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Original article

Conformationally restrained analogues of sympathomimetic catecholamines
Synthesis and adrenergic activity of 5,6- and 6,7-dihydroxy-3,4-dihydrospiro[naphthalen-1(2H)-2′,5′-morpholines]☆

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Abstract

The 5,6- (**10a**) and 6,7-dihydroxy-3,4-dihydrospiro[naphthalen-1(2H)-2′,5′-morpholine] (**11a**) and their *N*-isopropyl derivatives (**10b** and **11b**) (DDSNMs), which can be viewed as the result of the combination of the structure of the 2-(3,4-dihydroxyphenyl)morpholine **5a** or **5b** (DPMs) with the structure of the corresponding 1-(aminomethyl)-5,6-dihydroxy- (**8a** or **8b**) or 1-(aminomethyl)-6,7-dihydroxy-1,2,3,4-tetrahydro-1-naphthalen-ol (**9a** or **9b**) (1-AMDTNs) were synthesised. The new compounds DDSNMs **10a**,**b** and **11a**,**b** were assayed for their α- and β-adrenergic properties by means of binding experiments and functional tests and the results were compared with those obtained for catecholamines **1a**, **b** and the previously described morpholine (**5**) and tetrahydronaphthalene (**8**, **9**) derivatives. The affinity and activity indices thus obtained indicate in general a low ability of the new compounds **10** and **11** to interact with the α- and β-adrenoceptors, which, in all cases, was lower than that of the corresponding morpholine (**5**) and tetrahydronaphthalene (**8**, **9**) analogues.

Keywords: catecholamines; α- and β-adrenoceptors; morpholine; *N*-isopropyl derivatives

1. Introduction

One of the methods most frequently used in order to obtain information about the pharmacophoric conformation of conformationally mobile drugs consists of the synthesis and the study of the pharmacological properties of their analogues in which the presumed pharmacophoric groups are introduced into rigid or semirigid structures [2–14].

Natural catecholamines and the structurally related adrenergic drugs are flexible molecules which, at the moment of interacting with the receptor, may assume

Abbreviations: NE, norepinephrine; ISO, isoprenaline; 1-AMDICs, 1-(aminomethyl)-5,6- and 1-(aminomethyl)-6,7-dihydroxyisochromane; 3-DPPs, 3-(3,4-dihydroxyphenyl)-3-piperidinols; 2-DPMs, 2-(3,4-dihydroxyphenyl)morpholines; 2-ADTNs, 2-amino-5,6- and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalen-1-ol; 1-AMDTNs, 1-(aminomethyl)-5,6- and 1-(aminomethyl)-6,7-dihydroxy-1,2,3,4-tetrahydro-1-naphthalen-ol; DDSNMs, 5,6- and 6,7-dihydroxy-3,4-dihydrospiro[naphthalen-1(2H)-2′,5′-morpholine]; DHA, dihydroalprenolol; NOE, nuclear Overhauser effect.

☆ 21st paper in the series *Conformational effects in the activity of drugs*. For preceding paper see Ref. [1].

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different spatial arrangements of the presumed active groups (aryl moiety, amino nitrogen and alcoholic hydroxyl) by rotation around the single C_α–C_1 and/or C_1–C_2 bond [15–19]. The exploitation of the method of the conformationally restrained analogues on this class of drugs has led to the synthesis of numerous semirigid and rigid compounds, some of which have been found to maintain, towards one or more subtypes of adrenergic receptors, the adrenergic properties of the corresponding conformationally free drugs [3,4,7,8,11–14]. Our contribution in this context has been the synthesis and the study of some types of semirigid analogues of norepinephrine (NE, **1a**) and isoproterenol (ISO, **1b**), in which the conformational freedom of catecholamines **1a** and **1b** was limited around the single carbon–carbon C_α–C_1 bond, as in 1-AMDIC derivatives **2** and **3** by means of an isochromanic structure [12], around the C_1–C_2 bond, as in 3-DPP derivatives **4** by means of a piperidinic structure [11], and in 2-DPM derivatives **5** with a morpholinic structure [11], or around both the C_α–C_1 and C_1–C_2 bonds, as in 2-ADTN derivatives **6** and **7**, by means of a tetrahydronaphthalenic structure [11] (Fig. 1).

By superimposing the stereostructures of the molecules that proved to be biopharmacologically active in their low-energy conformations allowing the spatial coincidence of the pharmacophoric groups, it was possible to construct steric models for interaction with the α_1-, α_2- and β-adrenergic receptors [11,12] (Fig. 2).

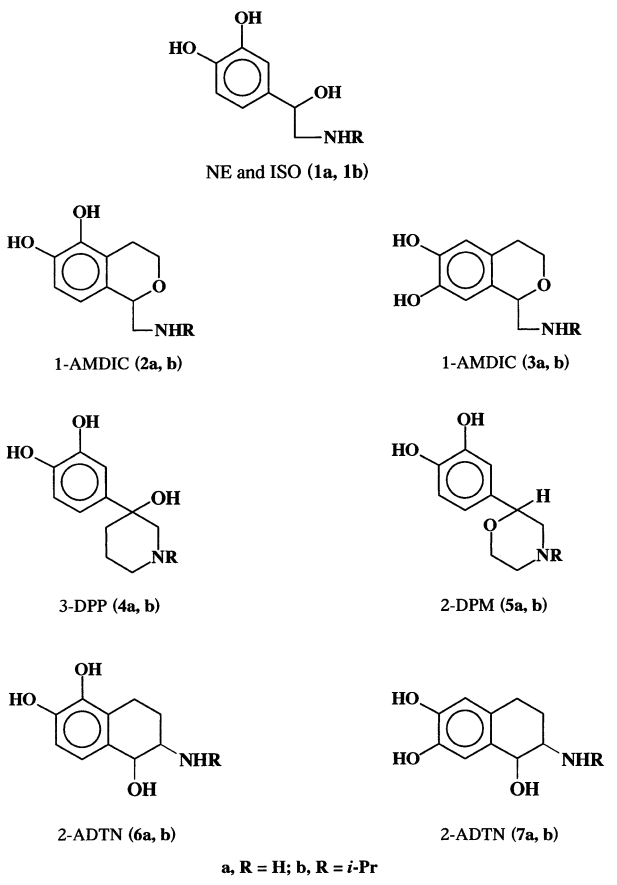


Fig. 1. Structures of catecholamines NE and ISO and of some their semirigids analogues.

Following this study, another semirigid analogue of the tetrahydronaphthalenic type, the 1-(aminomethyl)-5,6-dihydroxy-1,2,3,4-tetrahydro-1-naphthalen-1-ol (1-AMDTN, **8a**) was found to be active at the level of the α_2 receptor ($pD_2 = 5.78$ and $K_i = 46$ nM) [20–23]. For this compound, the arrangement of the pharmacophoric groups, with the exception of the phenyl ring, which appears to be clearly in a different rotameric position, corresponds to the one indicated by the α_2 model [23].

5,6-Dihydroxy-3,4-dihydrospiro[naphthalen-1(2H)-2′, 5′-morpholine] (DDSNM, **10a**) (Fig. 3) may be viewed as the result of the combination of the structure of the 2-DPM **5a**, used for the construction of the α_2 model in Fig. 2, with the structure of the 1-AMDTN **8a**, in which the aryl is blocked in a conformation which, even if pharmacophoric, is different from the one indicated by the model in Fig. 2.

