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Synthesis and Preliminary Biological Characterization of a New Potential ^{125}I-Radioligand for Dopamine and Serotonin Receptors

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Abstract—The synthesis and a preliminary biological characterization of a new class of *N*-benzyl-aminoalcohols which have serotonin (5-HT_2) and dopamine (D_2) receptor affinity is described. In vitro competition binding studies were conducted with the new molecules and ^{3}H-spiperone on crude membrane preparation from rat striatum and frontal cortex. One of these compounds, 3-benzylamino-1-(4-fluoro-2-iodophenyl)-propan-1-ol (**6f**), whose IC_{50} values are in the micromolar range for both the D_2 and 5-HT_2 receptors, was prepared in iodine-125 labelled form (**6i**) by nucleophilic substitution of the bromine atom of 3-benzylamino-1-(2-bromo-4-fluorophenyl)-propan-1-ol (**6d**). In the in vivo studies, conducted on rats, the radiolabelled molecule **6i** shows a good capacity to cross the blood–brain barrier (BBB) with a mean value of first pass cerebral extraction (E) of ca. 50% when the regional cerebral blood flow, measured with microsphere technique, is in the experimental animal's physiologic range (0.8–1 mL/min/g). A preliminary in vitro autoradiographic distribution on coronal rat brain slices of the radioiodinated molecule showed that it was preferentially localized in the striatum and in the cerebral regions rich in dopamine- and serotonin receptors, even if a high non-specific binding was observed.

Introduction

The expanding sector of in vivo imaging devices,[1,2] with the efficient implementation of single photon and positron emission tomography (SPECT and PET respectively), stimulates radiopharmaceutical research to synthesize new radiolabelled molecules allowing the evaluation of brain functions. The use of radioligands to assess in vivo receptor concentrations has enabled researchers to investigate the extent to which specific neurotransmitter systems are involved in the pathogenesis and progression of mental illness and has provided the basis for the design of better therapies and for quantitative monitoring of therapeutic response.[3,4]

In this paper we report the synthesis of new molecules, *N*-benzyl-aminoalcohol derivatives, and their preliminary characterization as ligands for brain receptors by in vitro competition binding studies on crude rat brain membrane preparations. Moreover we have taken into account the possibility to introduce, by nucleophilic substitution, a radioisotopic atom in a functionally suitable position of a molecule of this class and to ensure the possibility for the analytical discrimination and separation between the radiolabelled molecule and the non-labelled precursor so as to obtain a radioligand with high specific activity. The radiolabelled molecule was then tested in in vivo experiments to value its ability to cross the blood–brain barrier in the experimental animals and in vitro autoradiographic assays to determine its cerebral distribution.

Results

The synthesis of a series of new secondary *N*-benzyl-β-aminoalcohol derivatives of general formula **6a**–**6h** is shown in Scheme 1. β-Chloroketones **1a**–**1e**, commercially available or prepared by Friedel–Craft acylation,[5,6] were transformed, by reduction with $NaBH_4$,

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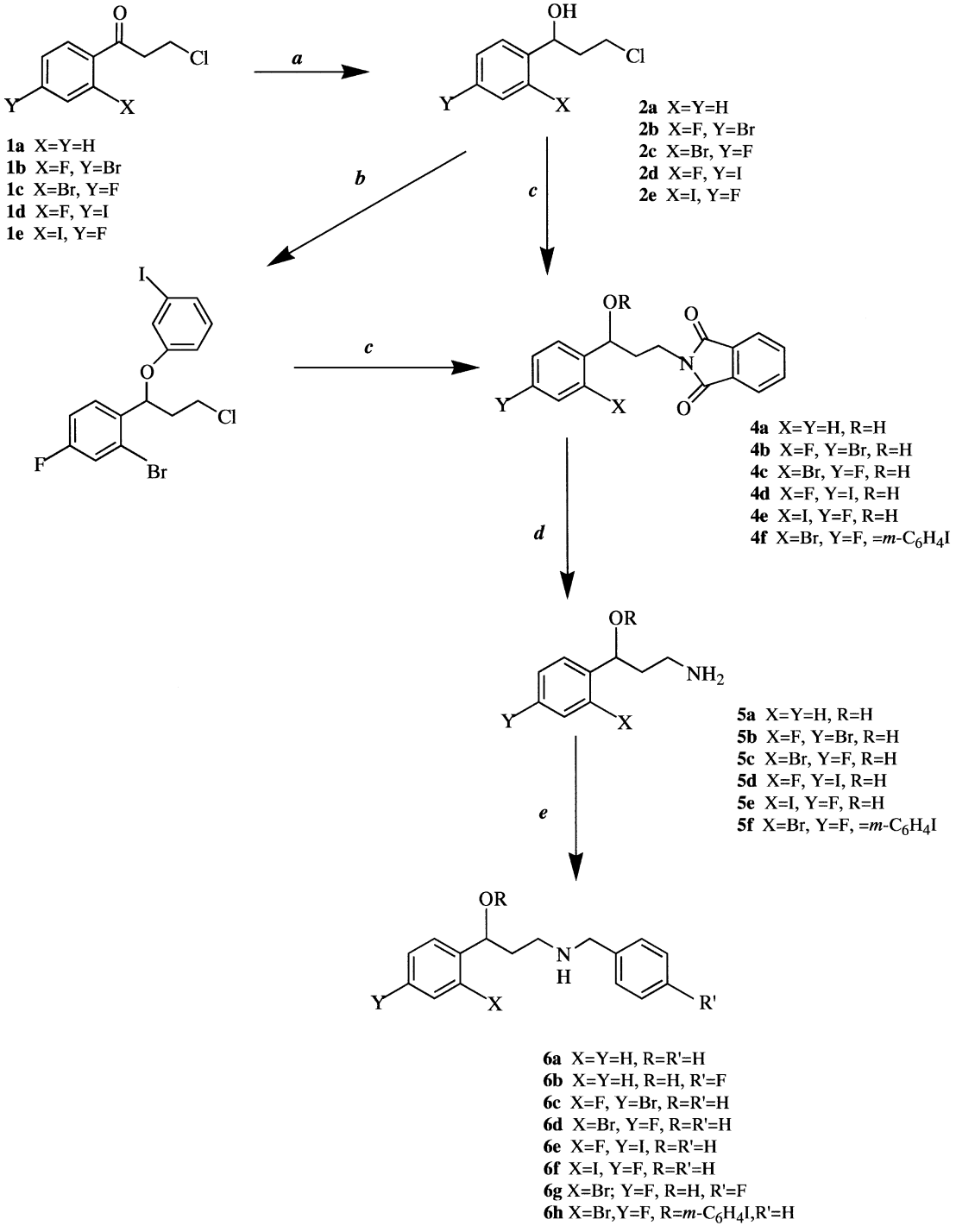
into the corresponding chloroalcohols (**2a**–**2e**) which were coupled with phthalimide in DMF at 120 °C for 8 h in the presence of KF[7,8] to give imides **4a**–**4e**. Imide **4f** was obtained from the product **3** prepared by the Mitsunobu reaction[9] from β-chloroalcohol **2c** with 3-iodophenol. Imide derivatives **4a**–**4f** were hydrolyzed to the corresponding amines (**5a**–**5f**) in high yield by hydrazine in MeOH at reflux in the presence of HCl.[6,10] These amines, sufficiently pure to be used without further purification, were transformed into the target compounds **6a**–**6h** by treatment with an equivalent of benzaldehyde or *p*-fluorobenzaldehyde in the presence of $NaBH_3CN$ at room temperature for 24 h.[11]

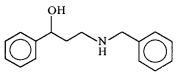
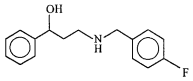
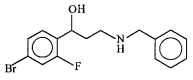
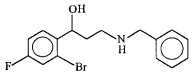
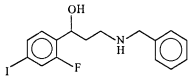
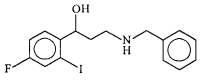
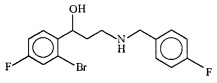
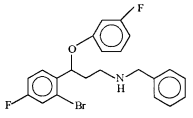
The capacity of the synthesized molecules to interact with the D_2- and 5-HT_2 receptors was evaluated using inhibition experiments of the specific ^{3}H-spiperone binding in homogenates of striate and frontal cortex rat brain tissue, respectively. All the substances were tested at a final concentration of 10 μM and the inhibition



