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Short communication

Synthesis and β-adrenergic properties of tetrahydronaphthalene analogs of dichloroisoproterenol*

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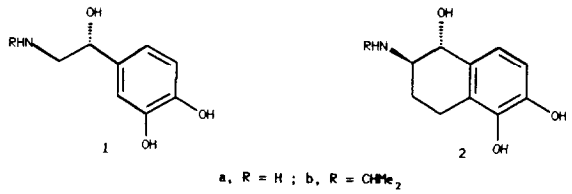
Summary — The tetrahydronaphthalene analogs **4** and **5** of DCI (**3b**) and of its *N*-unsubstituted derivative (**3a**) were synthesized and tested for their β-adrenergic properties by means of both binding tests and functional tests. Compounds **4** and **5** proved to possess a good affinity for β-adrenergic receptors accompanied by an appreciable β-blocking activity, thus indicating that the formal insertion of a β-blocking drug such as DCI into a tetrahydronaphthalene structure is able to provide compounds which still possess a β-blocking activity.

adrenergic drugs / β-blocking agents / dichloroisoproterenol cyclic analogs / tetrahydronaphthalene derivatives

Introduction

Within the sphere of the studies carried out on the influence of the conformational situation of adrenergic drugs on their activity, there have been some publications [1–10] dealing with cyclic analogs of catecholamines and of drugs that can be structurally correlated with them, in which the 3 pharmacophoric groups (aryl moiety, amine nitrogen and alcoholic oxygen) [11–17] are incorporated in conformationally restricted structures. If the examination is limited to those compounds which are active on the β-adrenergic receptor, it may be seen that while there are a certain number of conformationally restricted analogs of β-stimulating drugs which present the same properties as the corresponding open-chain compounds[4, 9, 10], no analogs of β-blocking drugs have been described which possess to any appreciable degree the adrenergic activity of the corresponding aminoalcohols. Among the semi-rigid analogs of agonist drugs, the tetrahydronaphthalene derivatives **2** [1, 4] have been shown to possess the high stimulating activity of catecholamines **1** towards both the β_1- and the β_2-adrenergic receptors. The catecholic structure characteristic of catecholamines **1**, which their agonist properties are known to be linked to, is present in compounds **2**. In these tetrahydronaphthalenic compounds (**2**), the pharmacophoric groups [11–17] present a spatial arrangement [18, 19] which corresponds to that found, for the same groups, in catecholamines **1** in their preferred conformation [20].



On the basis of these data, we hypothesized that the substitution of the catecholic portion of a structure possessing a high capacity to interact with the β-adrenergic receptor, such as that of **2**, with a portion typical of β-blocking drugs, might represent a means to obtain semi-rigid compounds which in turn possess β-blocking properties.

*Fourteenth paper in the series: 'Conformational effects on the activity of drugs'. For preceding paper, see [1]. A preliminary account of this work was presented at the Interdivisional Meeting of the Italian Chemical Society CISCI 90, San Benedetto del Tronto, Sept 1990, abstr FP 49.

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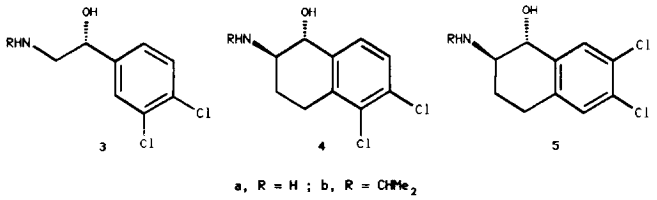