This paper describes the synthesis and the adrenergic properties of the DDSNM **10a**, together with those of its *N*-isopropyl derivative **10b** and their respective regioisomers characterised by the different position of one of the two catecholic hydroxyls (**11a** and **11b**). DDSNMs **11a** and **11b** may be considered as conformationally restrained analogues of the previously described 1-AMDTNs **9a** and **9b**, respectively.

2. Chemistry

The DDSNMs **10a** and **11a** and their *N*-isopropyl derivatives **10b** and **11b** were synthesised as outlined in Fig. 4. The reaction of aminoalcohols **12** [20] and **13** [21] with chloroacetyl chloride yielded the chloroacetamides **14** and **15**. Base-catalysed (KO*t*-Bu) cyclisation of **14** and **15** gave the spiromorpholones **16** and **17**, which were reduced with $LiAlH_4$ to the amines **18** and **19**. While catalytic hydrogenolysis of **18** and **19** with palladium on charcoal yielded the catecholic compounds **10a** and **11a**, respectively, alkylation of the same compounds with isopropyl bromide in the presence of potassium hydroxide afforded the *N*-isopropyl derivatives **20** and **21**, which by catalytic hydrogenolysis gave the *N*-isopropyl DDSNMs **10b** and **11b**.

3. Pharmacology

The affinity of DDSNMs **10** and **11** for α-adrenoceptors was determined by binding tests carried out on rat brain membrane preparations. [^{3}H]Prazosin and

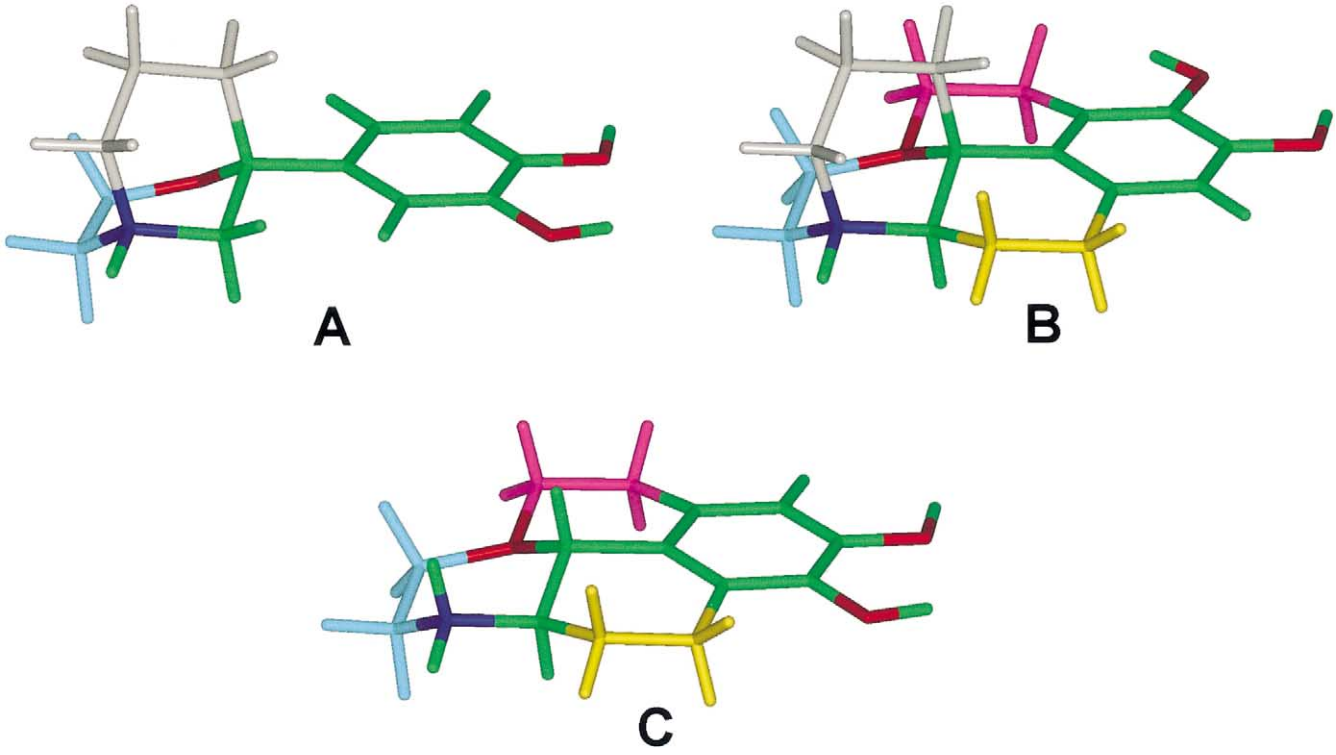


Fig. 2. Steric models for the interaction with the α_1- (A) α_2- (B) and β- (C) adrenergic receptors. The models arise from the superimposition of the pharmacophoric groups (aryl moiety, aminic nitrogen, alcoholic or ethereal oxygen) of adrenergic agonist catecholamine derivatives in the conformations in which they should interact with the receptors. The common arylethanolaminic portion is colored in green; the other portions are colored depending on which drug they arise from (see [12]).

[^{3}H]rauwolscine were used as specific tritiated ligands for α_1 and α_2 receptors, respectively. The affinity for β_1- and β_2-adrenergic receptors of **10** and **11** was determined by binding tests on rat brain for β_1- and on bovine lung membranes for β_2-receptors, respectively. [^{3}H]CGP 26505 was used as the specific tritiated ligand for rat brain β_1 receptors, while [^{3}H]DHA in the presence of 50 nM CGP 26505 which displaced [^{3}H]DHA binding from the β_1-adrenoceptor subpopulation, which represented 17% in the bovine lung, was used to label bovine lung β_2 receptors. The binding affinity indices of DDSNMs **10** and **11** are reported in Table 1, together with those obtained in the same tests with NE and ISO (**1**) and 2-DPM (**5**) and 1-AMDTN (**8** and **9**).

The activity of DDSNMs **10** and **11** was tested on isolated vas deferens for α_1-adrenoceptors, and on isolated, electrically-stimulated guinea-pig ileum for α_2-adrenoceptors. The activity of DDSNMs **10** and **11** was tested on isolated guinea-pig atria and on isolated guinea-pig tracheal strips for β_1- and β_2-adrenoceptors, respectively. The activity indices towards α- and β-adrenoceptors are shown in Table 2, together with those obtained in the same functional tests for catecholamines 1, 2-DPM (**5**) and 1-AMDTN (**8** and **9**).

4. Results

As for α_1-receptors (Table 1), the DDSNM analogue of **8a** (**10a**) was found to be completely devoid of any affinity, while **8a** and 2-DPM **5a** showed affinity indices, respectively 55- and 16-fold higher than that of NE. Also the regioisomer of **10a** (**11a**) proved to be completely devoid of affinity for this α_1-receptor, while 1-AMDTN **9a** was practically devoid of affinity, with a K_i value more than 150 times higher than that of NE. Both the *N*-isopropyl-substituted DDSNMs **10b** and **11b** and 1-AMDTN **9b** were completely lacking in affinity for α_1-receptors, while 1-AMDTN **8b** and 2-DPM **5b** showed affinity indices about 1.5 times higher or fivefold lower than that of ISO.

As for α_2-adrenoceptors (Table 1), DDSNM **10a** showed a certain affinity, with an index about 60 or 110 times higher than that of 1-AMDTN **8a** or 2-DPM **5a**, respectively. These last compounds in turn showed a lower affinity for this receptor by about half or one order of magnitude than NE. The regioisomer of the DDSNM derivative **10a** (**11a**) showed a K_i value 20- or 13-fold higher than that of **10a** and of the corresponding 1-AMDTN **9a**, respectively.