Scheme 1. Preparation of aminoalcohols **6a**–**6h**: (a) $NaBH_4$ in CH_3OH at 0 °C; (b) 3-iodophenol, DEAD/PPh_3 in THF, 21 h at rt; (c) phthalimide and anhydrous KF in DMF at 120 °C for 8 h; (d) hydrazine in H_2O/CH_3OH/HCl, reflux, 3 h; (e) benzaldehyde or *p*-fluorobenzaldehyde, $NaBH_3CN$ in CH_3OH, 24 h at rt.

data are reported in Table 1. Molecule **6a**, which is the reference molecule for our study, shows poor affinity for the D_2 receptors and a very low affinity towards the 5-HT_2 receptors. Molecules **6c**–**6f**, containing the 3-aryl group substituted with two halogen atoms, which can be either fluorine and bromine, or, alternatively fluorine and iodine atoms, show an affinity increment especially for 5-HT_2 receptors and, when the substituent in position 2 of the benzene ring is an iodine atom as in **6f**, there is a relevant affinity increment also towards the D_2 receptors. The introduction of a fluorine atom on the *N*-benzyl ring, as in compounds **6b** and **6g**, causes a slight increase of the affinity for the D_2 receptor binding site with respect to the analogous molecules (**6a** and **6d**), thus reducing their selectivity towards the studied receptors. Compound **6h**, which has a iodophenoxy group, shows a slightly lower affinity with respect to the hydroxyl derivative analogue **6d**.

Table 1. Compounds **6a**–**6h** were tested at concentrations of 10 μM against ^{3}H-spiperone (2.5 nM). D_2 receptor assay was performed on rat striate tissue homogenates added with ketanserine (4×10^{-8} M fin), to block 5-HT_2 receptors, and with sulpiride (10^{-5} M fin), to determine the non-specific binding. 5-HT_2 receptor assay was performed on rat frontal cortex tissue homogenates added with sulpiride (10^{-6} M fin) to block the D_2 receptors, and with ketanserine (10^{-5} M), to determine the non-specific binding

Ligand		D_2	5-HT_2
	(**6a**)	6±5	24±8
	(**6b**)	11±1	29±3
	(**6c**)	26±9	60 ±7
	(**6d**)	19±5	48±7
	(**6e**)	18±2	48±10
	(**6f**)	51±14	50±10
	(**6g**)	39±12	46±5
	(**6h**)	14±4	42±6

The best ligand, amongst those containing an iodine atom, turned out to be the compound 3-benzylamino-1-(4-fluoro-2-iodophenyl)-propan-1-ol (**6f**) (Table 1), that in the in vitro assays displayed a dose-related inhibition of ^{3}H-spiperone specific binding with IC_{50} values in the micromolar range (about 20 μM in the 5-HT_2 assay, frontal cortex homogenates, and about 30 μM in the D_2 assay, striate homogenates).

To study the in vivo cerebral extraction of this new ligand, we prepared the compound **6f** in the ^{125}I-iodine labelled form [3-benzylamino-1-(4-fluoro-2-[^{125}I]-iodophenyl)-propan-1-ol (**6i**)], by Cu(I)-assisted nucleophilic substitution of the bromine atom with ^{125}I-iodine on 3-benzylamino-1-(2-bromo-4-fluorophenyl)-propan-1-ol **6d** (Scheme 2). This procedure allowed the separation of the radiolabelled ligand **6i** from the precursor **6d**. The exchange of bromine with the ^{125}I radioisotope was performed by heating compound **6d** at 100 °C for 90 min in an ethanol/water mixture containing Na^{125}I and catalyzed by an organo-copper (I) complex formed in situ.[12–14] The resulting mixture was separated from the unreacted ^{125}I-iodide by elution on an ion-exchange column and then, compound **6i** was separated by reversed-phase HPLC from the starting compound **6d**, obtaining the radiochemically and chemically pure compound **6i** (Fig. 1) (specific activity 2.2 Ci/μmol) which was used for in vivo rat brain uptake measurement.

The octanol/water partition coefficient experimentally determined according to Leo et al.[15] was shown to be

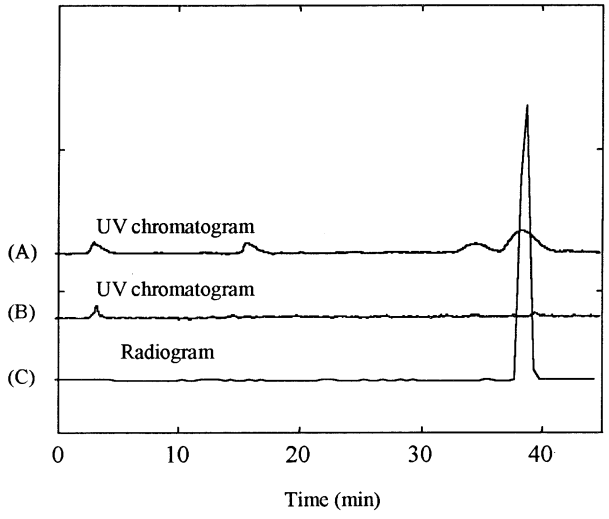
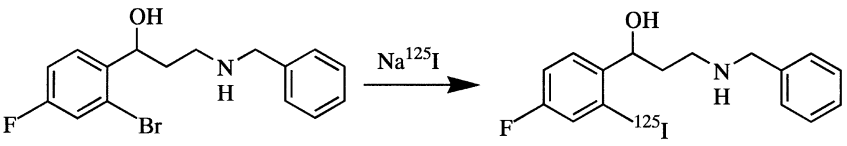


Figure 1. Radiogram and ultraviolet chromatograms to document the radiochemical and chemical purity of compound **6i**. (A): UV chromatogram of a mixture of **6f** and **6d**; (B) and (C): Radiogram and UV chromatogram of **6i** after HPLC purification.



Scheme 2. Preparation of aminoalcohol **6i**: Na^{125}I, Cu(I)-organo complexes in EtOH–H_2O, 100 °C, 90 min.

4.10 which is a value in the range useful for a good extraction in the brain.[16]

The cerebral extraction fraction (E), calculated as the ratio between the unidirectional influx constant K_i (mL/min/g) and the regional cerebral blood flow rCBF (mL/min/g), was 0.56 ± 0.14 (mean $\pm$ S.D.) (a range of K_i values between 0.6 and 2.1 mL/min/g, were obtained varying the rCBF values between 0.8 and 4.8 mL/min/g with hypercapnia). The experimental data of the molecule **6i** tested in different tissues were compared with those previously obtained[17] using three well-known receptor-binding tracers, IBZM,[18] Epidepride[19] and NCQ298,[20] by fitting each tracer data-set in a 4-parameter converging function (based on a saturable recruitment model of the cerebral capillaries[21]) (Fig. 2). The cerebral extraction fraction of **6i** is close to that of IBZM, the most diffusible molecule in the group. In vitro autoradiography performed on rat coronal brain slices showed accumulation of the radioligand in those regions of the rat brain known to be rich in dopamine- and serotonin receptors, thus confirming the affinity towards this type of receptors already observed in the in vitro binding experiments. However it is present a high non-specific binding, even after extensive washing of the slices, which does not allow to have a well definite auotradiographic image.

Discussion and Conclusions

We report the synthesis of the compounds **6a**–**6h**, which belong to a class of *N*-benzyl aminoalcohols; the molecules (**6c**, **6d**, **6g** and **6h**), containing a bromine atom on the benzene ring, can be substituted with a radioactive iodine atom obtaining pure labelled compounds.

In the preliminary in vitro characterization studies as brain receptor ligand, compound **6f**, tested in racemic form, showed a detectable affinity at micromolar level for both the 5-HT_2 and D_2 receptor populations. The corresponding ^{125}I-iodine labelled compound **6i**, prepared radiochemically and chemically pure, showed a high first-pass cerebral extraction (E) value, according to its octanol/water partition coefficient value of 4.10. Indeed the molecule easily reaches the cerebral tissue crossing the BBB after its iv administration. The ability to cross the BBB is of fundamental importance for a cerebral tracer, but the useful range of this transfer is defined also by the other kinetic and pharmacological characteristics of the tracer. Indeed, if a molecule with a very high affinity toward a given cerebral binding site has also a very high capacity to cross the BBB, then the tracer uptake will reflect more cerebral blood flow dependent delivery than the density of its cerebral binding sites.[22] On the other hand, also a molecule with high affinity and low cerebral extraction may have kinetics which are too slow to reach a sufficient concentration in the brain to be actually used in an emission tomographic study. As a matter of fact, the extraction values shown by **6i** (mean about 50%) are in a range similar to that of IBZM, a benzamide derivative clinically used for imaging the D_2 dopamine receptors (Fig. 2).

