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Synthesis of halogenated analogs of 5-(4-chlorophenyl)-2,3-dihydro-5-hydroxy-5*H*-imidazo[2,1-*a*]-isoindole or mazindol for exploration of the dopamine transporter

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Summary — In order to study the presynaptic dopamine transporter in the human brain by single photon emission tomography (SPET) or positron emission tomography (PET) we have developed new halogenated mazindol derivatives using a pathway involving a reaction between a dilithio derivative of 2-phenyl-2-imidazoline and appropriate methyl or ethyl halogenobenzoates. After HPLC purification and chemical characterization, the affinity for the dopamine transporter was studied *in vitro* with [^{3}H]-GBR 12935 and compared to mazindol and nomifensine. Affinity potencies were determined as follow:- mazindol > brominated derivatives > iodinated derivatives > nomifensine > fluorinated derivatives. The properties were related to the structure of these compounds and showed the importance of the nature of the substituent on the primary phenyl ring of mazindol for affinity to the dopamine transporter.

mazindol / dopaminergic transporter / scintigraphy / structure–affinity relationship / PET–SPET

Introduction

Dysfunction of the central dopaminergic system is involved in several neurological disorders such as Parkinson's [1] and Alzheimer's [2] diseases and in psychiatric diseases such as schizophrenia [3]. Two facets of this dopamine system must be considered, the presynaptic uptake and the postsynaptic receptors. The D_2 dopamine receptors may be currently imaged by single photon emission tomography (SPET) with radioiodinated ligands [4, 5] or by positron emission tomography (PET) [6]. This exploration would be valuable for evaluating neuropsychiatric treatments by the measurement of occupancy of D_2 receptors by therapeutic drugs as demonstrated by PET. However, in some diseases such as Parkinson's disease [1] post and presynaptic neurons are not both affected, but only the presynaptic. A decrease in the number of dopamine uptake sites has been observed post mortem in brains of Parkinson's [1] and Alzheimer's disease [2] patients, which show 30 and 50% of control values, respectively. Moreover, dopamine uptake sites have been shown to decrease in rats [7] and in humans [8] as a function of age. Such *in vitro* determinations have been performed with [^{3}H]-GBR 12935 [9] or [^{3}H]-mazindol [10]. It would be of great value to also evaluate this presynaptic function *in vivo*. This would allow a better understanding of the role and the disturbance of this process in clinical psychopharmacology research, diagnosis and treatment evaluation. PET has been proposed [11, 12] for this purpose. In monkeys with unilateral dopaminergic lesions induced with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Leenders *et al* [12] showed a large decrease in the concentration of dopamine uptake sites using ^{11}C-nomifensine. Promising results indicate the possibility of *in vivo* exploration of the brain [13, 14]. PET is not available in a large number of nuclear medicine centers, so SPET would be more useful for the clinical application of this cerebral exploration. Currently, no specific photon emitter tracers are available. Some halogenated derivatives of GBR have been described by Van Dort *et al* [15], Hanson *et al* [16] and by the present study group [17] but, despite their *in vitro* affinity for the dopamine carrier, considerable nonspecific

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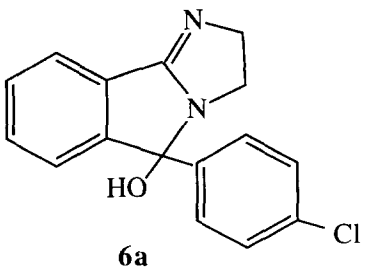
binding has been observed *in vivo*. Mazindol is a potent inhibitor of the dopamine uptake system. Indeed, *in vitro* studies with [^{3}H]-mazindol have shown that this compound binds to the dopamine carrier with high affinity [10] and is suitable to detect modifications of these sites in physiopathological situations [8]. Therefore we have prepared new halogenated derivatives of mazindol and tested their affinity for the dopamine transporter *in vitro* on rat striatal membranes. Additionally, structure–affinity relationship studies have been performed to describe the best structure for binding to the dopamine uptake system. For this, physical parameters have been determined using computer-aided design or using HPLC, and these parameters have been related to affinity properties.