Both the *N*-isopropyl-substituted DDSNM **10b** and ISO (**1b**) exhibited a similar affinity index for this

receptor that is about half an order of magnitude higher than 1-AMDTN **8b** and 2-DPM **5b**. The regioisomer of DDSNM **10b** (**11b**) was completely devoid of any affinity, while the corresponding 1-AMDTN **9b** showed an affinity similar to that of ISO.

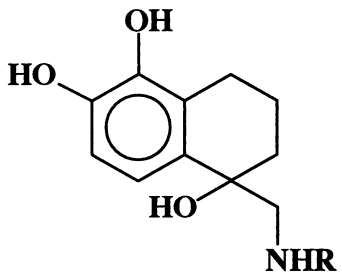
At the level of β_1-adrenoceptors (Table 1), both DDSNMs **10a** and **10b** and their regioisomers **11a** and **11b** were practically lacking in affinity. On the contrary, on the same β_1-adrenoceptors, both 2-DPM **5** and 1-AMDTNs **8** and **9** proved to possess a certain affinity, even if considerably lower than that of the corresponding catecholamines **1a** and **1b**.

As regards β_2-adrenoceptors (Table 1), the DDSNM **10a** appeared to possess an affinity index is about six times higher than those of the 2-DPM **5a** and the corresponding 1-AMDTN **8a**. 2-DPM **5a** and 1-AMDTN **8a** exhibited affinity indices similar to that of the natural catecholamine NE (**1a**). The regioisomer of DDSNM **10a** (**11a**) was found to be lacking in affinity; the same result was obtained with the corresponding 1-AMDTN **9a**.

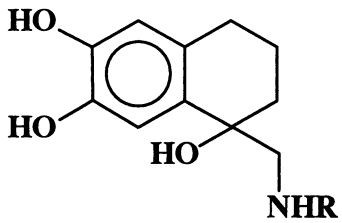
Neither of the *N*-isopropyl-substituted DDSNMs **11a** and **11b** showed an appreciable affinity for β_2-adrenoceptors; also 1-AMDTN **9b** appeared to be devoid of affinity on these adrenoceptors, whereas the 2-DPM **5b** and the 1-AMDTN **8b** exhibited a certain affinity, albeit considerably lower than that of ISO (**1b**).

As regards the results of the functional tests (Table 2), on α_1-adrenoceptors the DDSNM **10a** appeared to be practically inactive with a pD_2 value ≤ 3.5; the 2-DPM **5a** and 1-AMDTN **8a**, on the contrary, proved to possess an appreciable activity, with pD_2 values of 4.88 and 4.12, respectively, and an intrinsic activity (i.a.) close to 1.00, while the natural catecholamine NE **1a** exhibited a pD_2 value of 5.12. Also the regioisomer of **10a** (**11a**) showed a pD_2 value ≤ 3.5, while the corresponding 1-AMDTN **9a** was completely inactive. On the same α_1-adrenoceptors, both the *N*-isopropyl-substituted DDSNMs **10b** and **11b** were completely inactive, like 2-DPM **5b** and 1-AMDTNs **8b** and **9b**, which had proved to be practically inactive.

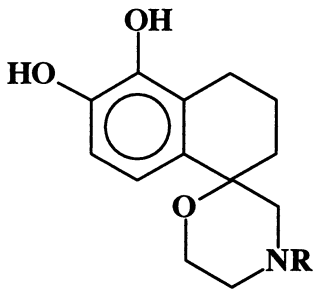
As for the activity on α_2-adrenoceptors (Table 2), DDSNM **10a** showed a pD_2 value of 5.01 and an intrinsic activity of 1.00, while the 2-DPM (**5a**) and the corresponding 1-AMDTN **8a** exhibited a pD_2 value of 7.34 and 5.78, and an i.a. of 1.08 and 0.73, respectively. On the same adrenoceptor, NE **1a** showed a pD_2 of 6.56. The regioisomer of **10a** (**11a**) showed a pD_2 value of 3.50, with a low i.a. value (0.60), while the corresponding 1-AMDTN **9a** was slightly more active, with a pD_2 value and an i.a. index of 4.31 and 1.00, respectively.



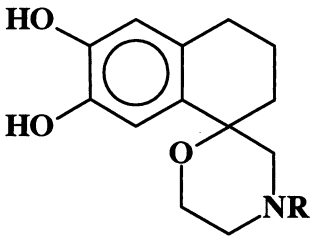
1-AMDTN (**8a, b**)



1-AMDTN (**9a, b**)



DDSNM (**10a,b**)



DDSNM (**11a, b**)

a, R = H; b, R = *i*-Pr

Fig. 3. Structure of title compounds.

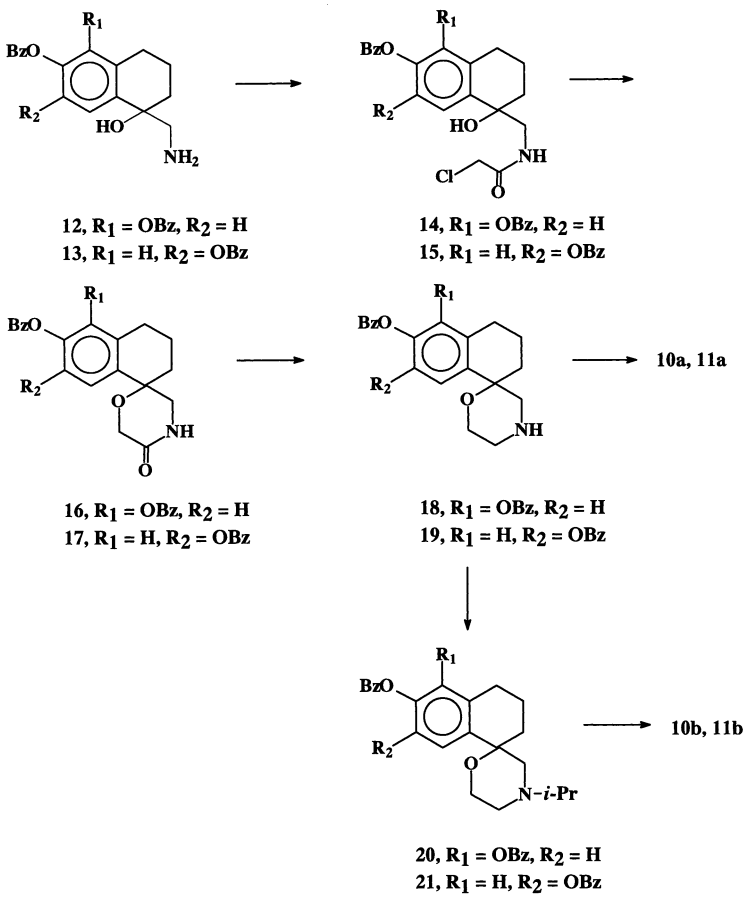


Fig. 4. Synthesis of title compounds.

Table 1
Radioligand binding affinities of compounds **1**, **5**, **8**–**11**.