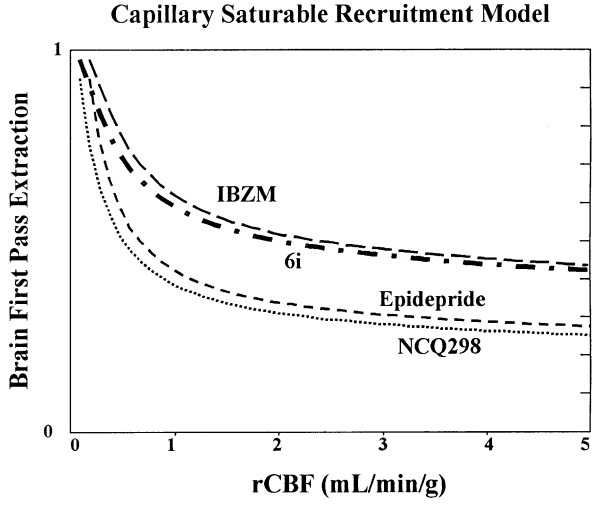


Figure 2. First pass cerebral extraction (experimental data fitting based on the capillaries recruitment model) of compound **6i**, IBZM, Epidepride and NCQ298.

A preliminary in vitro autoradiographic study on rat cerebral coronal section revealed an accumulation of the radioligand **6i** in those cerebral regions rich in dopamine and serotonin receptors (about 15% of activity was displaced from the specific binding sites by adding (+)-butaclamol and ketanserine); however there was also a very high fraction of radioactivity not specifically bound. This non-specific binding was higher than that obtained in the rat brain homogenate assays. We hypothesize that this fact could be attributed to a binding of the radioligand **6i** with tissue components that are discarded during the homogenate preparation procedure. Indeed the high lipophilicity of the molecule, which allows a better penetration through the BBB, also could increase the non-specific binding interaction with tissue components.[23]

The main characteristic usually required for an in vivo receptor-binding tracer is to have a high specificity and a high affinity for the receptor population type under study, but that is not the case of **6i**. Nevertheless it is worthwhile to mention that the increasing sensitivity of PET and SPECT instrumentation is encouraging researchers to study not only the receptor population concentrations and their state of affinity, but also the different receptor populations interaction, and, actually, also the influence of the endogenous ligand; such studies, allowing the elucidation of the action mechanism of drugs which are active on different type of receptors,[24–27] or the monitoring of pharmacological therapies, can motivate tracers with multiple specificity or moderate affinity, respectively.

For this reason a ligand having moderate affinity and specificity as those showed by **6i,** could have a role in the in vivo studies where the presence of the endogenous transmitter and the complexity of the neuronal transmission should be addressed; however the very high

non-specific binding of the compound **6i** does not allow yet to propose its use as in vivo radiotracer.

In conclusion, although radiolabelled ligand **6i** doesn't possess all the desired characteristics for its use as in vivo cerebral receptor tracer, the chemical structure of the class to which it belongs together with the other compounds of this study, has several promising features, mainly due to the fact that it could be easily modified and labelled with radioisotopes. Future efforts will be devoted to extend this synthetic approach to new compounds of this class, in order to optimize their affinity and specificity and to reduce their non-specific binding, using appropriate structural changes.

Experimental

Chemistry

Melting points were determined with a Büchi 510 apparatus without corrections. Chromatographic separations were performed under pressure on silica gel using flash-column techniques; R_f values refer to TLC carried out on 25 mm silica gel plates (Merck F254), with the same eluent indicated for column flash chromatography.

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 200 and 50.33 MHz, respectively, unless otherwise stated, on a Varian Gemini instrument. EI mass spectra were carried out at 70 eV ionizing voltage with a Carlo Erba QMD 1000 spectrometer. IR spectrum was recorded with a Perkin–Elmer 881 spectrophotometer. HPLC analyses were performed on a Shimadzu instrument, model SCL-6B, equipped with a Waters Spherisorb S5C8 column (4.6×250 mm) and with a UV detector, model UVICORD VW 2251-Pharmacia, operating at 254 nm and radioactivity detector Gaby-Raytest. Analytical data is reported as retention time (t_R) in minutes. Dowex 1X8–200 ion-exchange resin (Sigma–Aldrich), previously activated, was used to eliminate the inorganic salts and the unreacted $Na^{125}I$.

THF was distilled from Na/benzophenone. DMF was distilled under a nitrogen atmosphere (bp. 102 °C, 100 mmHg).

3-Chloropropiophenone (**1a**) was an Aldrich product.

$Na^{125}I$ in 0.2N NaOH solution was purchased from Nycomed Amersham Sorin. (10 μL, 3.7–18.5 GBq (100–500 mCi)/mL, ^{125}I 97%, ^{126}I < 3%, radiochemical purity > 99%, carrier free).

1-(4-Bromo-2-fluorophenyl)-3-chloro-propan-1-one (1b) and 1-(2-Bromo-4-fluorophenyl)-3-chloropropan-1-one (1c). Anhydrous $AlCl_3$ (21.0 g, 157.5 mmol) was added to a solution of 1-bromo-3-fluorobenzene (15.67 g, 89.5 mmol) and 3-chloropropionyl chloride (15.96 g, 126 mmol) in CS_2 (40 mL). The mixture was refluxed for 8 h, stirred at room temperature overnight and then added to ice (100 g) and adjusted to pH 2 with HCl. The resulting solution was extracted with Et_2O; the organic phase was washed with aqueous 5% NaOH and brine, dried over Na_2SO_4. Removal of the solvent gave a 2:3 mixture of **1b** and **1c** as a light yellow oil (21.14 g). Purification by flash chromatography (petroleum ether–EtOAc, 20:1) afforded **1b** (7.48 g, 31%, R_f 0.34) and **1c** (9.98 g, 42%, R_f 0.48). **1b**: GC–MS m/z 229 $(M–Cl)^+$; ^{1}H NMR: δ 7.80 (t, J = 7.3 Hz, 1H), 7.30–7.41 (m, 2H), 3.87 (t, J = 6.6 Hz, 2H), 3.40 (td, J = 6.6, 3.3 Hz, 2H); ^{13}C NMR: δ 193.7 (s, J_F = 3.7 Hz), 161.7 (s, J_F = 259.1 Hz), 131.8 (d, J_F = 3.7 Hz), 128.8 (s, J_F = 10.0 Hz), 128.3 (d, J_F = 3.7 Hz), 123.8 (s, J_F = 12.8 Hz), 120.4 (d, J_F = 27.5 Hz), 46.0 (t, J_F = 8.2 Hz), 38.1 (t, J_F = 2.8 Hz). **1c**: GC–MS m/z 229 $(M–Cl)^+$; ^{1}H NMR: δ 7.47 (dd, J = 8.4, 5.9 Hz, 1H), 7.30 (dd, J = 8.1, 2.5 Hz, 1H), 7.05 (td, J = 8.1, 2.2 Hz, 1H), 3.84 (t, J = 6.4 Hz, 2H), 3.38 (t, J = 6.4 Hz, 2H); ^{13}C NMR: δ 200.7 (s), 163.5 (s, J_F = 256.4 Hz), 130.9 (d, J_F = 9.1 Hz), 128.3 (s), 121.2 (d, J_F = 24.7 Hz), 120.5 (s, J_F = 18.3 Hz), 114.7 (d, J_F = 11.0 Hz), 44.9 (t), 38.5 (t).