Chemistry

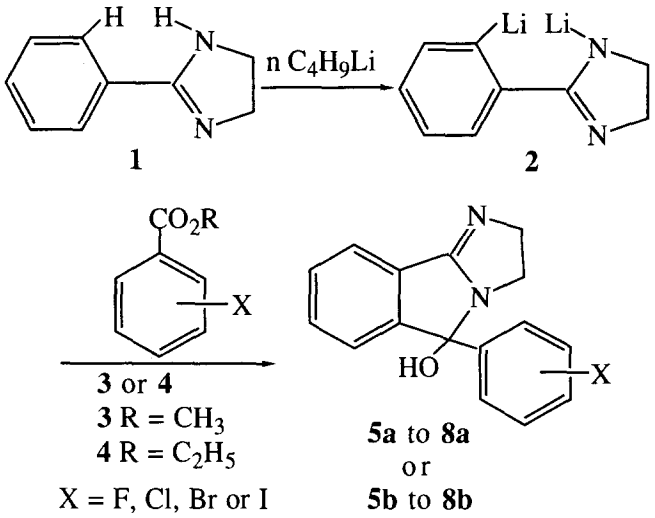
Several methods have been described for the preparation of mazindol **6a** (fig 1) and its analogs [18–21]. One of these methods, described by Houlihan [20], is a 3-step synthesis which starts by a reaction between an *ortho* benzoyl benzoic acid and ethylene diamine, followed by a reduction with $LiAlH_4$ before air oxidation. Another method (scheme 1), reported by Houlihan [21], is a 1-pot synthesis. It has permitted, for the first time, the synthesis of new halogenated derivatives of mazindol such as brominated **7a–b** and iodinated **8a–b** derivatives; we therefore chose this method with minor modifications.

The procedure consists of first preparing the dilithioderivative **2** by double hydrogen–metal exchange between 2-phenyl-2-imidazoline **1** and an excess of butyllithium. Then the organolithium derivative **2** is allowed to react with the appropriate methyl **3** or ethyl **4** halogenobenzoate. When we hydrolyzed the reaction mixture, excess of starting materials were solubilized, whereas the expected products were insoluble and precipitated. The crude products were separated by filtration. These compounds were recrystallized from 1-pentanol before use. The reaction



6a

Fig 1. Mazindol **6a**.



Scheme 1. One-pot synthesis of fluorinated, chlorinated, brominated, and iodinated analogs of mazindol.

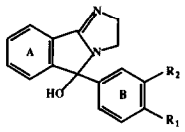
afforded *para* **5a** to **8a** or *meta* **5b** to **8b** halogenated derivatives in 40 to 70% yields and high chemical purity was determined from elemental analysis (see table I). Nevertheless, the products **7b**, **8a** and **8b** respectively present a chemical purity of 96.5, 97.0 and 98.5%; so they were re-purified by HPLC before biological utilization. The purity of **5a** to **8a** and **5b** to **8b** was finally controlled on the basis of their elution profile in a HPLC procedure according to the conditions reported in the *Experimental protocols*: the chromatogram exhibited the signal of the expected compound and the product corresponding to this signal was used for biological tests.

The reaction failed to obtain the *ortho* isomer from the corresponding methyl or ethyl 2-fluoro, 2-chloro, 2-bromo and 2-iodobenzoates. We hypothesize that the steric hindrance of halogen in the *ortho* position of the aromatic ring is the cause of the absence of reaction in this case.