Compound	K_i (nM) [a]			
	α-adrenergic binding affinity		β-adrenergic binding affinity	
	Rat brain (α_1)	Rat brain (α_2)	Rat brain (β_1)	Bovine lung (β_2)
1a (NE)	450 (390–520) [b]	4.8 (4.5–7.1) [b]	126 (108–144) [b]	6000 (5200–6600) [b]
5a (2-DPM)	7400 (7000–7800) [b]	26 (17–28) [b]	15 000 (12 000–18 000) [b]	9200 (8500–9800) [b]
8a (1-AMDTN)	25 000 (23 000–27 000) [c]	46 (43–48) [c]	27 000 (24 000–29 000) [c]	11 000 (10 000–13 000) [c]
9a (1-AMDTN)	75 000 (68 500–81 000)	4300 (3500–5000)	54 000 (47 500–60 500)	>100 000
10a (DDSNM)	>100 000	2900 (2200–3500)	>100 000	53 000 (46 000–61 000)
11a (DDSNM)	>100 000	58 000 (50 000–65 000)	>100 000	>100 000
1b (ISO)	35 500 (32 000–39 500) [b]	21 000 (16 000–24 000) [b]	80 (53–100) [b]	110 (100–130) [b]
5b (2-DPM)	6700 (6000–7500) [b]	5100 (4300–5600) [b]	48 000 (42 000–53 500) [b]	36 000 (32 000–40 000) [b]
8b (1-AMDTN)	52 000 (47 000–56 000) [c]	3000 (2700–3300) [c]	24 000 (22 000–26 000) [c]	9000 (8400–9700) [c]
9b (1-AMDTN)	>100 000	29 000 (21 000–36 000)	47 000 (39 000–54 000)	>100 000
10b (DDSNM)	>100 000	22 000 (17 000–27 000)	>100 000	>100 000
11b (DDSNM)	>100 000	>100 000	>100 000	>100 000

[a] Geometric means of five separate determinations with confidence limits shown in parentheses.
[b] Ref. [11].
[c] Ref. [20].

The *N*-isopropyl-substituted DDSNM **10b** exhibited a pD_2 value and an i.a. index similar to those of 2-DPM **5b** (5.27 and 0.97 vs. 5.00 and 1.02), while ISO showed a pD_2 value of 4.95 and an i.a. of 0.74. The DDSNM regioisomer of **10b** (**11b**) was slightly less active than DDSNM **10b** (pD_2 and i.a. 4.53 and 0.94, respectively).

Table 2
Adrenergic activities [a] of compounds **1**, **5**, **8**–**11** on isolated preparations.

Compound	α-adrenergic activity [a]				β-adrenergic activity [a]			
	Isolated rat vas deferens (α_1)		Isolated guinea-pig ileum (α_2)		Isolated guinea-pig atria (β_1)		Isolated guinea-pig tracheal strip (β_2)	
	pD_2	i.a. [b]	pD_2	i.a. [b]	pD_2	i.a. [b]	pD_2	i.a. [b]
1a (NE)	5.12 (±0.10) [c]	1.00 [c]	6.56 (±0.11) [c]	1.00 [c]	6.32 (±0.07) [c]	1.00 [c]	6.03 (±0.06) [c]	1.00 [c]
5a (2-DPM)	4.88 (±0.09) [c]	0.91 [c]	7.34 (±0.12) [c]	1.08 [c]	5.31 (±0.30) [c]	0.91 [c]	4.76 (±0.20) [c]	0.70 [c]
8a (1-AMDTN)	4.12 (±0.05) [d]	1.01 [d]	5.78 (±0.10) [e]	0.73	4.04 (±0.18) [d]	0.98 [d]	<3.50 [d]	0.60 [d]
9a (1-AMDTN)	–	–	4.31 (±0.06)	1.00	4.08 (±0.08)	0.73	4.08 (±0.11)	0.61
10a (DDSNM)	≤3.50	–	5.01 (±0.17)	1.00	≤3.50		4.10 (±0.11)	0.48
11a (DDSNM)	≤3.50	–	3.50 (±0.26)	0.60	–		≤3.50	
1b (ISO)	3.50 (±0.14) [c]	0.83 [c]	4.95 (±0.14) [c]	0.74 [c]	8.45 (±0.12) [c]	1.00 [c]	8.33 (±0.18) [c]	1.00 [c]
5b (2-DPM)	– [c]	–	5.00 (±0.21) [c]	1.02 [c]	4.62 (±0.08) [c]	0.77 [c]	5.91 (±0.91) [c]	0.87 [c]
8b (1-AMDTN)	– [d]	–	4.16 (±0.01)	0.86	– [d]	–	– [d]	
9b (1–AMDTN)	≤3.50	–	–		–	–	4.45 (±0.35)	0.36
10b (DDSNM)	–	–	5.27 (±0.11)	0.97	–	–	≤3.50	
11b (DDSNM)	–	–	4.53 (±0.22)	0.94	–	–	≤3.50	

[a] The values represent the mean of 4–6 experiments for each drug ± standard error in parentheses.
[b] Intrinsic activity, i.e. the ratio between the maximal response elicited by the compound under test and that elicited by the full agonist, namely NE and ISO for α and β adrenoceptors, respectively.
[c] Ref. [11].
[d] Ref. [20].
[e] Ref. [23].

On the same adrenoceptors, 1-AMDTN **8b** exhibited an activity slightly lower than that of **11b**, while **9b** appeared to be inactive.

Regarding β_1-adrenoceptors (Table 2) both DDSNMs **10a** and **11a** proved to be practically devoid of activity, while 1-AMDTNs **8a** and **9a** showed a certain activity (a pD_2 of about 4 and an i.a. of 0.98 and 0.73, respectively). The 2-DPM **5a** exhibited a pD_2 value about one order of magnitude lower than that of NE (5.31 vs. 6.32 and an i.a. slightly lower (0.91 vs 1.00).

On the same β_1-adrenoceptors, both *N*-isopropyl-substituted DDSNMs **10b** and **11b** were completely inactive, as were the corresponding 1-AMDTNs **8b** and **9b**; *N*-isopropyl- substituted 2-DPM **5b** appeared to possess an activity much lower than that of ISO (**1b**), with a pD_2 and an i.a. value of 4.62 and 0.77 vs 8.45 and 1.00 of **1b**. On β_2-adrenoceptors (Table 2), the DDSNM **10a**, like the 2-DPM **5a**, proved to possess a modest activity (a pD_2 of 4.10 vs 4.76 and an i.a. of 0.48 vs 0.70, respectively); the regioisomer of **10a** (**11a**) and the corresponding 1-AMDTN (**9a**) exhibited still lower values both of pD_2 and of i.a. Also the *N*-isopropyl-substituted DDSNMs **10b** and **11b**, like the 1-AMDTN **8b**, were practically inactive on this adrenoceptor; only the 1-AMDTN **9b** showed a certain activity, albeit characterised by a very low i.a. value. The 2-DPM **5b** exhibited pD_2 and i.a. values of 5.81 and 0.87, respectively, considerably lower than those of ISO **1b**, i.e. 8.33 and 1.00, respectively.

5. Conformational studies

With the aim of determining the real conformational situation of the DDSNM **10a** and thus understanding the spatial relationship between its active groups and those of its conformationally more free analogues 2-DPM **5a** and 1-AMDTN **8a**, conformational studies were carried out by means of both experimental and theoretical methods.