3-Chloro-1-(2-fluoro-4-iodiophenyl)-propan-1-one (1d) and 3-Chloro-1-(4-fluoro-2-iodophenyl)-propan-1-one (1e). Prepared as reported for **1b** and **1c**. Starting from 1-fluoro-3-iodiobenzene (13.23 g, 59.6 mmol), a 2:3 mixture of **1d** and **1e** as a light yellow oil (18.0 g) was recovered. Purification by flash chromatography (petroleum ether–EtOAc, 20:1) afforded **1d** (4.63 g, 26%, R_f 0.33) and **1e** (7.8 g, 44%, R_f 0.51). **1d**: GC–MS m/z 314 (M^+, 30%) and 312 (M^+, 70%); ^{1}H NMR: δ 7.62–7.46 (m, 3H), 3.88 (t, J = 6.2 Hz, 2H), 3.42 (td, J = 6.6, 3.4 Hz, 2H); ^{13}C NMR: δ 194.2 (s, J_F = 0 Hz), 161.4 (s, J_F = 255.9 Hz), 135.4 (s), 134.5 (d, J_F = 3.0 Hz), 131.8 (d, J_F = 3.0 Hz), 124.4 (d, J_F = 26.2 Hz), 100.9 (s, J_F = 8.6 Hz), 46.3 (t, J_F = 8.2 Hz), 38.3 (t, J_F = 2.5 Hz). **1e**: GC–MS m/z 314 (M^+, 30%) and 312 (M^+, 70%); ^{1}H NMR: δ 7.68 (dd, J = 5.8, 2.6 Hz, 1H), 7.50 (dd, J = 5.8, 2.9 Hz, 1H), 7.11 (td, J = 8.0, 2.5 Hz, 1H), 3.88 (t, J = 6.4 Hz, 2H), 3.37 (t, J = 6.4 Hz, 2H); ^{13}C NMR: δ 200.4 (s), 164.4 (s, J_F = 257.8 Hz), 140.3 (s), 131.4 (d, J_F = 9.0 Hz), 129.7 (d, J_F = 23.7 Hz), 116.7 (d, J_F = 21.2 Hz), 93.0 (s, J_F = 8.0 Hz), 45.3 (t), 39.9 (t).

3-Chloro-1-phenyl-propan-1-ol (2a). $NaBH_4$ (2.00 g, 53.37 mmol) was slowly added to a stirred solution of 3-chloropropiophenone (**1a**) (5.0 g, 29.65 mmol) in MeOH (100 mL) cooled at 0 °C. The reaction mixture was left 10 min at room temperature and was then adjusted to pH 7 with a saturated solution of NH_4Cl. The organic phase was extracted with Et_2O, washed with brine and dried over Na_2SO_4. Pure **2a** (3.65 g, 72%) was obtained by flash chromatography (CH_2Cl_2–petroleum ether 2:1, R_f 0.30). GC–MS m/z 170 (M^+); ^{1}H NMR: δ 7.42–7.23 (m, 5H), 4.74 (d, J = 7.2 Hz, 1H), 3.87–3.65 (m, 2H), 2.31–2.17 (m, 2H); ^{13}C NMR: δ 148.5 (s), 130.6 (d), 128.3 (d), 126.1 (d), 56.4 (d), 41.7 (t), 39.4 (t).

1-(4-Bromo-2-fluorophenyl)-3-chloro-propan-1-ol (2b). Prepared as reported for **2a**. Starting from **1b** (1.54 g, 4.91 mmol), after purification by flash chromatography (CH_2Cl_2–petroleum ether 2:1, R_f 0.35), pure **2b** (0.69 g, 53%) was obtained. GC–MS m/z 268 (M^+, 30%) and 266 (M^+, 70%); ^{1}H NMR: δ 7.71 (dd, J = 8.8, 6.3 Hz, 1H), 7.35–7.30 (m, 1H), 7.22–7.17 (dd, 1 H), 5.19 (t, J = 6.4 Hz,

1H), 3.77–3.45 (m, 2H), 2.10–2.00 (m, 2H); ^{13}C NMR: δ 159.6 (s, J_F = 250.8 Hz), 130.1 (s, J_F = 13.6 Hz), 128.7 (d, J_F = 50.0 Hz), 128.0 (d, J_F = 3.5 Hz), 121.8 (s, J_F = 10.1 Hz), 119.4 (d, J_F = 5.2 Hz), 65.6 (d), 41.5 (t), 40.2 (t).

1-(2-Bromo-4-fluorophenyl)-3-chloro-propan-1-ol (2c). Prepared as reported for **2a**. Starting from **1c** (2.80 g, 10.56 mmol), after flash chromatography (CH_2Cl_2–petroleum ether 2:1, R_f 0.35), pure **2c** (1.8 g, 64%) was recovered. GC–MS *m*/*z* 268 (M^+, 30%) and 266 (M^+, 70%); ^{1}H NMR: δ 7.55 (dd, J = 8.8, 6.0 Hz, 1H), 7.30 (d, J = 2.5 Hz, 1H), 7.07 (td, J = 8.5, 2.6 Hz, 1H), 5.27 (d, J = 8.8 Hz, 1H), 3.82–3.69 (m, 2H), 2.15 (d, J = 3.7 Hz, 1H), 2.10–2.02 (m, 2H); ^{13}C NMR: δ 161.6 (s, J_F = 249.4 Hz), 138.8 (s), 128.3 (d, J_F = 11.0 Hz), 121.4 (s, J_F = 9.1 Hz), 119.8 (d, J_F = 24.6 Hz), 114.9 (d, J_F = 21.0 Hz), 69.5 (d), 41.5 (t), 38.8 (t).

3-Chloro-1-(2-fluoro-4-iodiophenyl)-propan-1-ol (2d). Prepared as reported for **2a**. Starting from **1d** (0.63 g, 2.02 mmol), purification by flash chromatography (CH_2Cl_2–petroleum ether 2:1, R_f 0.37) gave pure **2d** (0.44 g, 70%). GC–MS *m*/*z* 316 (M^+, 30%) and 314 (M^+, 70%); ^{1}H NMR δ 7.50 (dd, J = 8.0 Hz, 1H), 7.40 (d, J = 9.8 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1 H), 5.18 (t, J = 6.2 Hz, 1H), 3.81–3.55 (m, 2H), 2.24–2.20 (m, 2H).

3-Chloro-1-(4-fluoro-2-iodophenyl)-propan-1-ol (2e). Prepared as reported for **2a**. Starting from **1e** (4.05 g, 12.97 mmol), purification by flash chromatography (CH_2Cl_2–petroleum ether 2:1, R_f 0.33) gave pure **2e** (2.73 g, 67%). GC–MS *m*/*z* 316 (M^+, 30%) and 314 (M^+, 70%); ^{1}H NMR: δ 7.56–7.50 (m, 2H), 7.11 (td, J = 8.4, 2.6 Hz, 1H), 5.11 (dt, J = 9.5, 3.5 Hz, 1H), 3.89–3.66 (m, 2H), 2.10–2.00 (m, 2H); ^{13}C NMR: δ 161.7 (s, J_F = 251.8 Hz), 141.8 (s, J_F = 3.0 Hz), 128.0 (d, J_F = 8.0 Hz), 126.4 (d, J_F = 23.7 Hz), 116.0 (d, J_F = 20.6 Hz), 96.2 (s, J_F = 8.0 Hz), 74.2 (d), 41.7 (t), 40.3 (t).

2-Bromo-1-[3-chloro-1-(3-iodophenoxy)-propyl]-4-fluorobenzene (3). To a stirred solution of **2c** (1.8 g, 6.73 mmol), 3-iodophenol (1.6 g, 7.27 mmol) and triphenylphosphine (1.94 g, 7.4 mmol) in anhydrous THF (50 mL), at 0 °C and under nitrogen atmosphere, diethyl azodicarboxylate (DEAD) (1.4 g, 8.04 mmol) was added. The mixture was stirred at 0 °C for 1 h and for 24 h at room temperature; the solvent was distilled off under reduced pressure and the residue was washed with petroleum ether and then purified by flash chromatography (petroleum ether, R_f 0.30) to give pure compound **3** (1.4 g, 44%). GC–MS *m*/*z* 470 (M^+, 30%) and 468 (M^+, 70%); ^{1}H NMR: δ 7.41–7.30 (m, 2H), 7.22 (m, 2H), 7.00 (td, J = 8.1, 2.6 Hz, 1H), 6.90 (t, J = 7.7 Hz, 1H), 6.71–6.65 (m, 1H), 5.65 (t, J = 5.9 Hz, 1H), 3.87–3.65 (m, 2H), 2.31–2.17 (m, 2H); ^{13}C NMR: δ 161.9 (s, J_F = 250.0 Hz), 157.8 (s), 135.3 (s, J = 3.5 Hz), 130.9 (d), 130.6 (d), 128,5 (d, J_F = 8.2 Hz), 125.4 (d), 121.7 (s, J_F = 9.1 Hz), 120.2 (d, J_F = 24.6 Hz), 115.6 (d, J_F = 21.9 Hz), 114.4 (d), 94.4 (s), 75.0 (d), 40.8 (t), 39.6 (t).

