentyl]imidazole ***15c***
Compound **14c** was treated with 66% aqueous acetic acid (30 ml) and this mixture was heated to reflux until complete dissolution. The solution was then cooled in an ice bath and the precipitate which formed was filtered. The filtrate was neutralized with 5% aqueous sodium bicarbonate and extracted 3 times with portions (50 ml) of ether. The combined organic extracts were dried over magnesium sulfate and evaporated to dryness under reduced pressure. The resulting solid was recrystallized from acetonitrile. Yield: 0.81 g (23%). Mp: 161–163°C. Anal ($C_{21}H_{24}N_2O$) C, H, N.

4(5)-(2,2-Diphenylacetyl)imidazole ***18***
CrO_3 (2.77 g, 27.7 mmol) was added to a solution of pyridine (4.47 ml, 56 mmol) in acetonitrile (400 ml) and stirred for 0.5 h. 4-(1,1-Diphenyl-2-hydroxyethyl)-1-tritylimidazole **11a** (0.5 g, 1 mmol) was added along with Celite (14 g) and the suspension was stirred for an additional 1.5 h. Sodium bicarbonate (6.9 g, 66.3 mmol) was then added and the reaction mixture was filtered. The yellow filtrate was evaporated under reduced pressure, dissolved in dichloromethane (100 ml) and then silica gel (40 g) and magnesium sulfate (40 g) added. The resulting mixture was stirred for 5 min and filtered. The filtrate was evaporated and the resulting solid was recrystallized from heptane. This ketone (340 mg, 0.7 mmol) was dissolved in 90% acetic acid (20 ml) and brought to reflux for one min. The solvent was evaporated to dryness under reduced pressure, dissolved in water saturated with sodium carbonate and extracted with 3 portions of dichloromethane (10 ml). After evaporation of the solvent, the residue dissolved in ether was precipitated as the hydrochloride salt by addition of a HCl saturated ether solution. The solid was recrystallized twice from isopropanol–water. Yield: 110 mg (49%). Mp: 245°C. Anal ($C_{17}H_{14}N_2O{\cdot}HCl{\cdot}H_2O$) C, H, N.

Route 4

4-(2-Chloroethyl)-1-tritylimidazole ***19***
4(5)-(2-Chloroethyl)imidazole [44] (7 g, 54 mmol) was dissolved in DMF (60 ml) containing triethylamine (8 ml) and trityl chloride (15 g, 54 mmol). After stirring for 4 h at room temperature, the reaction mixture was poured onto ice-water and the resulting solid was filtered, dissolved in dichloromethane (100 ml), washed with 2 portions (30 ml) of water, dried over magnesium sulfate and evaporated under reduced pressure. The resulting semi-solid was purified by chromatography on silica gel (300 g) by elution with dichloromethane followed by a mixture of dichloromethane–methanol 5%. The product which eluted with this solvent mixture was recrystallized from methanol. Yield: 12 g (60%).

4-(3,3-Diphenylpropyl)-1-tritylimidazole ***20***
$CuBr{\cdot}Me_2S$ (1.2 g, 5.7 mmol) was suspended in ether (10 ml). This mixture become homogeneous when Me_2S (7 ml) was added. When the solution was cooled to –55°C, a white precipitate appeared. A diphenylmethyllithium solution (23 ml, 0.5 M) was then added. The orange red solution turned green and **19** (2.13 g, 5.7 mmol) in THF (10 ml) was slowly added dropwise. The reaction mixture was warmed to –15°C. The green color faded and a black solid formed. The reaction was quenched by addition of a saturated aqueous ammonium chloride solution (10 ml). The 2 phases were filtered through a Celite layer. The black solid was washed with water. The 2 phases were separated and the ether phase was washed with 2 portions (10 ml) of concentrated ammonia and 1 portion of water. The organic phase was dried over calcium sulfate, evaporated and purified by chromatography on silica gel (300 g) with a gradient elution of hexane/acetone from 9/1 to 9/3. The pure product was isolated as an oil (yield: 1.41 g, 50%).

4(5)-(3,3-Diphenylpropyl)imidazole ***21***
20 (800 mg, 1.6 mmol) was brought to reflux in 90% acetic acid (50 ml) for 2 min. The reaction mixture was poured onto ice (100 ml). The solid was filtered and the filtrate made basic by addition of 1 N NaOH. The aqueous phase was extracted by 3 portions of dichloromethane (25 ml), the organic solution was dried over potassium carbonate and evaporated under reduced pressure. The solid obtained was crystallized once from toluene and once from acetonitrile. Yield: 90 mg (21%). Mp: 120°C. Anal ($C_{18}H_{18}N_2$) C, H, N.