Results and discussion

For PET or SPET exploration of presynaptic uptake, halogenated congeners of mazindol **6a** were synthesized (fluorinated **5a–b**, chlorinated **6b**, brominated **7a–b** and iodinated **8a–b**). The brominated **7a–b** and iodinated **8a–b** are novel analogs. These compounds are prepared using a reaction between a dilithio derivative of 2-phenyl-2-imidazoline **2** produced *in situ* and the appropriate methyl or ethyl halogenobenzoate [21]. This pathway is used to synthesize mazindol **6a** and its analogs, thus obtaining products with acceptable yields: 40 to 70%, of correct chemical purity (table I).

Table I. Halogenated analogs of mazindol.



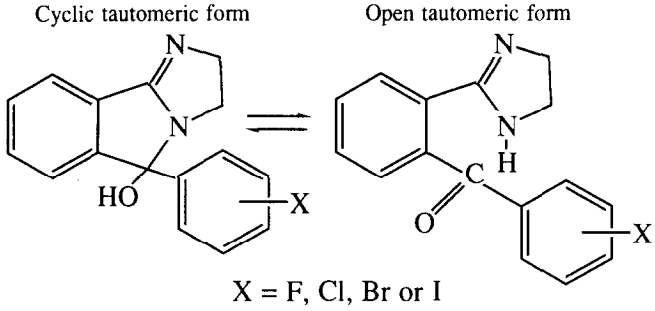
Compounds	R_1	R_2	*Formula*[a]	*mp, °C (1-pentanol)*	*Yield*[b] *(%)*	*Chemical purity*[c] *(%)*
5a (4-F)	F	H	$C_{16}H_{13}FN_2O$	198–200	60	99.0
5b (3-F)	H	F	$C_{16}H_{13}FN_2O$	200–202	70	99.0
6a (4-Cl)	Cl	H	$C_{16}H_{13}ClN_2O$	202–204	60	99.0
6b (3-Cl)	H	Cl	$C_{16}H_{13}ClN_2O$	208–210	40	99.5
7a (4-Br)	Br	H	$C_{16}H_{13}BrN_2O$	216–218	50	99.5
7b (3-Br)	H	Br	$C_{16}H_{13}BrN_2O$	218–220	60	96.5
8a (4-I)	I	H	$C_{16}H_{13}IN_2O$	206–208	60	97.0
8b (3-I)	H	I	$C_{16}H_{13}IN_2O$	224–226	50	98.5

[a]C, H, N analysis were within ±0.4% of the calculated values; [b]yield after recrystallization; [c]calculated from elemental analysis results.

Currently the development of radiopharmaceuticals is directed by empirical methodology consisting of the modification of ligands with known activity [22]. The most common pathway used to date in radiopharmaceutical development requires the synthesis and biological study of many compounds. Using structure–affinity relationship studies, it was possible to reduce the number of compounds to be synthesized and to find the best ligand for exploration of presynaptic uptake by PET or SPET.

In order to perform these studies we compared the affinity for the carrier and the physicochemical parameters of halogenated analogs to those of the reference compound mazindol **6a**. According to Barcza *et al* [23], mazindol **6a** and its analogs can exist under 2 tautomeric forms (see scheme 2). Moreover, the same authors showed that the mazindol base form is the cyclic form. When we heated these compounds in acetic acid medium we obtained the benzophenone tautomeric form (see scheme 2). Indeed, the chromatogram exhibited 2 signals, the first corresponding to the cyclic tautomeric form and the second (with a higher retention time) to the open form. Therefore, before biological analysis, we checked the tautomeric form of these products. HPLC analyses showed that the products were under their cyclic tautomeric form and that the biological experimental conditions did not modify this cyclic form.