2-[3-Hydroxy-3-phenyl-propyl]-isoindole-1,3-dione- (4a). Phthalimide (4.31 g, 29.3 mmol) and anhydrous KF (10.7 g, 73.2 mmol) were added, under nitrogen atmosphere, to a solution of **2a** (2.5 g, 14.64 mmol) in anhydrous DMF (120 mL). The reaction mixture was heated at 120 °C for 6 h and stirred overnight at 25 °C, then 240 mL of H_2O was added and the resulting solution extracted with Et_2O. The organic phase was washed with H_2O, with aqueous 5% NaOH solution and brine and dried over Na_2SO_4. After evaporation of the solvent, the crude product was first washed with Et_2O and then crystallized from acetone recovering pure compound **4a** (2.1 g, 54%). GC–MS *m*/*z* 281 (M^+); ^{1}H NMR: δ 7.80 (m, 4H), 7.45–7.15 (m, 5H), 4.85 (d, J = 8.2 Hz, 1H), 3.98–3.71 (m, 2H), 2.18–1.98 (m, 2H); ^{13}C NMR: δ 166.3 (s), 156.1 (s), 148.9 (s), 132.6 (d), 129.5 (d), 127.1 (d), 59.8 (d), 34.5 (t), 32.5 (t).

2-[3-(4-Bromo-2-fluorophenyl)-3-hydroxypropyl]-isoindole-1,3-dione (4b). Prepared as reported for **4a**. Starting from **2b** (0.600 g, 2.24 mmol), after crystallization from acetone pure **4b** (0.246 g, 29%) was collected. GC–MS *m*/*z* 378 (M^+); ^{1}H NMR: δ 7.80 (m, 4H), 7.42 (t, J = 8.0 Hz, 1H), 7.29 (d, 1H), 7.15 (dd, J = 9.5, 1.8 Hz, 1H), 4.90 (d, J = 8.8 Hz, 1H), 3.96–3.69 (m, 2H), 2.12–1.94 (m, 2H); ^{13}C NMR: δ 168.9 (s), 159.1 (s, J_F = 248.5 Hz), 134.2 (d), 132.0 (s), 130.0 (s, J_F = 13.7 Hz), 128.4 (d, J_F = 4.5 Hz), 127.7 (d, J_F = 2.7 Hz), 123.4 (d), 121.1 (s, J_F = 9.1 Hz), 118.8 (d, J_F = 24.6 Hz), 64.9 (d, J_F = 1.8 Hz), 36.5 (t), 34.6 (t).

2-[3-(2-Bromo-4-fluorophenyl)-3-hydroxypropyl]-isoindole-1,3-dione (4c). Prepared as reported for **4a**. Starting from **2c** (1.08 g, 4.03 mmol), after crystallization from acetone pure compound **4c** (0.71 g, 47%) was recovered. GC–MS *m*/*z* 378 (M^+); ^{1}H NMR: δ 7.81 (m, 4H), 7.59 (dd, J = 8.4, 6.2 Hz, 1H), 7.20 (dd, J = 8.4, 2.6 Hz, 1H), 7.05 (td, J = 8.4, 2.6 Hz, 1H), 4.94 (d, J = 11.4 Hz, 1H), 4.08–3.84 (m, 2H), 3.05 (d, J = 4.4 Hz, 1H), 2.23–2.08 (m, 1H), 1.89–1.72 (m, 1H); ^{13}C NMR: δ 169.1(s), 163.0 (s, J_F = 255.4 Hz), 137.8 (s), 134.4 (d), 132.2 (s), 128.4 (d, J_F = 8.6 Hz), 123.6 (d), 119.8 (d, J_F = 25.3 Hz), 115.1 (d, J_F = 21.2 Hz), 99.2 (s), 69.7 (d), 36.8 (t), 35.0 (t).

2-[3-(2-Fluoro-4-iodophenyl)-3-hydroxypropyl]-isoindole-1,3-dione (4d). Prepared as reported for **4a**. Starting from **2d** (0.59 g, 1.88 mmol), crystallization from acetone gave pure **4d** (0.40 g, 50%). GC–MS *m*/*z* 425 (M^+); ^{1}H NMR: δ 7.79 (m, 4H), 7.46 (dd, J = 8.5, 1.9 Hz, 1H), 7.34 (d, J = 1.8 Hz, 1H), 7.32 (d, J = 1.4 Hz, 1H), 4.90 (m, 1H), 3.95–3.88 (m, 2H), 3.17 (d, J = 4.7 Hz, 1H), 2.10–1.93 (m, 2H); ^{13}C NMR: δ 168.9 (s), 158.8 (s, J_F = 249.5 Hz), 134.2 (d), 133.6 (d, J_F = 2.7 Hz), 131.9 (s), 130.8 (s, J_F = 13.6 Hz), 128.7 (d, J_F = 4.5 Hz), 124.4 (d, J_F = 23.6 Hz), 123.4 (d), 91.8 (s, J_F = 8.5 Hz), 64.9 (d), 36.5 (t), 34.6 (t).

2-[3-(4-Fluoro-2-iodophenyl)-3-hydroxypropyl]-isoindole-1,3-dione (4e). Prepared as reported for **4a**. Starting from **2e** (0.77 g, 2.56 mmol), after crystallization from acetone pure **4e** (0.31 g, 28%) was collected. GC–MS *m*/*z* 425 (M^+); ^{1}H NMR: δ 7.79 (m, 4H), 7.55–7.41 (m, 2H), 7.07 (td, J = 8.4, 2.6 Hz, 1H), 4.74 (d, J = 8.4 Hz, 1H), 4.01–3.85 (m, 2H), 3.04 (d, J = 4.0 Hz, 1H), 2.15–2.03 (m, 1H), 1.79–1.67 (m, 1H); ^{13}C NMR: δ 169.0 (s), 161.2 (s, J_F = 249.4 Hz), 141.4 (d, J_F = 2.75 Hz), 134.2

(d), 132.0 (s), 127.5 (d, J_F=8.2 Hz), 125.8 (d, J_F=22.8 Hz), 123.4 (d), 115.7 (d, J_F=20.0 Hz), 96.0 (s, J_F=9.2 Hz), 73.7 (d), 36.8 (t), 34.7 (t).

2-[3-(2-Bromo-4-fluorophenyl)-3-(3-iodophenoxy)-propyl]-isoindole-1,3-dione (4f). Prepared as reported for **4a**. Starting from **3** (1.4 g, 2.98 mmol), after purification by flash chromatography (petroleum ether–ethyl acetate 10:1, 0.1% NEt_3, R_f 0.50), pure compound **4f** (1.1 g, 63%) was obtained. GC–MS *m*/*z* 580 (M^+); ^{1}H NMR: δ 7.77 (m, 4H), 7.38–7.30 (m, 2H), 7.20–7.17 (m, 1H), 7.00 (s, 1H), 6.96–6.91 (m, 1H), 6.84 (t, J=8.1 Hz, 1H), 6.57–6.53 (m, 1H), 5.49 (dd, J=8.8, 3.0 Hz, 1H), 4.06–3.85 (m, 2H), 2.29–2.12 (m, 2H).

3-Amino-1-phenyl-propan-1-ol (5a). A suspension of **4a** (1.3 g, 4.87 mmol) and hydrazine (0.47 mL, 0.49 g, 9.74 mmol) in MeOH (30 mL) was stirred at reflux for 3 h. After cooling at 0 °C, H_2O (30 mL) and HCl concd to adjust the pH to 2–3, was added. The resulting mixture was refluxed for 1 h, the white solid formed was filtered off and the pH of the solution was adjusted to 9 with aqueous 50% NaOH. The obtained solution was extracted with CH_2Cl_2 and dried over Na_2SO_4. Removal of the solvent gave **5a** (0.59 g, 80%) as an oil sufficiently pure to be used without further purification. GC–MS *m*/*z* 151 (M^+); ^{1}H NMR: δ 7.38–7.27 (m, 5H), 4.85–4.93 (m, 1H), 3.02 (m, 2H), 1.87–1.82 (m, 2H); ^{13}C NMR: δ 145.1 (s), 128.3 (d), 127.2 (d), 125.7 (d), 53.8 (d), 40.3 (t), 37.3 (t).

3-Amino-1-(4-bromo-2-fluorophenyl)-propan-1-ol (5b). Prepared as reported for **5a**. Starting from **4b** (0.195 g, 0.56 mmol), pure **5b** (0.10 g, 71%) was recovered. GC–MS *m*/*z* 249 (M^+); ^{1}H NMR: δ 7.48 (t, J=8.0 Hz, 1H), 7.27 (d, J=10.0 Hz, 1H), 7.15 (d, J=10.0 Hz, 1H), 5.20 (d, J=8.0 Hz, 1H), 3.08 (m, 2H), 1.93 (m, 1H), 1.65 (m, 1H).