a, R = H ; b, R = $CHMe_2$

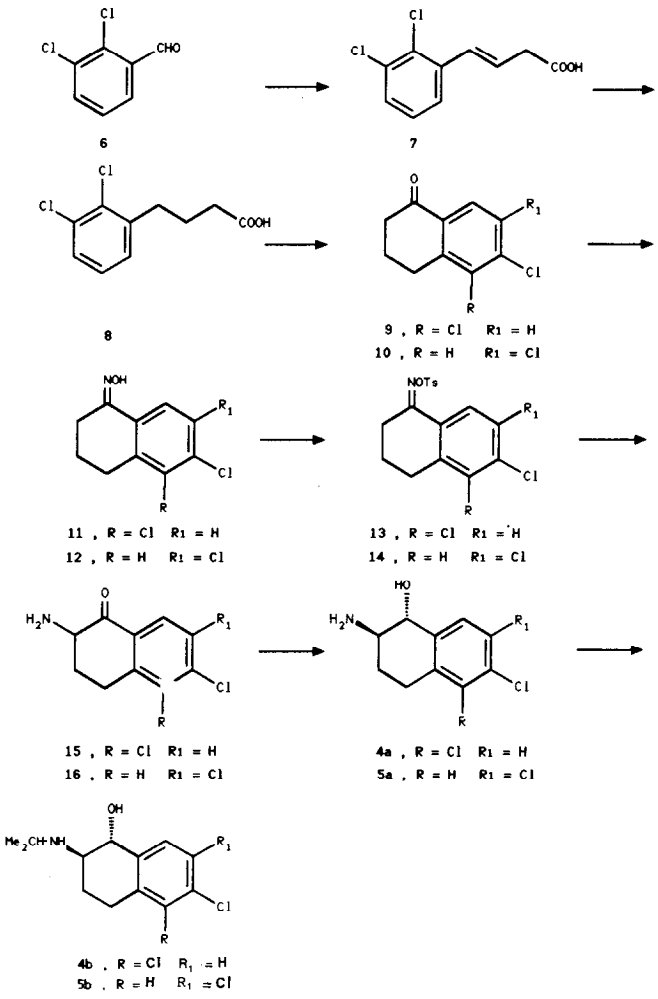
The present paper describes the synthesis and the β-adrenergic properties of the tetrahydronaphthalene derivatives **4b** and **5b** and of their analogs which are not substituted on the nitrogen (**4a** and **5a**)*, which present the dichlorophenyl group of a known β-blocking drug such as dichloroisoproterenol (DCI, **3b**) in the place of the catecholic portion present in the β-stimulating tetrahydronaphthalene derivatives of type **2**. DCI was the first drug shown to produce a selective blockade of the β-adrenergic receptors [21], but it was not of therapeutic interest as it appeared to have sympathomimetic properties [22].

The semi-rigid compounds of type **4** and **5** differ in the position of a chlorine atom in the aromatic system, and may be considered as formally obtained from DCI (**3b**) and its derivative which is not substituted on the nitrogen (**3a**), by means of the insertion of an ethylene bridge between the C(2) of the ethanolamine side chain and either of the 2 carbon atoms of the aromatic ring in the *ortho* position with respect to the side chain. Thus, **4** and **5** represent conformationally restricted analogs of 2 opposite rotameric forms around the C(α)-C(1) bond of the corresponding aminoalcohols of type **3**.

Chemistry

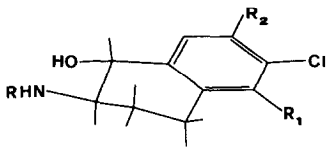
The synthesis of the *trans*-2-amino-5,6-dichloro-1,2,3,4-tetrahydronaphthalen-1-ol **4a** and the *trans*-2-amino-6,7-dichloro-1,2,3,4-tetrahydronaphthalen-1-ol **5a** and of their *N*-isopropyl derivatives (**4b** and **5b**) is shown in scheme 1. The 5,6-dichlorotetrahydronaphthalenone (**9**) was obtained by Wittig reaction of 2,3-dichlorobenzaldehyde (**6**) with 2-carboxyethyltriphenylphosphonium chloride [23, 24] followed by treatment of the crude acid **7** with hydrogen in the presence of 10% platinum on charcoal. The acid thus obtained (**8**) was cyclized to the 5,6-dichloronaphthalenone **9** using polyphosphoric acid and phosphorous pentoxide. The 5,6-dichloro (**9**), and the 6,7-dichloronaphthalenone (**10**) [25] were converted to their oximes (**11**, **12**) by treatment with hydroxylamine hydrochloride in dry pyridine. Reaction of **11** and **12** with *p*-toluenesulfonyl chloride in pyridine afforded the tosyl derivatives **13** and **14** which, by Neber rearrangement with potassium ethoxide in benzene and subsequent treatment with hydrochloric acid, yielded the amino ketones **15** and **16** as hydrochlorides.

Compounds **15** and **16** were stereoselectively reduced with sodium borohydride to the corresponding *trans*-aminoalcohols **4a** and **5a**. Reductive alkylation of **4a** and **5a** with acetone and sodium cyanoborohydride gave the 5,6-dichloro-**4b** and the 6,7-dichloro-2-(isopropylamino)-1,2,3,4-tetrahydronaphthalen-1-ol **5b**, respectively.



Scheme 1.

*All compounds were synthesized and tested as racemates. However, in the scheme and formulas, only the enantiomer in which the relative configuration on C(1) corresponds to that of the natural catecholamines is shown.