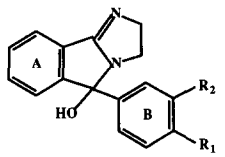
Inhibitory potencies (K_i) of all the compounds **5a** to **8a** and **5b** to **8b** were determined using the method of Cheng and Prusoff [24] by *in vitro* competition with [^{3}H]-GBR 12935 in comparison with mazindol **6a** and



Scheme 2. Tautomeric equilibrium between the cyclic tautomer form and the open tautomer form (benzophenone form).

nomifensine, which had a high affinity for the dopamine transporter. In our experiments, a mean K_d of 1.6 nM and a B_{max} of 10.6 pmol/mg protein were obtained for [^{3}H]-GBR 12935 on rat striatal membrane preparations. As can be seen in table II, the meta chlorinated analog **6b** had a similar activity (K_i = 30 nM) to that of mazindol **6a**. The *para* **7a** and the *meta* **7b** brominated analogs showed the best affinity to the dopamine transporter ($K_i \approx$ 35 nM). The affinity ($K_i \approx$ 100 nM) of the *para* **8a** and the *meta* **8b** iodinated analogs was weaker than that of mazindol **6a**. However, they exhibited better inhibitory potency than nomifensine (K_i = 270 nM). The *para* **5a** and the *meta* **5b** fluorinated analogs had a weak affinity ($K_i \approx$ 300 nM). Other authors [25] have found a lack of affinity of the non-halogenated derivative of mazindol to the dopamine transporter.

Table II. Inhibition constants for [^{3}H]-GBR 12935 binding, lipophilicity values and computational values.



Compounds	R_1	R_2	K_i *(nM)*[a]	*log* k_w[b]	$\sigma_{\pi B}$[c]	*rms*[c]
5a (4-F)	F	H	300 ± 50	0.95 ± 0.05	− 0.028	0.075
5b (3-F)	H	F	220 ± 40	1.28 ± 0.07	− 0.028	0.080
6a (4-Cl)	Cl	H	30 ± 7	1.82 ± 0.09	− 0.045	0.000
6b (3-Cl)	H	Cl	32 ± 5	1.85 ± 0.09	− 0.045	0.010
7a (4-Br)	Br	H	35 ± 6	1.90 ± 0.09	− 0.050	0.027
7b (3-Br)	H	Br	37 ± 3	1.97 ± 0.09	− 0.051	0.040
8a (4-I)	I	H	80 ± 15	2.20 ± 0.10	− 0.075	0.140
8b (3-I)	H	I	120 ± 20	2.30 ± 0.10	− 0.073	0.186

$\sigma_{\pi B}$: Average electronic density of phenyl ring B; rms: root mean square; [a]K_i values for dopamine uptake sites were determined by *in vitro* competition with [^{3}H]-GBR 12935 binding to rat striatal membranes, each value being the mean ± SE from 3 separate experiments; [b]these experiments were performed with a C-18 reverse phase chromatography column using MOPS buffer (20 mM, pH 7.5) containing 0.2% *n*-decylamine and methanol concentrations between 30 and 60% on a LKB isocratic liquid chromatograph fitted with UV detector at 254 nm; [c]calculations and molecular manipulations were carried out on a silicon graphics SG 25 workstation using an INSIGHT II program (Biosym).

To define the structure we chose electronic, lipophilic and steric parameters according to Hansch's studies [26].

The introduction of the halogen atom to the phenyl ring B involved a partial electronic modification, particulary in this ring, therefore we focused on this phenyl ring by determining its electronic density. Electronic density ($\sigma_{\pi B}$) is the average of calculated values of the precise load of each carbon atom to the ring B. We observed that K_i values and $\sigma_{\pi B}$ increased together in the case of chlorinated **6a–b**, brominated **7a–b** and iodinated **8a–b**, but not for fluorinated **5a–b** derivatives. According to Koe [27] and Terada [28], we can hypothesize that the ligand should bind to the receptor through a π–π bond. Indeed, taking mazindol **6a** as the template, electronic density diminution of ring B produced a considerable decrease in affinity to fluorinated analogs **5a–b**. This corroborates the results obtained by Heikkila *et al* [25] for the non-halogenated congener of mazindol **6a**. Consequently, an increase in electronic density for brominated **5a–b** and iodinated **8a–b** derivatives should lead to better affinity of these ligands, but these anticipated results were not observed. Therefore we can suggest that the other physicochemical factors interfere in the ligand–transporter interaction. In order to understand this fact better, it is necessary to take into account other structural parameters, both lipophilic and steric.