3-Amino-1-(2-bromo-4-fluorophenyl)-propan-1-ol (5c). Prepared as reported for **5a**. Starting from **4c** (0.61 g, 1.61 mmol), pure **5c** as oil (0.35 g, 87%) was obtained. GC–MS *m*/*z* 249 (M^+); ^{1}H NMR: δ 7.64 (dd, J=8.8, 6.6 Hz, 1H), 7.20 (d, J=2.2 Hz, 1H), 7.03 (td, J=8.4, 2.5 Hz, 1H), 5.20 (dd, J=8.8, 2.2 Hz, 1H), 3.20–2.90 (m, 2H), 1.99–1.87 (m, 1H), 1.60–1.40 (m, 1H); ^{13}C NMR: δ 161.5 (s, J_F=247.0 Hz), 140.0 (s, J_F=3.0 Hz), 128.8 (d, J_F=8.0 Hz), 121.3 (s, J_F=9.5 Hz), 119.7 (d, J_F=24.5 Hz), 114.8 (d, J_F=20.6 Hz), 74.3 (d), 40.8 (t), 37.7 (t).

3-Amino-1-(2-fluoro-4-iodophenyl)-propan-1-ol (5d). Prepared as reported for **5a**. Starting from **4d** (0.31 g, 0.74 mmol), pure **5d** as oil (0.19 g, 86%) was recovered. GC–MS *m*/*z* 295 (M^+); ^{1}H NMR: δ 7.48 (dd, J=8.4 Hz, 1H), 7.35 (d, J=7.7 Hz, 1H), 7.33 (dd, J=9.5, 1.5 Hz, 1H), 5.20 (d, J=6.6 Hz, 1H), 3.21–3.04 (m, 2H), 2.03–1.81 (m, 1H), 1.70–1.61 (m, 1H); ^{13}C NMR: δ 159.2 (s, J_F=251.0 Hz), 133.6 (d, J_F=3.6 Hz), 132.5 (s, J_F=13.7 Hz), 129.3 (d, J_F=5.0 Hz), 124.4 (d, J_F=24.0 Hz), 91.3 (s, J_F=8.0 Hz), 69.8 (d), 40.8 (t), 37.9 (t).

3-Amino-1-(4-fluoro-2-iodophenyl)-propan-1-ol (5e). Prepared as reported for **5a**. Starting from **4e** (0.21 g, 0.47 mmol), pure **5e** as oil (0.132 g, 96%) was obtained. GC–MS *m*/*z* 295 (M^+); ^{1}H NMR: δ 7.56 (dd, J=9.5, 3.0 Hz, 1H), 7.48 (dd, J=8.0, 5.5 Hz, 1H), 7.10 (td, J=8.4, 2.2 Hz, 1H), 5.05 (dd, J=8.8, 2.2 Hz, 1H), 3.14 (t, J=4.4 Hz, 1H), 3.07–2.95 (td, J=9.9, 2.9 Hz, 1H), 1.98–1.87 (m, 1H), 1.60–1.46 (m, 1H); ^{13}C NMR: δ 161.3 (s, J_F=250.0 Hz), 142.7 (s), 128.3 (d, J_F=8.1 Hz), 126.0 (d, J_F=23.2 Hz), 115.6 (d, J_F=20.7 Hz), 95.9 (s, J_F=8.1 Hz), 78.8 (d), 40.9 (t), 38.0 (t).

3-(2-Bromo-4-fluorophenyl)-3-(3-iodophenoxy)-propylamine (5f). Prepared as reported for **5a** Starting from **4f** (0.90 g, 1.55 mmol), after purification by flash chromatography (CH_2Cl_2–CH_3OH 10:1, NEt_3 0.1%, R_f 0.20), **5f** as oil (0.345 g, 49%) was recovered. GC–MS *m*/*z* 451 (M^+); ^{1}H NMR: δ 7.41–7.28 (m, 2H), 7.24–7.20 (m, 2H), 6.99 (td, J=8.0, 2.2 Hz, 1H), 6.89 (t, J=8.5 Hz, 1H), 6.67 (m, 1H), 5.53 (t, J=6.0 Hz, 1H), 2.99–2.93 (m, 2H), 2.02–1.99 (m, 2H); ^{13}C NMR: δ 161.7 (s, J_F=250.9 Hz), 157.9 (s), 136.0 (s, J_F=2.8 Hz), 130.8 (d), 130.3 (d), 128.5 (d, J_F=8.2 Hz), 125.3 (d), 121.6 (s, J_F=10.1 Hz), 120.0 (d, J_F=27.7 Hz), 115.6 (d, J_F=21.1 Hz), 114.5 (d), 94.4 (s), 76.2 (d), 40.0 (t), 38.5 (t).

3-Benzylamino-1-phenyl-propan-1-ol (6a). $NaBH_3CN$ (0.067 g, 1.066 mmol), in small amounts, was added to a solution of **5a** (0.126 g, 0.836 mmol) and benzaldehyde (0.085 mL, 0.087 g, 0.835 mmol) in MeOH (10 mL) at 0 °C; the pH of the solution was adjusted to 6 by adding glacial acetic acid. After 24 h at 25 °C, H_2O (20 mL) was added and solid Na_2CO_3 until a basic pH was reached. After saturation with solid NaCl, the aqueous phase was extracted with CH_2Cl_2 and dried over Na_2SO_4. The crude product was purified by flash chromatography (CH_2Cl_2–CH_3OH 10:1, NEt_3 0.1%, R_f 0.35) thus giving pure **6a** (0.052 g, 26%). GC–MS *m*/*z* 241 (M^+); ^{1}H NMR: δ 7.33 (s, 10H), 4.95 (dd, J=8.0, 3.5 Hz, 1H), 3.82 (s, 2H), 2.99–2.89 (m, 2H), 1.91–1.81 (m, 2H), ^{13}C NMR: δ 144.9 (s), 138.9 (s), 128.6 (d), 128.4 (d), 128.3 (d), 127.4 (d), 127.0 (d), 125.6 (d), 75.6 (d), 53.7 (t), 47.7 (t), 37.1 (t); IR (cm^{-1}, $CDCl_3$): 3200 (br), 1610, 1512, 1238. Anal. calcd for: $C_{16}H_{19}NO$: C, 79.69; H, 7.94; N, 5.80. Found: C, 79.42; H, 7.80; N, 5.95.

3-(4-Fluorobenzylamino)-1-phenyl-propan-1-ol (6b). Prepared as reported for **6a**. Starting from **5a** (0.100 g, 0.661 mmol) and *p*-fluorobenzaldehyde (0.071 mL, 0.082 g, 0.661 mmol), after purification by flash chromatography (CH_2Cl_2–CH_3OH 10:1, NEt_3 0.1%, R_f 0.40), **6b** (0.078 g, 45%) was recovered. GC–MS *m*/*z* 259 (M^+); ^{1}H NMR: δ 7.33 (m, 7H), 7.02 (t, J=8.6 Hz, 2H), 4.93 (dd, J=7.7, 2.6 Hz, 1H), 3.78 (s, 2H), 2.95–2.90 (m, 2H), 1.90–1.83 (m, 1H), 1.76–1.68 (m, 1H); ^{13}C NMR: δ 162.3 (s, J_F=245.3 Hz), 144.7 (s), 133.9 (s), 130.2 (d, J_F=8.2 Hz), 128.3 (d), 127.1 (d), 126.6 (d), 115.5 (d, J_F=21.1 Hz), 75.1 (d), 52.8 (t), 47.3 (t), 36.9 (t); IR (cm^{-1}, $CDCl_3$): 3200 (br), 3088, 1602, 1504, 1233. Anal. calcd for: $C_{16}H_{18}NOF$: C, 74.16; H, 7.00; N, 5.40. Found: C, 74.42; H, 7.12; N, 5.52.