4, R_1=Cl; R_2=H

5, R_1=H; R_2=Cl

Fig 1. Compounds **4** and **5** in their preferred conformation.

The *trans* configuration of the tetrahydronaphthalene derivatives **4** and **5** was determined on the basis of their ^{1}H-NMR spectral data. The relatively high value of the coupling constant between the protons linked to the C(l) and the C(2) carbons (≈9 Hz) is in agreement with a *trans*-diaxial relationship between them, thus indicating that the benzylic hydroxyl and the amino group are in the *trans*-diequatorial position.

Confirmation of this steric relationship between these 2 groups in compounds **4** and **5** was obtained by an examination of their IR spectra in the 3 μ range in a dilute solution, showing a strong absorption at ≈3 500 cm^{-1} that can be attributed to an intramolecular OH···N interaction [26, 28]. The existence of this intramolecular hydrogen bond in **4** and **5** is, for steric reasons, only possible if the OH and NHR groups are in a *gauche* relationship. On the basis of these results, it is possible to also assign to **4** and **5** the half-chair conformation shown in figure l; this conformation corresponds to the preferential one found for the dihydroxytetrahydronaphthalene derivatives **2** (see *Introduction*). Compounds **4** and **5**, in the conformation shown, present a spatial arrangement of the O-C(l)Ar-C(2)-N portion which corresponds to that found for the same portion in the aminoalcohols **3** [29] in their preferred conformation, except for small differences in the torsion angles due to the half-chair system.

Biological studies

Radioligand binding assay

The β-adrenergic affinity of compounds **3–5** (see table I) was checked by binding tests on rat brain membranes for β_1-receptors, and on bovine lung membranes for β_2-receptors. ^{3}H-CGP 26505 was used as a specific tritiated ligand for rat brain β_1-receptors. ^{3}H-DHA was used to label bovine lung β_2-receptors in the presence of 50 nM CGP 26505 which displaced ^{3}H-DHA binding from the β_1-adrenoceptor subpopulation, which represents 17% in the bovine lung.

Rat brain β_1-receptors

Among the compounds unsubstituted on the nitrogen, the cyclic derivatives **4a** and **5a** present a similar binding affinity which is higher than that of the corresponding open-chain compound **3a**. Also the cyclic *N*-isopropyl-substituted compounds **4b** and **5b** present a similar binding affinity, which, however, in this case is lower than that of DCI (**3b**).

Bovine lung β_2-receptors

The aminoalcohol **3a** and its cyclic analogs **4a** and **5a** show a limited capacity to interact with the β_2-adrenergic receptor, with K_i values that are essentially similar. Among the *N*-isopropyl-substituted com-

Table I. β-Adrenergic activity and radioligand binding affinity of compounds **3–5**.

Compds	*β-Adrenoreceptor activity*[a]				*β-Adrenergic affinity*[b]			
	Isolated guinea pig atria (β_1)		*Isolated guinea pig tracheal strips (β_2)*		*Rat brain (β_1)*		*Bovine lung (β_2)*	
	pD_2	*–log IC_{50}*	*pD_2*[c]	*–log IC_{50}*	*K_i (nM)*	*Hill coeff*	*K_i (nM)*	*Hill coeff*
3a	–	4.56 ± 0.13	3.91 ± 0.20 (0.32)	< 3.50	17 000 (15 700–18 300)	1.00	10 900 (9700–12 000)	1.00
4a	–	4.84 ± 0.02	4.03 ± 0.07 (1.00)	4.68 ± 0.04	990 (910–1080)	1.00	5000 (4700–5300)	0.96
5a	–	4.60 ± 0.10	4.11 ± 0.11 (1.00)	4.18 ± 0.22	1900 (1700–2100)	1.00	7100 (6400–7800)	0.99
3b	–	6.94 ± 0.23	4.41 ± 0.61 (1.00)	6.01 ± 0.55	51 (44–57)	1.00	140 (120–160)	0.99
4b	–	5.62 ± 0.05	4.43 ± 0.08 (1.00)	4.88 ± 0.22	1200 (1100–1300)	0.97	420 (390–440)	1.00
5b	–	4.74 ± 0.20	4.81 ± 0.26 (1.00)	4.90 ± 0.16	610 (550–670)	0.99	2200 (2000–2400)	0.99
Propranolol	–	7.46 ± 0.10	–	7.54 ± 0.15	4.9 (3.6–6.1)	0.99	1.7 (1.4–1.9)	1.00

[a]The values represent the mean of 4–6 experiments for each drug ± SDS. [b]Geometric means of 5–6 experiments with confidence 95% limits shown in parentheses. [c]Intrinsic activity shown in parentheses, *ie*, the ratio between the maximal responses elicited by the compound tested and by the full agonist, namely NE and *l*-isoprenaline for α and β adrenoreceptors, respectively.

pounds, the derivative **4b** exhibits a binding affinity similar to that of DCI (**3b**), whereas the positional isomer of **4b** (**5b**) reveals a lower binding affinity.