Lipophilic parameters were obtained by measuring the apparent lipophilicity factor (log k_ω) at pH = 7.5. For mazindol **6a** (*para* chlorinated compound) the apparent lipophilicity factor (log k_ω) is consistent with the water–octanol partition coefficient (log P_{oct}). Indeed, our measurement of log k_ω was 1.82 and the literature value of log P_{oct} is 1.809 [29]. These 2 parameters represent the same physical parameter in any given compound; this fact has been proved in studies of wide ranges of values [30]. We chose to measure log k_ω by an HPLC procedure because the experimental technique is easier. The lipophilicity increased in the order of fluorinated **5a–b**, chlorinated **6a–b**, brominated **7a–b** and iodinated **8a–b** products (F < Cl < Br < I). The affinity for the carrier decreased when the lipophilic values increased except for fluorinated compounds **5a–b**. As we demonstrated in electronic studies, the lipophilic factor, *ie* log k_ω of fluorinated analogs **5a–b**, is weak in comparison with other compounds and K_i values are the highest.

The superimposition of 2 products reveals the discordance of alignment of atoms, thus giving the steric parameters (rms values). Before the superimposing operation the energetically permissible conformations of all these compounds were ascertained. Thus for each product we minimized the energy of the most stable conformer obtained. Moreover, these low-energy structures are statistically populated to a large extent under physiological conditions [31]. In all cases, steric values were calculated using mazindol as the template. As shown in table II, for the chlorinated **6a–b**, brominated **7a–b** and iodinated **8a–b**, steric studies revealed that K_i values and steric values increased together; in this series for the

same halogen, steric values for compounds substituted in the *para* position were weaker than those substituted in the *meta* position. Consequently, *para* analogs are better than *meta* compounds except for fluorinated analogs **5a–b**. For these last products, we observed that steric values were weaker than those of iodinated analogs **8a–b** but K_i values were higher.

All these observations display a relationship between the structure of these halogenated congeners of mazindol **6a** and biological results and show the important role of the primary aromatic ring B during the interaction with the transporter.

Conclusion

The pathway used to prepare the whole series of products is valuable because it permits the synthesis of derivatives with appreciable yield and in only 2 steps. The structure–affinity relationship analysis shows the importance of the primary phenyl ring B in the affinity of the ligand to the dopamine transporter. This work proves that for PET studies brominated analogs **7a–b** and fluorinated compounds **5a–b** would be usable, although brominated analogs **7a–b** are preferable; their affinity to the dopamine transporter is better than that of fluorinated products **5a–b**. For the SPET studies, the *para* iodinated analog of mazindol **6a** labelled with 123 iodine is a potential tool.

Experimental protocols

Infrared spectra were recorded on a Perkin–Elmer IR 1310 spectrophotometer and ^{1}H-NMR spectra on a Brüker AC200 (200 MHz) spectrometer using $CDCl_3$/DMSO–d_6 as solvent and tetramethylsilane as internal standard; mass spectra were obtained on a VG 30F spectrometer (electronic impact at 70 eV). Elemental analyses were within ± 0.4% of theoretical values and were determined in the laboratory of the Service Central d'Analyse du CNRS (Vernaison, France). Melting points were not corrected. HPLC analyses were performed on a LKB isocratic liquid chromatograph fitted with a UV detector at 254 nm, using a reverse phase column 10 RP 18 (25 cm x 4.6 mm) from Chrompack, and a MeOH/1%, $Et_3N/CH_3CO_2NH_4$ buffer at pH 4.8 (50:50 v/v) as mobile phase (flow 1 ml/min). The method of El Tayar *et al* [30, 32] was utilized for estimating the lipophilicity of mazindol and its halogenated analogs. The compounds were analyzed on a C-18 reverse phase chromatography column using a 4-morpholino propanesulfonic acid (MOPS) buffer (20 mM, pH 7.5) containing 2 ml/l *n*-decylamine and methanol concentrations between 30 and 60%.