3-Benzylamino-1-(4-bromo-2-fluorophenyl)-propan-1-ol (6c). Prepared as reported for **6a**. Starting from **5b** (0.040 g, 0.16 mmol), purification by flash chromatography (CH_2Cl_2–CH_3OH 10:1, NEt_3 0.1%, R_f 0.40) gave pure

6c (0.045 g, 83%). GC–MS m/z 337 (M^+); ^{1}H NMR: δ 7.40–7.37 (m, 1H), 7.32 (m, 5H), 7.22 (m, 1H), 7.15 (dd, J=10.1, 1.5 Hz, 1H), 5.19 (dd, J=8.0, 2.5 Hz, 1H), 3.81 (s, 2H), 2.96–2.90 (m, 2H), 2.03–1.94 (m, 1H), 1.76–1.68 (m, 1H); ^{13}C NMR: δ 159.1 (d, J_F=250.0 Hz), 138.6 (s), 131.2 (s, J_F=12.8 Hz), 128.7 (d), 128.5 (d), 127.6 (d), 127.4 (d, J_F=3.7 Hz), 125.3 (s, J_F=25.6 Hz), 120.4 (d, J_F=9.1 Hz), 118.5 (d, J_F=24.7 Hz), 69.3 (d, J_F=1.8 Hz), 53.6 (t), 47.4 (t), 35.1 (t); IR (cm^{-1}, $CDCl_3$): 3180 (br), 3082, 1598, 1500, 1228. Anal. calcd for: $C_{16}H_{17}NOBrF$: C, 57.02; H, 7.94; N, 5.08. Found: C, 56.94; H, 7.86; N, 5.18.

3-Benzylamino-1-(2-bromo-4-fluorophenyl)-propan-1-ol (6d). Prepared as reported for **6a**. Starting from **5c** (0.245 g, 0.98 mmol), after purification by flash chromatography (CH_2Cl_2–CH_3OH 10:1, NEt_3 0.1%, R_f 0.38), **6d** (0.26 g, 79%) was recovered. GC–MS m/z 337 (M^+); ^{1}H NMR: δ 7.56 (dd, J=8.4, 6.2 Hz, 1H), 7.34 (m, 5H), 7.21 (dd, J=8.4, 2.6 Hz, 1H), 7.01 (td, J=8.4, 2.2 Hz, 1H), 5.19 (d, J=8.0 Hz, 1H), 3.83 (s, 2H), 2.98–2.92 (m, 2H), 2.07–1.98 (m, 1H), 1.68–1.54 (m, 1H); ^{13}C NMR: δ 160.9 (s, J_F=250.3 Hz), 139.6 (s, J_F=2.8 Hz), 139.1 (s), 128.8 (d), 128.7 (d), 128.5 (d, J_F=7.6 Hz), 127.8(d), 121.0 (d, J_F=7.8 Hz); 119.5 (d, J_F=22.3 Hz), 114.6 (s, J_F=21.0 Hz), 74.3 (d), 53.9 (t), 47.7 (t), 35.0 (t); IR (cm^{-1}, $CDCl_3$): 3090 (br), 2990, 1598. Anal. calcd for: $C_{16}H_{17}NOBrF$: C, 57.02; H, 7.94; N, 5.08. Found: C, 57.24; H, 7.98; N, 5.23.

3-Benzylamino-1-(2-fluoro-4-iodophenyl)-propan-1-ol (6e). Prepared as reported for **6a**. Starting from **5d** (0.110 g, 0.373 mmol), after chromatography (CH_2Cl_2–CH_3OH 10:1, CH_3OH–NEt_3 100:1, R_f 0.45), **6e** (0.108 g, 75%) was obtained. GC–MS m/z 385 (M^+); ^{1}H NMR: δ 7.51–7.44 (m, 2H), 7.34 (m, 5H), 7.03 (td, J=8.4, 2.6 Hz, 1H), 5.00 (dd, J=8.4, 2.3 Hz, 1H), 3.81 (s, 2H), 3.03–2.91 (m, 2H), 2.03–1.94 (m, 1H), 1.70–1.47 (m, 1H); ^{13}C NMR: δ 161.3 (s, J_F=250.3 Hz), 142.5 (s, J_F=3.0 Hz), 138.8 (s), 128.9 (d), 128.6 (d), 128.3 (d, J_F=7.5 Hz), 127.8 (d), 126.0 (d, J_F=23.7 Hz), 115.6 (d, J_F=20.6 Hz), 95.9 (s, J_F=8.0 Hz), 78.6 (d), 53.9 (t), 47.8 (t), 35.4 (t); IR (cm^{-1}, $CDCl_3$) 3240 (br), 3078, 1590, 1496, 1233. Anal. calcd for: $C_{16}H_{17}NOFI$: C, 49.91; H, 4.45; N, 3.64. Found: C, 50.24; H, 4.56; N, 3.77.

3-Benzylamino-1-(4-fluoro-2-iodophenyl)-propan-1-ol(6f). Prepared as reported for **6a**. Starting from **5e** (0.040 g, 0.133 mmol), purification by flash chromatography (CH_2Cl_2-CH_3OH 10:1, NEt_3 0.1%, R_f 0.50) gave pure **6f** (0.033 g, 64%). GC–MS m/z 385(M^+); ^{1}H NMR: δ 7.44 (d, J=8.4 Hz, 1H), 7.32 (m, 7H), 5.17 (d, J=5.6 Hz, 1H); 3.78 (s, 2H), 2.91 (m, 2H), 1.93 (m, 1H), 1.72–1.66 (m, 1H); ^{13}C NMR: δ 158.9 (s, J_F=250.9 Hz), 138.2 (s), 133.4(d), 129.5 (s), 129.0 (s), 128.7 (d), 128.5 (d, J_F=23.7 Hz), 127.6 (d), 124.4 (d), 123.9 (d), 69.3 (d), 53.5 (t), 47.3 (t), 34.9 (t); IR (cm^{-1}, $CDCl_3$): 3180 (br), 3076, 1590, 1510, 1240. Anal. calcd for: $C_{16}H_{17}NOFI$: C, 49.91; H, 4.45; N, 3.64. Found: C, 49.82; H, 4.32; N, 3.82.

1-(2-Bromo-4-fluorophenyl)-3-(4-fluorobenzylamino)-propan-1-ol (6g). Prepared as reported for **6a**. Starting from **5c** (0.120 g, 0.486 mmol) and *p*-fluorobenzaldehyde (0.052 mL, 0.060 g, 0.486 mmol), after purification by flash chromatography (CH_2Cl_2–CH_3OH 15:1, NEt_3 0.1%, R_f 0.40), **6g** (0.130 g, 76%) was recovered. GC–MS m/z 357 (M^+); ^{1}H NMR: δ 7.55 (m, 1H), 7.33–7.25 (m, 3H), 7.04 (t, J=8.6 Hz, 3H), 5.18 (t, J=8.4 Hz, 1H), 3.79 (s, 2H), 2.95 (m, 2H), 2.00 (m, 1H), 1.63 (m, 1H); ^{13}C NMR: δ 162.2 (s, J_F=245.0 Hz), 161.3 (s, J_F=249.0 Hz), 139.5 (s), 134.7 (s, J_F=2.8 Hz), 130.0 (d, J_F=7.4 Hz), 128.6 (d, J_F=8.3 Hz), 121.1 (s, J_F=9.1 Hz), 119.5 (d, J_F=24.87 Hz), 115.5 (d, J_F=21.1 Hz), 114.6 (d, J_F=21.1 Hz), 74.3 (d), 53.0 (t), 47.6 (t), 35.0 (t); IR (cm^{-1}, $CDCl_3$): 3200 (br), 3076, 1609, 1360, 1221. Anal. calcd for: $C_{16}H_{16}NOBrF2$: C, 54.08; H, 4.54; N, 3.94. Found: C, 53.94; H, 4.66; N, 3.87.

Benzyl-[3-(2-bromo-4-fluorophenyl)-3-(3-iodophenoxy)-propyl]-amine (6h). Prepared as reported for **6a**. Starting from **5f** (0.324, 0.72 mmol), purification by flash chromatography (CH_2Cl_2–CH_3OH 10:1, CH_3OH–NEt_3 100:1, R_f 0.32) gave pure **6h** (0.310 g, 79%). ^{1}H NMR: δ 7.38–7.24 (m, 8H), 7.20–7.16 (m, 2H), 7.02–6.94 (m, 1H), 6.92 (t, J=8.1 Hz, 1H), 5.54 (t, J=6.3 Hz, 1H), 3.80 (m, 2H), 2.87 (t, J=7.0 Hz, 2H), 2.06 (m, 2H); ^{13}C NMR: δ 161.7 (s, J_F=250.9 Hz), 158.1 (s), 140.1 (s), 136.3 (s, J_F=3.6 Hz), 130.8 (d), 130.3 (d), 128.7 (d, J_F=7.0 Hz), 128.4 (d), 128.2 (d), 127.0 (d), 125.4 (d), 121.6 (s, J_F=10.0 Hz), 120.0 (d, J_F=24.0 Hz), 115.5 (d, J_F=20.5 Hz), 114.5 (d), 94.4 (s), 76.6 (d), 53.8 (t), 45.4 (t), 37.2 (t); IR (cm^{-1}, $CDCl_3$): 3029, 2856, 1709, 1357. Anal. calcd for: $C_{22}H_{20}NOBrFI$: C, 49.03; H, 3.74; N, 2.60. Found: C, 49.24; H, 3.56; N, 2.77.