As a first approach, a ^{1}H-NMR study was carried out on the dibenzyloxy derivative of **10a** (**18**), chosen in view of the relatively limited stability of its free catecholic nuclei in solution. As regards the conformation of the tetrahydronaphthalene portion, the results obtained for compound **18** are in agreement with those found for type **8** and **9** analogues, for which a half-chair preferred conformation was found ([23] and reference therein citated). As regards the conformational situation of the spiromorpholine ring, studies on **18** were unsuccessful, due to the superimposition of the signals of the diagnostic $H_{6'a}$ and $H_{6'b}$ (Fig. 5) protons on the signals of the other ring protons. However, the signals of these two protons were well separated in the spectrum of the *N*-isopropyl derivative of **18** (**20**). In this compound, the saturation of the proton $H_{6'a}$ (2.28 ppm), which lies in the pseudoaxial position, produced not only the expected NOE (21%) on the signal of its geminal proton $H_{6'b}$ (2.68 ppm), but also an appreciable NOE (9%) on the signal at 7.45 ppm attributable to the aromatic proton H_8, thus indicating that the proton

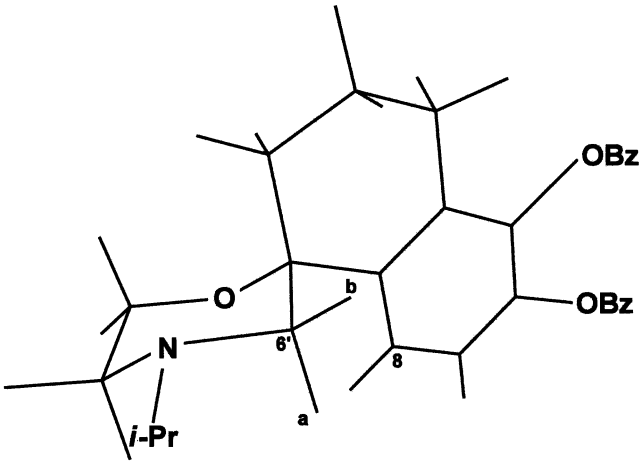


Fig. 5. Compound **20** in its preferred conformation.

$H_{6'a}$ is in the proximity of H_8. On the basis of these data, it is thus possible to assign to compound **20** the conformation shown in Fig. 5 and consequently to attribute the same type of conformation also to compound **10b**. This type of conformation is presumably extensible also to **10a**, **11a**, **11b**, which differ from **10b** only in the position of the phenolic hydroxyl (**11b**) or in the presence of an hydrogen in the place of the *N*-isopropyl (**10a** and **11a**).

As regards conformational analysis of **10a** carried out by theoretical methods, this was performed by molecular mechanics and dynamics calculations using the DISCOVER program [24] with the CVFF force field, in which partial atomic changes are also defined. The starting geometries of DTNSM **10a**, considered in their protonated form, were built by using the Insight programme [24] A molecular dynamics simulation was run at a temperature of 1000 K with a step of 1 fs for 50 ps after an equilibration of 1 ps; for every ps of the simulation, a conformation was sampled and minimised with 10^{-4} kcal mol^{-1} Å on RMS derivative as convergence criteria. The dielectric constant was fixed to 4, and was distance-dependent, in order to simulate an aq. environment. All the 50 minimised conformations were then compared and it was found that they can be reduced to four, corresponding to the two possible chair arrangements that both the cyclohexenic and morpholinic ring can assume. The conformation shown in Fig. 6, possessing the morpholinic oxygen in a pseudoequatorial position, is the preferred one, with energy differences of almost 3.4 kcal mol^{-1}, with respect to all others. These results are thus in agreement with those obtained by ^{1}H-NMR because the distance between the H_4 and H_8 in this conformation is only 2.7 Å.

6. Discussion

The DDSNM **10a** presents a certain affinity for the α_2-adrenoceptor, which, however, is lower than that of both 2-DPM **5a** and 1-AMDTN **8a**; the *N*-isopropyl derivative of **10a** (**10b**) showed a level of affinity of about one order of magnitude lower than that of both **10a** and of 2-DPM and the 1-AMDTN analogues **5b** and **8b**. The regioisomeric DDSNMs of **10a** and **10b** (**11a** and **11b**) are practically devoid of any affinity for the α_2-adrenoceptor; the same result was also found for the 1-AMDTN analogue **9b** while the 1-AMDTN analogue **9a** exhibited a modest affinity.

Although, the low potency values recorded by functional tests for several compounds can reflect a poor selectivity towards receptor subtypes and suggest a

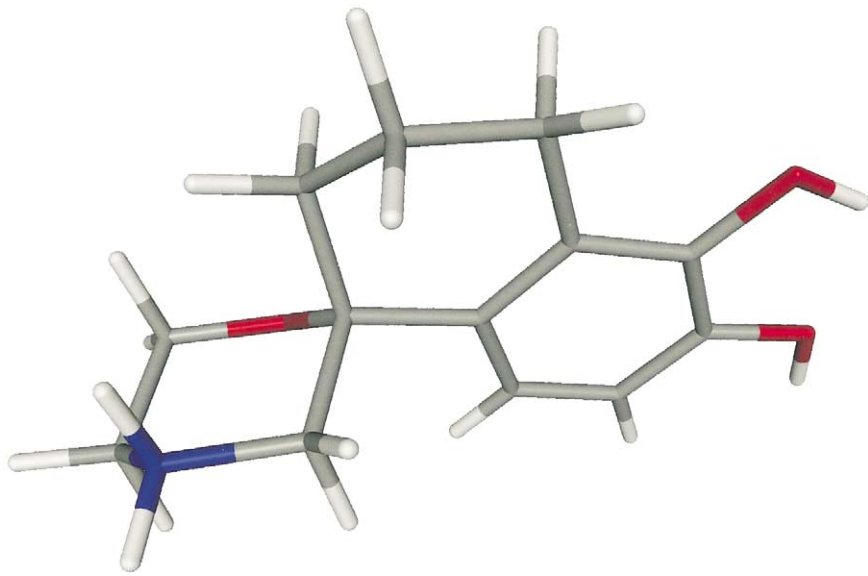


Fig. 6. Compound **10a** in its preferred conformation.

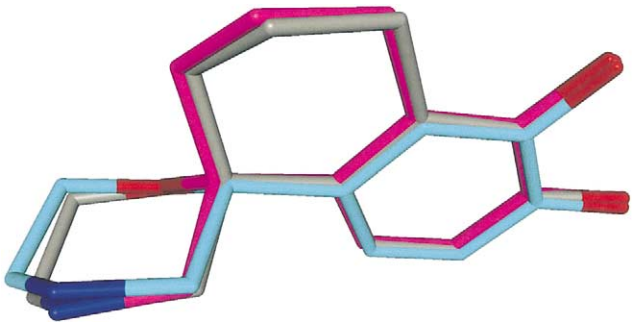


Fig. 7. Superimposition of the compound **10a** (grey) in its preferred conformation with compound **5a** (cyan) and compound **8a** (magenta) in low energy conformations; nitrogen and oxygen atoms are blue and red, respectively, the hydrogen atoms are not reported.

cautious interpretation of these experimental data, however it should be observed that the trend of the activity indices corresponded to that of the affinity indices. In particular, the DDSNM **10a** proved to possess a moderate agonistic activity towards the α_2-adrenoceptor, which was in all cases clearly lower than that of the 2-DPM **5a** and the 1-AMDTN analogue **8a**; the *N*-isopropyl-substituted DDSNM **10b** exhibited a similar activity to that of **10a**, whereas the DDSNM regioisomers of **10a** and **10b** (**11a** and **11b**) appeared to be almost completely inactive.