Functional tests

Compounds **3–5** were tested on isolated guinea pig atria and on isolated guinea pig tracheal strips for their activity on β_1- and β_2-receptors respectively (see table I). The results obtained by us in these functional assays for compounds **3** were essentially in agreement with those reported in the literature [30, 31].

Guinea pig atria β_1-receptors
All the compounds tested displayed β-blocking activity, evidenced by their ability to antagonize the isoprenaline inotropic response. Both the *N*-unsubstituted tetrahydronaphthalene derivatives **4a** and **5a** showed an activity on β_1-receptors that was practically identical to that of the open-chain compound **3a**. As regards the *N*-isopropyl-substituted compounds, the tetrahydronaphthalene derivative **4b** showed an appreciable $-\log IC_{50}$ value, even if ≈1 order of magnitude lower than that of DCI; the positional isomer of **4b** (**5b**) showed a lower $-\log IC_{50}$ value.

No stimulating properties were detected for the compounds examined.

Guinea pig tracheal strip β_2-receptors
Compounds **4** and **5**, tested on tracheal β_2-receptors, generally displayed both stimulating and blocking properties. The pD_2 values show that the cyclic compounds **4** and **5** maintain the ability to stimulate the β_2-receptors of the corresponding aminoalcohols **3**. All compounds, with the exception of **3a**, were able to inhibit β_2-receptors. The *N*-unsubstituted tetrahydronaphthalene derivative **4a** and the 2 *N*-isopropylsubstituted tetrahydronaphthalene derivatives **4b** and **5b** exhibited similar $-\log IC_{50}$ values which were ≈1 order of magnitude lower than that of DCI; the other *N*-unsubstituted tetrahydronaphthalene derivative **5a** showed a slightly lower $-\log IC_{50}$ value.

Conclusions

Results of both the binding and the functional tests indicate that the tetrahydronaphthalene compounds **4** and **5** are able to interact with β_1- and β_2-adrenergic receptors. Compounds **4a** and **5a** which are not substituted on the nitrogen, reveal an affinity and an activity which are higher than those of the corresponding open-chain compound **3a**. Also the *N*-isopropyl-substituted tetrahydronaphthalene derivatives proved to maintain a large part of the affinity and of the blocking activity of the corresponding aminoalcohol DCI (**3b**). Taken overall, these results indicate that in the tetrahydronaphthalene derivatives, the substitution of the catecholic group of **2** with a group typical of a β-blocking drug such as DCI leads to compounds (**4** and **5**) which still possess a good affinity for β-adrenergic receptors, accompanied by an appreciable β-blocking activity. It would therefore appear that also for β-blocking drugs of the DCI type, their formal insertion into a tetrahydronaphthalene structure in their preferential conformation does not impede the maintenance of the β-blocking activity. As regards the importance of the rotameric position of the aryl regarding the activity in compounds **3–5**, the biopharmacological data that can be obtained do not make it possible to advance any certain interpretation. No significant differences are found for the 2 cyclic *N*-unsubstituted compounds (**4a** and **5a**), either in the values of the affinity indices or in those of the receptor activity; on the contrary, for the 2 *N*-isopropyl-substituted tetrahydronaphthalene derivatives (**4b** and **5b**), the binding test results indicate that **4b** possesses a greater affinity for the β_2 receptor and **5b** for the β_1 receptor, whereas the results of the functional tests indicate a greater activity of **4b** on the β_1-receptor and comparable activities of the 2 compounds (**4b** and **5b**) on the β_2 receptor.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken on paraffin oil mulls or as liquid film on a Perkin–Elmer Model 1310 instrument, and those for determination of OH stretching band frequency of **4** and **5** were measured with a Perkin–Elmer Model 257 double-beam grating instrument in dried ($CaCl_2$) CCl_4, using the indene band at 3110 cm^{-1} as calibrator standard; a 2-cm optical length quartz cell was employed, and the concentration of the solution was $< 5 \times 10^{-3}$ M to prevent intermolecular associations. ^{1}H-MR spectra of all compounds were routinely detected with a Varian EM 360 A instrument 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 of compounds **4** and **5** as free bases were determined with a Bruker AC-200 instrument in *ca* 2% solution of $CDCl_3$, while the ^{1}H-NMR spectra of **4** and **5**, as salts, were detected in *ca* 2% D_2O solution with a Varian CFT-20 instrument operating at 80 MHz. Compounds **3a** and **3b** were synthesized following a method previously described for analogous compounds [32]. Evaporation was carried out *in vacuo* (rotating evaporator). $MgSO_4$ was used as the drying agent in all cases. Elemental analyses were performed by our analytical laboratory and agreed with the theoretical values to within ±0.4%.