3-Benzylamino-1-(4-fluoro-2–^{125}iodophenyl)-propan-1-ol (^{125} I-6i). To a solution of **6d** (1.070 mg, 3.164 μmol) in EtOH (350 μL), under nitrogen atmosphere, 320 μL of a solution A were added. The solution A was prepared by adding into a vial, under a nitrogen atmosphere, 1.2 mg of copper (II) sulphate, 1.2 mg of tin (II) sulphate, 30 mg of gentisic acid, 42 mg of citric acid, 30 μL of glacial acetic acid and 2.7 mL of deionized water. After 10 min 10 μL of $Na^{125}I$ in 0.2 N NaOH (10 μL, 1 mCi) and 10 μL of deionized water were added. The pH was adjusted at 1–2 with glacial acetic acid and the reaction mixture heated at 100 °C for 90 min under a stream of N_2; the residue was cooled down and taken up in 500 μL of deionized water. The solution was eluted through a Dowex 1X8–200 ion-exchange column to eliminate the inorganic salts and the unreacted $Na^{125}I$.

HPLC analyses (eluting with a linear gradient of 25–90% acetonitrile in 0.1% aqueous TFA over 50 min at a flow rate of 1 mL/min) showed two peaks: the first having t_R 33 min (UV detector) as **6d** compound, the latter having t_R 40 min (radioactivity detector) as 3-Benzylamino-1-(4-fluoro-2–^{125}iodophenyl)-propan-1-ol. Fractions containing the chemically pure ^{125}**I-6i** were pooled together (130 μCi, yield 13%) and, after evaporation of the solvent, used for in vivo experiments.

Biological Studies

Animal care, surgical preparation and experimental procedures were carried out in compliance with the

Italian Law (D.L. 116/92) regulating detention and practice with experimental animals.

The experimental animals studies were all conducted on male Sprague-Dawlley rats (350–400 g b/w). To measure the cerebral extraction albumin microspheres, Sferotec-S (Sorin Biomedica, Italy), were used to prepare ^{99m}Tc labelled microspheres (MI). ^{131}I-Hippuran (Sorin Biomedica, Italy) specific activity = 4 MBq/mL was used to evaluate the tested molecule intravascular content. Briefly, the rats were rapidly tracheotomized under light ether anaesthesia and local anaesthesia (lidocaine 2%), paralyzed with tubocurarine (0.5 mg/Kg iv) and passively ventilated with a mixture of halothane and O_2 (3 mL halothane in 32 L of O_2) by a small-animal respirator (Basile, Milano Italy). Atropine (0.5 mg/Kg) was injected iv to minimize tracheobronchial secretion and to control arterial hypotension due to halotane. Body temperature was monitored by a rectal probe and maintained between 37–39 °C with a heating lamp. With the animal positioned under the guillotine, both femoral arteries were catheterized; one catheter was connected to a pression transducer for monitoring arterial pressure and heart rate, the other one to a peristaltic pump (Pharmacia) set to withdraw blood at a rate of 0.5 mL/min, and utilised like the 'reference organ'. Eparinization was performed with 100 I.U. iv of eparine, and the left ventricle was catheterised via the right common carotid artery with a catheter (0.96–0.58 mm external and inner diameter respectively) connected with a pression transducer to ascertain its positioning into the left heart ventricle. A range of rCBF values were obtained varying the ventilatory rate and the tidal volume (between 58–89 min^{-1} and 2.5–6 mL respectively).

For the in vitro binding studies [^{3}H]spiperone (103 Ci/mmol) was obtained from Amersham (UK) and used at 0.25 nM final concentration; (+)-butaclamol, ketanserine and sulpiride were purchased from Alexis Biochemical (Switzerland). For the membrane preparations and the reagents dilutions Tean buffer (Tris–HCl buffer 15 mM, NaEDTA 5mM, ascorbic acid 1.1 mM, nialamide 12.5 μM) was used. Tissue samples were measured in a 1282-Compugamma CS-Wallac.

For the in vitro autoradiography the rat brain slices were incubated in buffer Tris–HCl 50 mM pH 7.4 added of 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and molecule **6i** at the concentration of 2.25 nM final concentration. Adjacent sections were incubated in the same medium containing (+)-butaclamol and ketanserine (both at 1μM final concentration) additionally. Once dried, the slides were put in contact with ScopixRD (Agfa) films which was developed in a Curix 242S (Agfa-Geavert). Slice autoradiographic images were qualitatively analyzed using the scanner ArcusII-Agfa and an image analyzing system: ImageJ 1.18x (National Institutes of Health, USA).

In vitro binding assays

The new molecules were tested in vitro on rat brain membrane preparations in ^{3}H-spiperone specific binding competition experiments[28] following previously described procedures.[29,30] Briefly, the D_2 receptor assays were performed on rat striate tissue homogenates, to which were added ketanserine (4.10^{-8} M fin), to block 5-HT_2 receptors, and (+)-butaclamol (10^{-6} M fin) and /or sulpiride (10^{-5} M fin), to determine the non-specific binding. The 5-HT_2 receptor assays were performed on rat frontal cortex tissue homogenates, to which was added sulpiride (10^{-6} M fin) to block the D_2 receptors, and ketanserine (10^{-5} M fin), to determine the non-specific binding. In the 5-HT_2 assays the non-specific binding was determined also in presence of (+)-butaclamol (10^{-6} M fin) obtaining non-specific binding values similar to those obtained using ketanserine alone. After 60 min of incubation at 20–22 °C, 0.5 mL of each sample were vacuum-filtered through Whatman GF/B filters and washed with 2×5 mL of Tris–HCl buffer. The filters were dissolved in a scintillation cocktail and counted the day after. All the experiments were carried out in triplicate.

In vivo kinetic studies

Data from three male Sprague–Dawley rats (300–350 g) are reported.

The cerebral extraction of the molecule was obtained measuring, simultaneously, the regional cerebral blood flow, rCBF (mL/min/g), and the blood to brain unidirectional influx constant, K_i (mL/min/g). Indeed, the K_i of a freely diffusible blood-brain barrier molecule is related to the rCBF by the following: $K_i = rCBF \cdot E$, where E is the single pass cerebral extraction of the molecule.

The used method[17,31] calls for the injection of a mixture of tracers: ^{99m}Tc-labelled albumin microspheres (to measure rCBF), ^{131}I-Hippuran (to estimate the intravascular content of the tracer) and the ^{125}I-labelled test molecule, into the left heart ventricle.

Briefly, under strict control of physiological parameters, arterial blood was withdrawn at a rate of 0.5 mL/min using a peristaltic pump and, with the withdrawal pump on, the injection mixture was loaded into the cardiac catheter and rapidly flushed (<0.5 s) into the left ventricle with a bolus of saline. Six seconds following the injection, the rat was decapitated and the withdrawing pump simultaneously stopped by a switch triggered by the guillotine. The brain was rapidly removed and dissected on ice. Samples of cortex, cerebellum and striate (about 100 mg each) were weighed and solubilized (Protosol, Dupont, USA). Blood and tissue samples were counted in a γ-counter and the measured activity corrected for the spill-over.

In vitro autoradiography

In vitro autoradiography was performed on serial coronal rat brain section of 20 μm thickness prepared by criocutting as previously described.[32] Sections were thaw-mounted onto glass slides and incubated with a solution of **6i** (Specific Activity 2.2 Ci/μmol) dissolved

in ethyl alcohol and diluted in buffer Tris–HCl 50 mM pH 7.4 added of 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$. Adjacent sections were incubated in the same medium containing (+)-butaclamol and ketanserine (1 μM final concentration) additionally. After 1 h of incubation, at room temperature, slides were given 3 rinses of 20 s each in ice cold buffer followed by a short dip in distilled water to remove salts and finally dried in a stream of dry air. Ultimately the slides were put in contact with films which were developed at several times of exposure. Slice autoradiographic images were qualitatively analyzed using an image analyzing system.

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