The logarithm of the capacity factor (log k_w) was plotted *versus* methanol concentration and the log k_w obtained by linear extrapolation to 0% methanol concentration. This value represents the apparent lipophilicity at pH 7.5.

The molecular structures were built and optimized with an INSIGHT II program (Biosym). Superimposing calculations were carried out using mazindol as the template. Electronic calculations were performed by an AMPAC–MOPAC program (INSIGHT II module). Calculations and molecular manipulations were carried out on a Silicon Graphics SG 25 workstation.

Preparation of 1,2'-dilithio-2-phenyl-2-imidazoline ***2***

According to the method described by Houlihan [21], 2-phenyl-2-imidazoline **1** from Aldrich (2.92 g, 20 mmol) in dry THF (45 ml), blanketed under nitrogen gas, was treated dropwise with 1.6 M butyllithium in hexane from Aldrich (40 ml, 64 mmol) at room temperature for 15 min. Then the mixture was stirred at 50°C for 3 h. The dilithio derivative **2** was used *in situ* for synthesis of compounds **5a** to **8a** and **5b** to **8b**.

5-(4-Fluorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***5a***

Ethyl 4-fluorobenzoate from Lancaster (7.87 g, 47 mmol) in dry THF (45 ml) was added to a solution of the dilithio derivative **2** (20 mmol). The mixture was stirred at 50°C for 5 h, then cooled at 10°C and treated dropwise with a saturated NH_4Cl solution (20 ml). After stirring at room temperature for 2 h, the precipitate was filtered off to separate the product **5a** (3.10 g, 60% yield). HPLC, Rt = 7 min; mp: 198–200°C (1-pentanol); IR (KBr) 2950, 2620, 1655, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2) 4–4.2 (br, m, 2H, CH_2) 7–8 (m, 8H arom); MS *m/z* 268 (M^+, 1.9%), 250 (68%, $C_{16}H_{11}FN_2$), 239 (100%, $C_{15}H_{12}FN_2$). Anal $C_{16}H_{13}FN_2O$ (C, H, F, N); calc: C: 71.63, H: 4.88, F: 7.08, N: 10.44; found: C: 71.93, H: 4.75, F: 6.81, N: 10.38.

5-(3-Fluorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***5b***

Using the procedure described for **5a**, ethyl 3-fluoro benzoate from Lancaster (7.87 g, 47 mmol) was added to the dilithio derivative **2**. The reaction led to the derivative product **5b** (3.80 g, yield: 70%). HPLC, Rt = 8 min; mp: 200–202°C (1-pentanol); IR (KBr) 2950, 2620, 1655, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2) 4–4.2 (br m, 2H, CH_2) 7–8 (m, 8H arom); MS *m/z* 268 (M^+ 2.1%), 250 (100%, $C_{16}H_{11}FN_2$), 239 (58.9%, $C_{15}H_{12}FN_2$). 231 (1.9%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}FN_2O$ (C, H, F, N); calc: C: 71.63, H: 4.88, F: 7.08, N: 10.44; found: C: 71.73, H: 4.97, F: 6.72, N: 10.74.