5, 6-Dichloro-3, 4-dihydronaphthalen-1(2H)-one **9**
A solution of (2-carboxyethyl)-triphenylphosponiumchloride [23, 24] (41.2 g, 0.11 mol) in anydrous THF (200 ml) was

added dropwise under nitrogen to a stirred suspension of $NaNH_2$ (8.4 g; 0.215 mol). After completion of the addition, the reaction mixture was stirred at room temperature for 1 h, cooled at 0°C and then treated dropwise with a solution of **6** (12 g, 68.6 mmol) in anydrous THF (170 ml). The reaction mixture was stirred at room temperature overnight, poured into ice and washed with Et_2O. The aqueous solution was acidified with 36% HCl and extracted with Et_2O. The organic layer was extracted with $NaHCO_3$ solution and the aqueous phase was acidified with 36% HCl and then extracted with Et_2O. The organic extracts were dried and evaporated to yield an oil consisting essentially of 4-(2,3-dicholorophenyl)-3-butenoic acid **7** [16.0 g; ^{1}H-NMR δ 3.38 (d, 2H, J = 7 Hz)], which was used for the following reaction without further purification.

A solution of **7** (16.0 g, 69.2 mmol) and 3 N HCl (21 ml) in a 4:1 CH_2Cl_2/MeOH mixture (100 ml) was shaken under hydrogen at room temperature and atmospheric pressure in the presence of 10% Pt on charcoal (1.60 g). When the absorption stopped, the catalyst was filtered off and the solution was concentrated and washed with water. Evaporation of the organic layer yielded an oily residue consisting of practically pure 4-(2,3-dichlorophenyl)butanoic acid **8** [15.8 g; ^{1}H-NMR δ 1.63–2.9 (m, 6H)].

The crude **8** (15.8 g; 0.068 mol) was added to a suspension of polyphosphoric acid (158 g, 0.46 mol) and P_2O_5 (15.8 g, 0.11 mol). The mixture was stirred at 100°C for 1.5 h and then cooled to room temperature and diluted with ice-cooled water (500 ml). The aqueous solution was extracted with AcOEt and the organic extracts were dried and evaporated to give a crude oil which was purified by column chromatography on 100–200 mesh Florisil eluting with 4:1 hexane/acetone mixture to yield pure **9** as an oil (2.8 g, 20%); ^{1}H-NMR δ 2.0–3.28 (m, 6H), 7.43 (d, 1H, J = 8 Hz), 7.97 (d, 1H, J = 8 Hz). Anal $C_{10}H_8C1_2O$ (C, H).

6,7-Dichloro-3,4-dihydronaphthalen-1(2H)-one ***10***
This compound was prepared following the synthetic route previously described [25]: mp: 104–106°C (cyclohexane), lit [25]: mp: 104–108°C (EtOH/H_2O).

5,6-Dichloro-3,4-dihydronaphthalen-1(2H)-one oxime ***11***
A mixture of **9** (3.5 g, 16.3 mmol) and NH_2·OH HCl (5.82 g, 83.8 mmol) in anhydrous pyridine (30 ml) was heated at 120°C for 1 h. After cooling at room temperature, the reaction mixture was poured into ice-cooled water and then was extracted with AcOEt dried and evaporated to give a solid which was crystallized from $CHCl_3$ to give **11** (2.8 g, 75%); mp: 138–140°C; ^{1}H-NMR δ 1.73–2.10 (m, 2H), 2,70–3.10 (m, 4H), 7.41 (d, 1H, J = 8.5 Hz), 7.91 (d, 1H, J = 8.5 Hz). Anal $C_{10}H_9Cl_2NO$ (C, H, N).

6,7-Dichloro-3,4-dihydronaphthalen-1(2H)-one oxime ***12***
This compound was prepared from **10** (5 g, 23.2 mmol) following the same procedure described above for the preparation of compound **11**. The crude product was purified by crystallization from EtOH to yield pure **12** (3.7 g, 69%); mp: 179–181 °C; ^{1}H-NMR δ 1.71–2.11 (m, 2H), 2.60–3.00 (m, 4H), 7.38 (s, 1H), 8.13 (s, 1H). Anal $C_{10}H_9Cl_2NO$ (C, H, N).