Route 5

4-Diphenylhydroxymethyl-1-methoxymethyl-2-phenylthioimidazole ***24***
Butyllithium (32 ml, 1.6 M in hexane) was added to diisopropylamine (7 ml, 50 mmol) in THF (150 ml). This solution was added dropwise to 1-methoxymethyl-2-phenylthioimidazole **22** [20] (8.8 g, 40 mmol) in THF (400 ml) held at –65°C by a dry ice–acetone mixture. After 30 min stirring, benzophenone (9.1 g, 50 mmol) was added at the same temperature. When the reaction temperature reached 20°C, a saturated aqueous ammonium chloride solution (50 ml) was added and the organic phase was separated and evaporated under reduced pressure. The oil was stirred into a mixture of 3 N HCl–ether. The solid which precipitated, was filtered. Yield: 8.92 g (50.7%). Mp: 126–127°C. The hydrochloride salt was transformed into the free base by dissolution in 10% NH_4OH and extraction with ether.

4-Diphenylhydroxymethyl-1-methoxymethyl-imidazole ***25***
24 (9.9 g, 24.6 mmol) was added portionwise very slowly to 27 g of aluminium amalgam suspended in 50% aqueous ethanol (700 ml). After 42 h, the suspension was filtered through a Celite layer and the filtrate evaporated to dryness under reduced pressure. The resulting solid was recrystallized from cyclohexane–toluene. Yield: 4.45 g (61.5%). Mp: 109–111°C.

4(5)-Diphenylhydroxymethylimidazole ***26***
25 (4.45 g, 15 mmol) was heated to reflux in 5 N HCl (100 ml) for 20 h. Upon cooling, a white solid appeared which was filtered and recrystallized from acetonitrile. Yield: 1.88 g (44.8%). Mp: 198–200°C. Anal ($C_{16}H_{14}N_2O{\cdot}HCl{\cdot}0.8\ H_2O$) C, H, N.

4(5)-Diphenylmethylimidazole ***27***
The hydrochloride salt of **26** (1.5 g, 5 mmol) was dissolved at room temperature in dichloromethane (60 ml). Triethylsilane (6.3 ml, 40 ml) was then added along with trifluoroacetic acid (12.6 ml, 160 mmol). The resulting clear solution was stirred

for 7 h. Water was added and the reaction mixture neutralized by adding solid sodium bicarbonate. The organic phase was separated, washed with water, dried over magnesium sulfate and evaporated. The solid residue was recrystallized from a minimum of toluene. Yield: 630 mg (54.8%). Mp: 209–210°C. Anal ($C_{16}H_{14}N_2$) C, H, N.

Biology

α_1 and α_2 Binding assay procedure

Male or female Sprague–Dawley rats were decapited, the brain was removed and the forebrain (whole brain minus cerebellum) immediately dissected and homogenized with an Ultraturrax in 10 vol 20 mM Tris–HCl pH 7.4 containing 1 mM dithioerythritol and 0.25 M sucrose. After filtration through 2 layers of sterilized gauze, the homogenate was centrifuged at 17000 rpm (35000 *g*) for 10 min in a SS-34 superspeed Sorvall rotor, cooled to 4°C. After homogenization with an Ultraturrax, the pellet was washed with the same buffer and centrifuged again at 35000 *g* for 10 min. The pellet was suspended using a Potter homogenizer in 4 vol of the above buffer and kept frozen in 5 ml aliquots at –20°C. Incubation was performed as follows: to each tube (12 x 7 mm) is added successively 0.6 ml of 20 mM Tris–HCl pH 7.4 containing 20 mM of $MgCl_2$, 0.36 ml bidistilled water, 0.08 ml of the [^{3}H]-ligand (24 nM [^{3}H]-WB-4101 for α_1 and 10.5 nM [^{3}H]-*p*-aminoclonidine for α_2), 0.12 ml of the compound to be tested at a concentration 10-fold higher than the final concentration, and finally 0.04 ml of the thawed crude forebrain preparation kept at 0°C. Each sample (1.2 ml) was then incubated immediately for 15 min at 25°C. The incubation mixture was diluted with 3 ml ice-cold homogenization buffer (20 mM Tris–HCl pH 7.4, 1 mM dithioerythritol, 0.25 M sucrose, enriched with 1% bovine serum albumin), filtered on glass fiber filter GF/C (Whatman) and rinsed twice with 3 ml of the ice-cold buffer. The filters were dried for 20 min at 60°C and counted for radioactivity in 10 ml of non-aqueous liquid scintillation cocktail (Lipoluma, Lumac) with an efficiency of 50–55%. The IC_{50} were estimated graphically from inhibition curves comprising about 5–6 points, each representing the mean value of duplicates. K_i values were calculated according to the Chen and Prussof [45] equation, using IC_{50} values from at least 3 independent assays. The results are expressed as $pK_i = -\log K_i$. In some cases enough experiments ($N > 5$) were conducted to calculated the corresponding standard deviations which were in the ± 54% of the mean value.