At the level of the other adrenoceptors studied (α_1, β_1 and β_2), the DDSNM **10a**, like its *N*-isopropyl derivative **10b** and their regioisomers **11a** and **11b**, proved to be almost completely devoid of any affinity or activity, in line with the fact that on these receptors, the 1-AMDTNs **8a** and **8b**, and to an even greater extent their regioisomers **9a** and **9b**, had not revealed any adrenergic properties.

7. Conclusions

This study, and in particular the synthesis of DDSNM **10a**, was undertaken with the aim of verifying the effects on the α_2-adrenergic properties, of the combination in a single molecule of the structure of two semirigid analogues of NE, both possessing appreciable properties on this receptor, like 2-DPM **5a**, and 1-AMDTN **8a**. The results shown in Tables 1 and 2 indicate that, while partly maintaining the affinity and the activity with respect to 2-DPM **5a**, and the corresponding 1-AMDTN **8a**, the new DDSNM analogue of NE (**10a**) appears to possess a lower ability to interact with this receptor at the molecular level.

A possible explanation for these results might have lain in differences in the spatial arrangement of the pharmacophoric groups of DDSNM **10a**, compared with the positions that the same groups occupy in the conformationally less rigid analogues 2-DPM **5a** and 1-AMDTN **8a**. Fig. 7 shows a superimposition of the structure of **10a**, arranged in the low-energy conformation resulting from the combination of both ^{1}H-NMR studies and theoretical calculations, on those of **5a** and **8a**, arranged in the low-energy conformations found in previous studies.

The good coincidence of the common molecular portions of these compounds allows us to exclude the possibility that the differences in the α_2-adrenergic properties between **10a** and **5a**, and **8a** may be due to differences in the spatial arrangement of the active groups. On the contrary, more probable explanations may be sought in the greater rigidity of the DDSNM system of **10a** with respect to the 2-DPM or 1-AMDTN of **5a** and **8a**, respectively, and/or in the steric hindrance due to the additional atoms that are necessary to reduce the conformational freedom of the same 2-DPM **5a** and 1-AMDTN **8a**, which may partially hinder a good fit with the α_2-adrenoceptor.

8. Experimental

8.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra for comparison between compounds were recorded with a Perkin–Elmer mod. 1310, as nujol mulls in the case of solid substances, or as liquid film in the case of liquids. ^{1}H-NMR spectra were routinely recorded with a CFT 20 spectrometer operating at 80 MHz in ca. 5% solution of $CDCl_3$ (for neutral compounds or the free bases) or D_2O (for the salts), using Me_4Si or $Me_3Si(CH_2)_3SO_3Na$ as the internal standard, respectively. The ^{1}H-NMR spectra for compounds **10a**,**b** and **11a**,**b** (as salts) were detected in ca. 2% D_2O with a Bruker AC-200 instrument. The ^{1}H-NMR for compounds **18** and **20** were recorded using a Varian VXR-300 spectrometer in ca. 0.08 M $CDCl_3$ solution, and the temperature was controlled to ± 0.1 °C. All the solutions were accurately degassed by freeze-pump-thaw cycles. Evaporations were made in vacuo (rotating evaporator). Magnesium sulphate was always used as the drying agent. Elemental analysis were performed by our analytical laboratory and agreed with theoretical values to within $\pm 0.4\%$.

8.1.1. 1-Hydroxy-1′-(chloroacetaminomethyl)-5,6-dibenzyloxy-1,2,3,4-tetrahydronaphthalene (**14**)

A solution of NaOH (0.74 g, 18.5 mmol) in H_2O (4 mL) was added to a solution of aminoalcohol **12** [20] (1.20 g, 3.08 mmol) in CH_2Cl_2 (33 mL) Chloroacetylchloride (0.695 g, 6.16 mmol) was then added dropwise and the mixture was stirred for 2 h at room temperature (r.t.). The mixture was then poured into

water (18 mL). The separated aq. layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with water, dried and evapored to yield a solid that was crystallised from EtOAc to afford **14** (0.95 g, 66%): m.p. 118–119 °C; IR ν 1660 cm^{-1}(CONH); ^{1}H-NMR δ 1.50–2.06 (m, 4H), 2.36–2.51 (br, 1H), 2.56–2.90 (m, 2H), 3.40–3.76 (m, 2H), 4.05 (s, 2H), 5.01 (s, 2H), 5.13 (s, 2H), 6.83–7.53 (m, 12H). $C_{27}H_{28}ClNO_4$ (C, H, N).

*8.1.2. 1-Hydroxy-1-(chloroacetaminomethyl)-6,7-dibenzyloxy-1,2,3,4-tetrahydronaphthalene (**15**)*

This compound was prepared from **13** [21] (1.10 g, 2.82 mmol) following the same procedure described above for the preparation of compound **14**. The crude product was an oil consisting essentially of **15** (1.10 g, 84%) which was used for the following reaction without further purification; IR ν 1660 cm^{-1} (CONH); ^{1}H-NMR δ 1.56–2.46 (m, 5H), 2.53–2.85 (m, 2H), 3.33–3.70 (m, 2H), 4.05 (s, 2H), 5.15 (s, 4H), 6.70 (s, 1H), 7.16 (s, 1H), 7.43 (br, 10H). $C_{27}H_{28}ClNO_4$ (C, H, N).

*8.1.3. (5,6-Dibenzyloxy)-3,4-dihydrospiro-[naphthalene-1(2H)-2′,5′-morpholin-4′-one] (**16**)*

KO*t*-Bu (1.20 g, 10.70 mmol) was added portionwise over 1 h to a stirred solution of the chloroacetamide **14** (0.95 g, 2.04 mmol) in anhydrous C_6H_6 (46 mL). The reaction mixture was stirred at r.t. for 1.5 h, and then diluted with water. The separated organic layer was washed with H_2O, dried and evaporated to give a solid that was crystallised from EtOAc to yield **16** (0.50 g, 57%): m.p. 192–194 °C; IR ν 1670 cm^{-1} (CONH); ^{1}H-NMR δ 1.63–2.96 (m, 6H), 3.33–3.73 (m, 2H), 4.26 (s, 2H), 5.02 (s, 2H), 5.13 (s, 2H), 6.76–7.56 (m, 12H). $C_{27}H_{27}NO_4$ (C, H, N).

*8.1.4. (6,7-Dibenzyloxy)-3,4-dihydrospiro-[naphthalene-1(2H)-2′,5′-morpholin-4′-one] (**17**)*

The chloroacetamide **15** (0.90 g, 1.93 mmol) was converted to the spiromorpholone **17** as described above for preparation of compound **16**. The crystallisation of the crude solid from AcOEt gave **17** (0.66 g, 80%): m.p. 171–173 °C; IR ν 1670 cm^{-1} (CONH); ^{1}H-NMR δ 1.50–2.83 (m, 6H), 3.20–3.60 (m, 2H), 4.31 (s, 2H), 5.18 (s, 4H), 6.63 (s, 1H), 7.16 (s, 1H), 7.36 (br, 10H). $C_{27}H_{27}NO_4$ (C, H, N).