5,6-Dichloro-3,4-dihydronaphthalen-1(2H)-one O-p-toluensulfonyloxime ***13***
A solution of *p*-toluensulfonyl chloride (4.82 g) in anhydrous pyridine (17.4 ml) was added dropwise to an ice-cooled solution of **11** (2.8 g, 12.2 mmol) in anhydrous pyridine (17.4 ml). The reaction mixture was stirred at 0°C for 30 min and for a further 1 h at room temperature, poured into water and then extracted with $CHCl_3$. Evaporation of the dried organic layers afforded a residue which was submitted to column chromatography on 70–230 mesh silica gel, using $CHCl_3$ as eluant. The solid obtained was crystallized from EtOH to give pure **13** (1.7 g, 36%); mp: 123–125°C; ^{1}H-NMR δ 2.36 (s, 3H). Anal $C_{17}H_{15}Cl_2NO_3S$ (C, H, N).

6,7-Dichloro-3,4-dihydronaphthalen-1(2H)-one O-p-toluensulphonyloxime ***14***
This compound was prepared from **12** (3.54 g, 15.4 mmol) following the same procedure described above for the preparation of compound **13**. Crystallization from EtOH yielded pure **14** (4 g, 67%); mp: 169–170°C; ^{1}H-NMR δ 2.48 (s, 3H). Anal $C_{17}H_{15}Cl_2NO_3S$ (C, H, N).

2-Amino-5,6-dichloro-3,4-dihydro-1(2H)-naphthalenone ***15***
A solution of EtOK, prepared from K (290 mg, 7, $42 \cdot 10^{-3}$ gatom) and absolute EtOH (7 ml), was added dropwise to a solution of **13** (2.54 g, 6.6 mmol) in anhydrous benzene (40 ml). The resulting mixture was stirred at 0°C for 5 h and then allowed to stand at 5°C for 5 d. The insoluble material was removed by filtration and the solution was treated with 36% HCl (2.5 ml) to yield a solid precipitate which was crystallized from MeOH/Et_2O to give pure **15**·HCl (1.0 g, 56%); mp: 230°C dec; IR (nujol) ν 1700 cm^{-1} (C=O). Anal $C_{10}H_{10}Cl_3NO$ (C, H, N).

2-Amino-6,7-dichloro-3,4-dihydronaphthalen-1(2H)-one ***16***
This compound was prepared from **14** (2 g, 5.2 mmol) following the same procedure described above for the preparation of compound **15**. Crystallization of the crude product from EtOH/Et_2O yielded **16**·HCl (0.45 g, 30%); mp: 179°C dec; IR (nujol) ν 1718 cm^{-1} (C=O). Anal $C_{10}H_{10}Cl_3NO$ (C, H, N).

trans-2-Amino-5,6-dichloro-1,2,3,4-tetrahydronaphthalen-1-ol ***4a***
$NaBH_4$ (1.02 g, 26.9 mmol) was added portionwise to a solution of **15** (0.61 g, 2.28 mmol) in MeOH (57 ml) and the mixture was stirred at room temperature for 20 min, then diluted with water and extracted with $CHCl_3$. Evaporation of the washed (H_2O) and dried organic layer yielded a solid residue which was crystallized from AcOEt to give pure **4a** (0.29 g, 54%); mp: 176–178°C; IR (CCl_4) ν 3510 cm^{-1} (OH···N); ^{1}H-NMR δ 4.32 (d, 1H, J = 8.9 Hz), 7.35 (d, 1H, J = 8.4 Hz), 7.46 (d, 1H, J = 8.4 Hz). Anal $C_{10}H_{11}Cl_2NO$ (C, H, N).

The maleate salt of **4a** melted at 194–195°C; ^{1}H-NMR δ 1.90–2.40 (m, 2H), 2.77–3.12 (m, 2H), 3.22–3.63 (m, 1H), 6.28 (s, 2H), 7.45 (s, 2H). Anal $C_{14}H_{15}Cl_2NO_5$ (C, H, N).

trans-2-Amino-6,7-dichloro-1,2,3,4-tetrahydronaphthalen-1-ol ***5a***
Compound **5a** was obtained from **16** (0.4 g, 1.5 mmol) following the same procedure described above for the preparation of compound **4a**. Crysrallization of the crude solid from AcOEt afforded pure **5a** (0.2 g, 57%); mp: 163–164°C; IR ($CC1_4$) ν 3520 cm^{-1}; ^{1}H-NMR δ 4.29 (d, 1H, J = 9Hz), 7.17 (s,1H), 7.65 (s, 1H). Anal $C_{10}H_{11}Cl_2NO$ (C, H, N).