LD_{50}

The acute toxicity of the compounds was studied after oral administration to mice. The compounds to be tested were suspended in a 1% tragacanth gum mucilage. They were administered by means of an intragastric tube to groups of 3 male CD1 mice, weighing between 22–33 g, housed in the same environment for at least 1 week and fasted overnight. The doses tested were selected between 3000 and 3 mg/kg based on the effect observed. Mice were observed regularly for 15 days. The LD_{50} were calculated according to the Lichtfield and Wilcoxon [46] method and expressed in mg/kg.

Guinea pig ileum

Male guinea pigs weighing between 300–400 g were killed by cervical dislocation and exsanguination. The ileum was quickly removed, placed in oxygenated Krebs solution and the luminal content carefully washed. Four 3 cm segments were cut from the distal end of the ileum and were individually mounted between platinum wire electrodes in 25 ml baths containing Krebs solution at 37°C and gassed with 95% O_2–5% CO_2. Both ends of the segments remained open. Each segment was fixed to a Gould Statham UC3 strain gauge connected to a Kipp and Zonen recorder. The resting tension was adjusted to 1 g throughout the experiment. After a 45 min stabilization period, the preparation was exposed to electrical field stimulation (0.1 Hz, 3 ms). When twitch responses to electrical stimulation had stabilized, a maximal concentration of clonidine (300 nM) was added to the bath and the effect was allowed to stabilize. The bath was then rinsed and after the twitch responses had recovered, a cumulative con-centration–response curve to clonidine was constructed (0.1–300 nM). The bath was rinsed again, the compound was added and a new concentration–response curve to clonidine was constructed in the presence of the compound. This procedure was repeated twice with increasing concentrations of the compound. The displacement to the left or to the right of the dose-response curve of clonidine is considered as indicative respectively of an agonist or antagonist behavior. The pA_2 was calculated according to Van Rossum [47] with maximum standard deviation of ± 1%.

Uptake inhibition

Sprague–Dawley CD rats weighing between 180–220 g were used. The animals were decapited and the brain removed. The brain tissue was homogenized in 0.32 M sucrose (pH 7.4 by the addition of Tris–HCl) at 0°C with a Teflon glass homogenizer. All further procedures were carried out at 0–5°C. Tissue homogenates (10% W/V) were centrifuged at 1 000 *g* for 10 min. The supernatant was decanted and centrifuged at 10 000 *g* for 15 min. The pellet was resuspended in 2 ml of the sucrose solution and recentrifuged at 10 000 *g* for an additional 15 min. The pellet corresponding to each brain was gently suspended in 35 ml of a phosphate buffer (pH 7) containing Na_2HPO_4 (15 mM), NaH_2PO_4 (15 mM), NaCl (100 mM), KCl (5 mM), glucose (10 mM), sucrose (40 mM), pargiline (12.5 µM), ascorbic acid (142 µM). To 1 ml of this suspension, the test compound was added (0.02 ml of DMSO solutions ranging from 10^{-5} to 10^{-8} M) and the tube preincubated at 37°C for 5 min. A solution of [^{3}H] amine (5-HT or NA, 0.02 ml of a 2.6 10^{-6} M solution in water) was then added and the incubation was pursued for 5 min. 2 ml of ice-cold phosphate buffer were then added and the suspension was filtered on glass filter (GF/B). The filter was washed twice with the buffer and dried at 60°C for 30 min in the scintillation vials. 10 ml of scintillation cocktail (Lipoluma) was then added and the vials counted. The concentration of these test compounds producing 50% inhibition of neurotransmitter uptake (IC_{50}) was estimated graphically from log dose–response curves based on at least 5 concentrations, with at least 2 determinations for each concentration. The data given in table VI represent the mean of 2–3 runs with a standard deviation of ± 17%.

Inhibition of clonidine-induced hypomotility

Thirty min after administration of 0.15 mg/kg of clonidine, the mice were placed in a rectangular open field of 47 x 53 cm, whose floor was divided into 36 boxes of ≈ 8 x 9 cm each. The number of boxes through which the animal went in 3 min and the number of rearing episodes were noted. The compounds to be tested were administered at doses of 1–10 mg/kg *po* to groups of 5 CD1 mice, weighing between 22–33 g and fasted overnight. The results are expressed as % inhibition of the clonidine effect on either the locomotor activity or the rearings. The results were analyzed using variance analysis and Dunnett's *t*-test or two-tailed Student's *t*-test, when group size was unequal.

Potentiation of 5-HTP induced head twitches
Nialamide (a MAO inhibitor, 50 mg/kg ip) was administered, 22 h before L-5-HTP, to groups of 10 CD1 mice, weighing between 22 and 33 g and fasted overnight. The compounds to be tested were administered at doses of 1–30 mg/kg *po* 2 h before the administration of L-5-HTP (10 mg/kg ip). The head twitches were recorded for 45 min starting 15 min after the I-5-HTP challenge. The results are expressed as % increase in the number of head twitches over control values. Statistical significance was determined by the 2-tailed Student's *t*-test.

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