*8.1.5. (5,6-Dibenzyloxy)-3,4-dihydrospiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**18**·$H_2C_2O_4$)*

A solution of the morpholone **16** (0.60 g, 1.40 mmol) in anhydrous THF (15 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (0.21 g, 5.54 mmol) in anhydrous THF (9 mL). The reaction mixture was heated at reflux for 3 h and then cooled to 10 °C. The excess of $LiAlH_4$ was destroyed by careful addition of H_2O, 10% NaOH solution, H_2O. The mixture was filtered and the solid washed with ether. The organic solvents were dried and evaporated to give an oil consisting of **18** (0.56 g, 96%): ^{1}H-NMR δ 1.56–1.88 (m, 4H), 2.13–3.12 (m, 6H), 3.48–4.12 (m, 2H), 5.00 (s, 2H), 5.16 (s, 2H), 6.94 (d, 1H, $J = 8.80$ Hz), 7.12–7.60 (m, 11H).

The resulting oil **18** was dissolved in a mixture of Et_2O–MeOH (8:2) and added of a solution of $H_2C_2O_4$ in the same mixture. The resulting oxalate salt was filtered and recrystallised from MeOH–Et_2O to give **18**·$H_2C_2O_4$; m.p. 153–155 °C. $C_{29}H_{31}NO_7$ (C, H, N).

*8.1.6. (6,7-Dibenzyloxy)-3,4-dihydrospiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**19**·$H_2C_2O_4$)*

Reduction of the morpholone **17** (0.66 g, 1.53 mmol) with $LiAlH_4$ as described above for **18** gave the desired morpholine **19** as an oil (0.58 g, 90%): ^{1}H-NMR δ 1.50–2.16 (m, 4H), 2.56–3.16 (m, 6H), 3.50–4.23 (m, 2H), 5.25 (s, 2H), 5.31 (s, 2H), 6.80 (s, 1H), 7.43 (s, 1H), 7.50–7.83 (m, 10H).

Compound **19** was converted to the oxalate salt and after recrystallisation from MeOH–Et_2O yielded **19**·$H_2C_2O_4$; m.p. 186–188 °C. $C_{29}H_{31}NO_7$ (C, H, N).

*8.1.7. (5,6-Dihydroxy)-3,4-dihydrospiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**10a**·$H_2C_2O_4$)*

A solution of **18**·$H_2C_2O_4$ (0.20 g, 0.40 mmol) in MeOH (20 mL) was hydrogenated at r.t. and atmospheric pressure in the presence of 10% Pd on charcoal (0.07 g). When the absorption stopped the catalyst was removed by filtration and the resulting solution was concentrated and anhydrous Et_2O was added. The resulting precipitate was filtered and then crystallised from MeOH–Et_2O to give **10a**·$H_2C_2O_4$ (0.08 g, 62%): m.p. 180–183 °C (dec.); ^{1}H-NMR δ 1.58–2.08 (m, 4H), 2.25–3.40 (m, 6H), 3.90–4.37 (m, 2H), 6.86 (d, 1H, $J = 8.77$ Hz), 7.10 (d, 1H, $J = 8.77$ Hz). $C_{15}H_{19}NO_7$ (C, H, N).

*8.1.8. (6,7-Dihydroxy)-3,4-dihydrospiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**11a**·$H_2C_2O_4$)*

This compound was prepared from **19**·$H_2C_2O_4$ (0.25 g, 0.60 mmol) dissolved in a mixture of MeOH (10 mL) and CH_2Cl_2 (3 mL) following the same procedure described for compound **10a**·$H_2C_2O_4$. The crude product was purified by crystallisation from MeOH–Et_2O to yield **11a**·$H_2C_2O_4$ (0.20 g, 66%): m.p. 164–167 °C. ^{1}H-NMR δ 1.54–2.09 (m, 4H), 2.30–3.51 (m, 6H), 3.85–4.27 (m, 2H), 6.69 (s, 1H), 7.10 (s, 1H). $C_{15}H_{19}NO_7$ (C, H, N).

*8.1.9. (5,6-Dibenzyloxy)-3,4-dihydro-5′-N-isopropyl-spiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**20**·$H_2C_2O_4$)*

A suspension of **18** (0.35 g, 0.84 mmol), 2-bromopropane (0.35 mL, 3.7 mol) and KOH (0.09 g, 6.2

mmol) in absolute EtOH (2 mL) was stirred at 60 °C for 4 days. Then the reaction mixture was supplemented with Et_2O, filtered and evaporated to dryness to give **20** as an oil (0.32 g, 83%): ^{1}H-NMR δ 1.00 and 1.03 (2d, 6H, $J = 6.40$ Hz), 1.13–1.76 (m, 4H), 2.16–2.93 (m, 7H), 3.68–4.12 (m, 2H), 4.99 (s, 2H), 5.13 (s, 2H), 6.91 (d, 1H, $J = 8.80$ Hz), 7.03–7.60 (m, 11H).

Compound **20** was converted to the corresponding oxalate salt of the amine which was recrystallised from MeOH–Et_2O to give **20·$H_2C_2O_4$**: m.p. 173–175 °C. $C_{32}H_{37}NO_7$ (C, H, N).

*8.1.10. (6,7-Dibenzyloxy)-3,4-dihydro-5′-N-isopropyl-spiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**21·$H_2C_2O_4$**)*

The reaction was carried out on compound **19** (0.65 g, 1.56 mmol) as described for compound **20** to obtain the desired *N*-isopropyl derivative **21** as an oil (0.68 g, 95%) ^{1}H-NMR δ 0.96 and 0.99 (2d, 6H, $J = 6.45$ Hz), 1.55–1.90 (m, 4H), 2.33–3.01 (m, 7H), 3.40–4.02 (m, 2H), 5.09 (s, 2H), 6.15 (s, 2H), 6.61 (s, 1H), 7.13 (s, 1H), 7.18–7.48 (m, 10H).

Conversion to the oxalate salt and recrystallisation from MeOH–Et_2O gave **21·$H_2C_2O_4$**; m.p. 164–168 °C. $C_{32}H_{37}NO_7$ (C, H, N).

*8.1.11. (5,6-Dihydroxy)-3,4-dihydro-5′-N-isopropyl-spiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**10b·$H_2C_2O_4$**)*

A solution of **20·$H_2C_2O_4$** (0.30 g, 0.55 mmol) in MeOH (28 mL) was hydrogenated at r.t. and atmospheric pressure in the presence of 10% Pd on charcoal (0.10 g). When the absorption stopped the catalyst was removed by filtration and the resulting solution was evaporated to dryness. The resulting solid was then crystallised from MeOH–Et_2O to give **10b·$H_2C_2O_4$** (0.15 g, 60%): m.p. 185–188 °C. ^{1}H-NMR δ 1.35 and 1.38 (2d, 6H, $J = 6.40$ Hz), 1.52–2.10 (m, 4H), 2.38–3.58 (m, 7H), 3.95–4.28 (m, 2H), 6.87 (d, 1H, $J = 8.40$ Hz), 7.06 (d, 1H, $J = 8.40$ Hz). $C_{18}H_{25}NO_7$ (C, H, N).

*8.1.12. (6,7-Dihydroxy)-3,4-dihydro-5′-N-isopropyl-spiro[naphthalene-1(2H)-2′,5′-morpholine] oxalate (**11b·$H_2C_2O_4$**)*

The reaction was performed on **21·$H_2C_2O_4$** (0.30 g, 0.55 mmol) as described for **10b·$H_2C_2O_4$**. The amine salt was then recrystallised from MeOH–Et_2O to give **11b·$H_2C_2O_4$** (0.16 g, 80%): m.p. 203–206 °C. ^{1}H-NMR δ 1.34 and 1.38 (2d, 6H, $J = 6.45$ Hz), 1.58–2.06 (m, 4H), 2.50–3.60 (m, 7H), 3.95–4.38 (m, 2H), 7.08 (s, 1H), 6.69 (s, 1H). $C_{18}H_{25}NO_7$ (C, H, N).