5-(4-Chlorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***6a*** *or mazindol*

Using the procedure described for **5a**, methyl 4-chloro benzoate from Lancaster (7.98 g, 47 mmol) was added to the dilithio derivative **2**. The reaction led to the derivative product **6a** (3.30 g, yield: 60%). HPLC, Rt = 12 min; mp: 198–199°C (1-pentanol); IR (KBr) 2950, 2620, 1655, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2), 4–4.2 (br, m, 2H, CH_2), 7–8 (m, 8H arom); MS *m/z* 284 (M^+, 2.7%), 266 (62.7%, $C_{16}H_{11}ClN_2$), 255 (100%, $C_{15}H_{12}ClN_2$), 231 (11.7%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}ClN_2O$ (C, H, Cl, N); calc: C: 67.49, H: 4.60, Cl: 12.45, N: 9.84; found: C: 67.56, H: 4.68, Cl: 12.68, N: 9.93.

5-(3-Chlorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***6b***

Using the procedure described for **5a**, ethyl 3-chloro benzoate from Lancaster (8.65 g, 47 mmol) was added to the dilithio derivative **2**. The reaction led to the derivative product **6b** (2.40 g, yield: 40%). HPLC, Rt = 11 min; mp: 208–210°C (1-pentanol); IR (KBr) 2950, 2620, 1655, 1615, 1490, 1100, 10 65 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2), 4–4.2 (br, m, 2H, CH_2), 7–8 (m, 8H arom); MS *m/z* 284 (M^+, 0.7%), 266

(100%, $C_{16}H_{11}ClN_2$), 255 (25.6%, $C_{15}H_{12}ClN_2$), 231 (13.6%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}ClN_2O$ (C, H, Cl, N); calc: C: 67.49, H: 4.60, Cl: 12.45, N: 9.84; found: C: 67.35, H: 4.57, Cl: 12.33, N: 9.62.

5-(4-Bromophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***7a***

Using the procedure described for **5a**, ethyl 4-bromo benzoate from Lancaster (10.72 g, 47 mmol) was added to the dilithio derivative **2**. The reaction led to the derivative product **7a** (3.44 g, 50% yield). HPLC, Rt = 13 min; mp: 216–218°C (1-pentanol); IR (KBr) 2950, 2870, 2620, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2) 4–4.2 (br, m, 2H, CH_2), 7–8 (m, 8H, arom); MS *m/z* 330 (M^+, 1.8%, ^{81}Br), 328 (M^+, 1.4%, ^{79}Br), 312 (43.1%, $C_{16}H_{11}{}^{81}BrN_2$), 310 (43.2%, $C_{16}H_{11}{}^{79}BrN_2$), 301 (98.4%, $C_{15}H_{12}{}^{81}BrN_2$), 299 (100%, $C_{15}H_{12}{}^{79}BrN_2$), 231 (12.2%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}BrN_2O$ (C, H, Br, N); calc: C: 58.38, H: 3.98, Br: 24.27, N: 8.51; found: C: 58.52, H: 3.87, Br: 24.13, N: 8.47.

5-(3-Bromophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***7b***

Using the procedure described for **5a**, ethyl 3-bromo benzoate from Lancaster (10.72 g, 47 mmol) was added to the dilithio derivative **2**. The reaction led to the derivative product **7b** (4.00 g, yield: 60%). HPLC, Rt = 13 min; mp: 218–220°C (1-pentanol); IR (KBr) 2950, 2620, 1655, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2), 4–4.2 (br, m, 2H, CH_2), 7–8 (m, 8H, arom); MS *m/z* 330 (M^+, 0.4%, ^{81}Br), 328 (M^+, 0.5%, ^{79}Br), 312 (100%, $C_{16}H_{11}{}^{81}BrN_2$), 310 (99.3%, $C_{16}H_{11}{}^{79}BrN_2$), 301 (32.9%, $C_{15}H_{12}{}^{81}BrN_2$), 299 (33.7%; $C_{15}H_{12}{}^{79}BrN_2$), 231 (25%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}BrN_2O$ (C, H, Br, N); calc: C: 58.38, H: 3.98, Br: 24.27, N: 8.51; found: C: 57.07, H: 4.08, Br: 23.20, N: 8.09.