The maleate salt of **5a** melted at 182–183°C; ^{1}H-NMR δ 1.85–2.40 (m, 2H), 2.72–3.07 (m, 2H), 3.15–3.69 (m, 1H), 6.22 (s, 2H), 7.25 (s, 1H), 7.52 (s, 1H). Anal $C_{14}H_{15}Cl_2NO_5$ (C, H, N).

trans-2-(Isopropyl amino)-5, 6-dichloro-1,2,3,4-tetrahydronaphthalen-1-ol ***4b***
$NaBH_3CN$ (0.086 g, 1.38 mmol) was added portionwise to a stirred solution of **4a** (0.16 g, 0.69 mmol) in a mixture of Me_2CO (1.4 ml) and MeOH (6.6 ml). After 5 h, the solution

was poured into water, extracted with $CHCl_3$, dried and evaporated to yield a residue which was crystallized from AcOEt to afford pure **4b** (0.10 g, 50%); mp: 98–100°C; IR (CCl_4) ν 3490 cm^{-1}; ^{1}H-HMR δ 1.06 and 1.04 (2d, 6H, J = 6.2 Hz), 3.11 (m, 1H, J = 6.2 Hz), 4.35 (d, 1H, J = 9.1 Hz), 7.26 (d, 1H, J = 8.5 Hz), 7.35 (d, 1H, J = 8.5 Hz). Anal $C_{13}H_{17}Cl_2NO$ (C, H, N).

The maleate salt of **4b** melted at 177–178°C; ^{1}H-NMR δ 1.37 and 1.42 (2d, 6H, J = 6.5 Hz), 1.86–2,53 (m, 2H), 2.78–3.21 (m, 2H), 3.38–3.85 (m, 2H), 6.28 (s, 2H), 7.44 (d, 1H, J = 8.5 Hz), 7.50 (d, 1H, J = 8.5 Hz). Anal $C_{17}H_{21}Cl_2NO_5$ (C, H, N).

trans-2-(Isopropylamino)-6,7-dichloro-1,2,3,4-tetrahydronaphthalen-1-ol ***5b***

This compound was prepared from **5a** (0.12 g, 0.51 mmol) following the same procedure described above for the preparation of compound **4b**. Crystallization from AcOEt of the crude product afforded **5b** (0.08 g, 55%); mp: 126–128°C. IR (CCl_4) ν 3500 cm^{-1}; ^{1}H-NMR δ 1.14 and 1.23 (2d, 6H, J = 6.2 Hz), 3.15 (m, 1H, J = 6.2 Hz), 4.42 (d, 1H, J = 9.3 Hz), 7.16 (s, 1H), 7.66 (s, 1H). Anal $C_{13}H_{17}Cl_2NO$ (C, H, N).

The maleate salt of **5b** melted at 148–150°C; ^{1}H-NMR δ 1.35–1.40 (2d, 6H, J = 6.4 Hz), 1.84–2.40 (m, 2H), 2.74–3.05 (m, 2H), 3.23–3.87 (m, 2H), 6.28 (s, 2H), 7.35 (s, 1H), 7.60 (s, 1H). Anal $C_{17}H_{21}Cl_2NO_5$ (C, H, N).

Radioligand binding methods

Rat brain β_1-receptors

β_1-Receptors were assayed in rat cortical membranes, as previously described [1], using ^{3}H-CGP 26505 (1-(2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino)-3-(4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy)-2-propanol as specific ligand (Du Pont de Nemours, New England Nuclear Division, spec act 28.5 Ci/mmol) [1].

Rat cortices were rapidly isolated and homogenized in 10 vol ice-cold 50 mM Tris–HCl buffer at pH 8. The homogenates were centrifuged at 48 000 *g* for 15 min at 4°C. This step was repeated 4 times, resuspending the pellets in 10 vol fresh Tris buffer. The final crude membranes were suspended in Tris–HCl buffer containing 0.1% ascorbic acid. Protein concentration, as assayed by the method of Lowry *et al* [33], amounted to 4 mg/ml for displacement studies.