8.2. Radioligand binding methods

8.2.1. Rat brain α_1 and α_2 receptors

α_1 and α_2 receptor binding was determined in rat cerebral cortex membranes as elsewhere reported [25].

8.2.2. Rat brain β_1 receptors

β_1 receptors were assayed in rat cortical membranes following the procedure previously described [11].

8.2.3. Bovine lung β_2 receptors

β_2 receptor binding was studied in bovine lung using [^{3}H]DHA (dihydroalprenolol) as the ligand (DuPont de Nemours, New England Nuclear Division, specific activity = 48.1 Ci mmol^{-1}).

Membranes were obtained by lung homogenisation in 1:20 volumes of 0.32 M sucrose, followed by centrifugation at 800 × *g* for 10 min at 5 °C. The resulting pellet was suspended in 50 mM phosphate buffer at pH 7.4 containing 0.02% ascorbic acid and then centrifuged. This step was repeated twice. Crude lung membranes were suspended in ($\cong$ 4 mg mL^{-1} proteins) and incubated with 1 nM [^{3}H]DHA in the presence of 50 nM CGP 26505. After incubation at 25 °C for 30 min, the samples were filtered on Whatman GF/B glass-fiber filters and washed with 3 × 5 mL of phosphate buffer, dried and added to 8 mL of Ready Protein Beckman scintillation cocktail. Non-specific binding was measured in the presence of 35 μM *l*-isoprenaline.

The affinity of drugs for specific binding sites was expressed as the molar concentration inhibiting the specific binding by 50% (IC_{50}). These values were calculated from the displacement curves by log probit analysis. The dissociation constant (K_i) was derived from the equation of Cheng Prusoff [26]. The ligand affinity (K_d) of [^{3}H]DHA was 1 nM.

8.3. Functional test methods

The assays were conducted on isolated mammalian preparations, in accordance with the legislation of the Italian Authorities (D.L. 27/01/92, no. 116) concerning animal experimentation. The animals, under light Et_2O anesthesia, were killed by cervical dislocation and bled. The thoracic and subsequently the abdominal cavities were opened by midline incision. The organs were immediately explanted and placed in Tyrode solution [composition (mM): NaCl (136.8), KCl (2.95), $CaCl_2$ (1.80), $MgSO_4 \cdot 7H_2O$ (1.05), NaH_2PO_4 (0.41), $NaHCO_3$ (11.9), Glucose (5.5)] at r.t. and gassed with carbogen (95% O_2–5% CO_2), or with O_2.

Adrenoceptor activity was assayed for α_1-receptors on rat *vas deferens*, for α_2-receptors on *guinea-pig ileum*, for β_1-receptors on *guinea-pig atria* and for β_2-receptors on *guinea-pig trachea*. When not otherwise described, agonist and antagonist activity was tested on resting tone of the organ.

8.3.1. Rat vas deferens

Vas deferens was taken from Sprague–Dawley male albino rats (200–250 g body weight). Both vasa deferentia were removed without stretching from the epididymis to the prostatic urethra, after moving the intestine to one side. The intact duct was carefully separated from extraneous surrounding tissues and placed in a 10 mL organ bath containing Tyrode solution (pH 7.4) at 37 °C, bubbled with carbogen. The preparation was suspended longitudinally between the organ holder and a force displacement transducer (Basile Model 7006), loaded with 0.5 g, connected to a unirecord microdynamometer (Basile Model 7050). The organ was left to stabilise for 30 min before beginning the experiment.

8.3.2. Guinea-pig ileum

Dunkin–Hartley male guinea pigs weighing 250–300 g, were deprived of food intake for 24 h before the experiments. Portions of ileum 2–3 cm in length, about 10 cm distal to the ileocecal valve, were carefully dissected, freed from the surrounding mesenteric tissue, attached with thread to the organ holder and to the recording system by opposite sides of their open ends, and suspended in a 10 mL organ bath containing Tyrode solution at 37 °C gassed with carbogen. The ileum preparations were placed between two platinum electrodes (4×45 mm) set at a distance of 7 mm in the bath. The tissues were preloaded with a tension of 0.5 g and left to stabilise for 45–60 min before beginning electrical stimulation, which was carried out with a digit stimulator (Biomedica Mangoni Model BM-ST3) using the following parameters: single rectangular pulses, 0.1 Hz frequency, 0.3 ms pulse width, 12 V supramaximal voltage. The activity of the tested drugs on α_2-adrenoceptors was evaluated as their ability to inhibit acetylcholine release evoked by electrical stimulation of nerve fibers. The effects of the released mediator on intestinal smooth muscle were recorded as longitudinal contractions by an isotonic transducer (Basile Model 7006) connected to a unirecord microdynamometer (Basile Model 7050).

8.3.3. Guinea-pig atria

The atria, like the tracheae, were obtained from the same animals employed in the previous preparation. After sacrifice, the heart was removed, both the atria were separated from the ventriculi and a strip was obtained. The ends of the specimen were tied and suspended in an organ bath containing Tyrode solution, at 32 °C aerated with pure O_2. The upper part of the organ was attached to an isometric transducer (Basile Model 7003) connected to a unirecord microdynamometer (Basile Model 7050). The organ was left to stabilise for 30 min before beginning the experiment.

8.3.4. Guinea-pig trachea

Tracheae were obtained from the same animals described above. The trachea was removed, freed from extraneous tissue, and after locating the smooth muscle tissue, the cartilage was cut on at the opposite side, obtaining a rectangular piece of tissue with the muscular layer in the middle. The trachea was then cut transversally at equally spaced intervals up to 1 mm from the left and the right border, resulting in zig–zag strips. The strip, tied by threads at the opposite ends, was placed in a 10 mL organ bath as described for previous preparations, filled with Krebs solution (composition (mM): NaCl (118), KCl (4.75), $CaCl_2$ (2.50), $MgSO_4 \cdot 7H_2O$ (1.19), KH_2PO_4 (1.19), $NaHCO_3$ (25), Glucose (11.5)) at 37 °C, and gassed with carbogen. The organs, held at a tension of 0.5 g, were tied to an isotonic transducer (Basile Model 7006) and the last in turn to a unirecord microdynamometer (Basile Model 7050) to record the response of the smooth musculature. After a 1 h stabilizing time interval, the organ was contracted with carbacol (5.5×10^{-6} M) to obtain a marked muscular tone. The agonistic action of the compounds under test was assessed as the ability to inhibit the constant level of tracheal smooth muscle tone induced by carbachol.

The concentration–response curves were obtained using the method of cumulative concentrations for all organs except the atria. Agonist activity was expressed in terms of pD_2 values ($-\log ED_{50}$ i.e. the negative logarithm of a drug molar concentration producing 50% of the maximal response) and intrinsic activity (the ratio between the maximal response of a test compound and that of the reference agonist, which was NE for a receptors and ISO for β receptors). Antagonist activity was evaluated as the ability of the compounds under test to reduce the response to a submaximal concentration of ISO after an incubation period of 30 min and expressed as $-\log IC_{50}$, i.e. the negative logarithm of the concentration that reduced the agonist response by 50%.

The following drugs were used as salts: **1a** (*l*-NE) as a bitartrate, **1b** (*l*-ISO) as a hydrochloride, compounds **10a**,**b** and **11a**,**b** as oxalates.

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