5-(4-Iodophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***8a***

Using the same procedure described for **5a**, ethyl 4-iodo-benzoate from Lancaster (12.92 g, 47 mmol) was added to the dilithio derivative **2**. The reaction afforded the product **8a** (4.63 g, yield: 60%). HPLC, Rt = 16 min; mp: 206–208°C (1-pentanol); IR (KBr) 2950, 2620, 1655, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2), 4–4.2 (br, m, 2H, CH_2), 7–8 (m, 8H, arom); MS *m/z* 376 (M^+, 0.5%), 358 (100%, $C_{16}H_{11}IN_2$), 347 (0.8%, $C_{15}H_{12}IN_2$), 231 (51.2%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}IN_2O$ (C, H, I, N); calc: C: 51.08, H: 3.48, I: 33.73, N: 7.45; found: C: 51.13, H: 3.77, I: 32.38, N: 7.81.

5-(3-Iodophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo[2,1-a]-isoindole ***8b***

By the same method used for preparing **5a**, ethyl 3-iodo-benzoate from Lancaster (12.92 g, 47 mmol) was added to the dilithio derivative **2**. The reaction gave the compound **8b** (3.79 g, yield: 50%). HPLC, Rt = 17 min; mp: 224–226°C (1-pentanol); IR (KBr) 2950, 2870, 2620, 1655, 1615, 1490, 1100, 1065 cm^{-1}; ^{1}H-NMR d 2.9–3.3 (br, m, 2H, CH_2), 4–4.2 (br, m, 2H, CH_2), 7–8 (m, 8H, arom); MS *m/z* 376 (M^+, 1.3%), 358 (100%, $C_{16}H_{11}IN_2$), 347 (72.9%, $C_{15}H_{12}IN_2$), 231 (49.1%, $C_{16}H_{11}N_2$). Anal $C_{16}H_{13}IN_2O$ (C, H, I, N); calc: C: 51.08, H: 3.48, I: 33.73, N: 7.45; found: C: 51.42, H: 3.59, I: 33.24, N: 7.10.

In vitro binding experiments

In vitro binding experiments were performed according to Bonnet *et al* [33] with minor modifications. Striatal membranes were prepared from the brains of Wistar rats. Tissues were homogenized in 10 vol 0.32 M sucrose using an Ultraturrax (Ultra-Turrax T 25). After 1000 *g* centrifugation for 10 min at 2°C, the supernatant was collected and the pellet homogenized and centrifuged as above. Supernatants were pooled and centrifuged at 17 500 *g* for 30 min at 2°C. Pellets were homogenized, suspended in 20 vol of a pH 7.5 bicarbonate buffer and centrifuged at 50 000 *g* for 10 min at 2°C. Final pellets were suspended in a small volume of buffer and protein concentration was determined according to Bradford [34] using bovine serum albumin as the standard. Membranes were incubated in the presence of 0.01% bovine serum albumin. Binding assays were run in duplicate in silicone-coated test tubes, in a final volume of 4 ml containing 2.4 ml incubation buffer, 0.4 ml [^{3}H]-GBR 12935 (NEN, specific activity 2TBq/mmol), 0.2 ml 0.5% ascorbate or competitors and 1 ml membrane suspension containing 0.1 mg protein. For saturation assays [^{3}H]-GBR 12935 was used at a concentration of 0.1 to 10 nM, and for competition assays [^{3}H]-GBR 12935 was used at a concentration of 1 nM. Samples were incubated at 25°C for 45 min and filtered under reduced pressure on glass fiber filters GF/B (Whatman). Filters were washed 3 times with 4 ml ice-cold buffer; radioactivity remaining on them was measured after addition of a scintillator (Optiphase Highsafe II, LKB) using a beta counter (LKB Rack Beta 1215). The specific binding was calculated by subtracting the nonspecific binding defined in the presence of 10^{-5} M nomifensine (RBI Bioblock) from the total binding.

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