Routine ^{3}H-CGP binding assays were run by incubating 0.1 ml crude rat brain membrane suspensions at 25°C for 60 min with 1 nM ^{3}H-CGP in a total vol of 0.5 ml Tris–HCl buffer. Incubations were terminated by rapid vacuum filtration through Whatman GF/B glass-fiber filters. Filters were washed with 3 x 5-ml portions of ice cold Tris–HCl buffer, dried, and added to 8 ml Ready Protein Beckman scintillation cocktail. The specific binding was determined as the excess over blanks containing 30 μM *l*-isoprenaline.

Bovine lung β_2-receptors

β_2-receptor binding was studied in bovine lung using ^{3}H-dihydroalprenolol (DHA) as ligand (Du Pont de Nemours, New England Nuclear Division, spec act 48.1 Ci/mmol).

Membranes were obtained by lung homogenization in 1:20 vol 0.32 M sucrose, and centrifugation at 800 *g* for 10 min at 5°C. The supernatant was centrifuged at 3 000 *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 phosphate buffer (~4 mg/ml 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 x 5 ml phosphate buffer, dried and added to 8 ml Ready Protein Beckman scintillation cocktail. Non-specific binding was measured in the presence of 35 μM *l*-isoprenaline.

The inhibition of the ^{3}H-CGP 26505 and ^{3}H-DHA specific binding were determined in the presence of various concentrations (6) of the compounds studied. The affinity of the compounds for the 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 inhibition constant (K_i) was derived according to the equation of Cheng and Prusoff [34]. The dissociation constants (K_d) of ^{3}H-CGP 26505 and ^{3}H-DHA were 0.7 and 1.0 nM, respectively.

Pharmacological methods

The activity of compounds **3–5** on β-adrenoceptors was evaluated on isolated preparations obtained from adult male Dunkin–Hartley guinea pigs weighing 300–350 g. Isolated guinea pig atria were employed to determine the activity of the compounds on β_1-adrenoceptors in accordance with [1]. The extremities of the strip consisting of both atria were tied with an inextensive thread. The first thread was used to tie the organ to a muscle holder fixed in place in a muscle chamber of an isolated organ bath; the second thread was used to connect atria to an isometric force-displacement transducer (Basile mod 7005) situated above the muscle chamber and connected to a microdynamometer (Basile mod 7050). The bathing fluid was Tyrode solution at 37°C gassed with pure O_2. The atria were left at rest for 45 min before starting the experiments and then they were submitted to increasing doses of *l*-isoprenaline (at least 5 concentrations) to obtain dose–response curves with the single dose method.

The agonistic actions of the compounds under testing were assessed as their ability to increase the inotropic activity of spontaneously beating atria. Antagonistic properties were evaluated as the progressive reduction of submaximal agonist responses to increasing concentrations of the drugs. The contact period for each antagonistic dose was 30 min.

Isolated guinea pig tracheal strips were used to evaluate the action of the compounds on β_2-adrenoreceptors. The organs were excised, cleaned of adhering fat and connective tissue and prepared following the method of Emmerson and Mackay [35]. Zigzag tracheal strips were tied at each end and suspended in a muscle chamber as described for atria. The perfusion fluid of the organs was Krebs maintained at 37°C and aerated with 95% O_2–5% CO_2. The tracheae were attached to an isotonic force displacement transducer (Basile mod 7006) and left to equilibrate for 1 h before administering drugs. The responses were registered by a microdynamometer Basile mod 7050. In order to evaluate the relaxant properties of β agonists, the preparations were contracted by carbachol (5.5×10^{-6} M). A dose–effect curve to *l*-isoprenaline was obtained in each organ and then the agonistic and antagonistic activities of the compounds under test were assessed. The dose–response curves were obtained out by using the method of cumulative doses [36]. The agonistic action was assessed as the ability of *l*-isoprenaline and the compounds under test to relax the tracheal smooth musculature precontracted with carbachol; the antagonistic effects of the same compounds were evaluated after 30 min incubation as their inhibitor properties on the relaxant effect of a submaximal dose of *l*-isoprenaline.

Agonism of the above drugs was indicated as pD_2, that is the negative logarithm of the drug molar concentration producing 50% of the maximal response; antagonism was expressed as

$-\log IC_{50}$, that is to say the negative logarithm of the drug molar concentration able to reduce by 50% the stimulating effect of *l*-isoprenaline.

Propranolol was taken as the reference antagonist.

The following drugs were used as salts: *l*-isoprenaline, dichloroisoproterenol, propranolol and compound **3a** as hydrochlorides, NE as bitartrate, carbachol as chloride and the cyclic compounds **4** and **5** as maleates.

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