Eur J Med Chem (1997) 32, 263–271

Sensitivity of the PCP receptor and the dopamine transporter to ligands bearing multiple asymmetric centres

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(Received 8 July 1996; accepted 23 September 1996)

Summary — Generation of one or two asymmetric carbons by means of a methyl substitution into cyclohexyl or piperidine moieties of 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) and 1-[1-(2-benzo[*b*]thiophenyl)cyclohexyl]piperidine (BTCP) structures has revealed improved affinity and/or selectivity for the PCP receptor and the dopamine (DA) transporter, respectively. Therefore, to get more information about the influence of chiral centres on affinity and selectivity, simultaneous methyl substitutions of cyclohexyl and piperidine moieties have been performed to generate three asymmetric carbons into the parent structures. Thus, *cis*-(Pip/Me)-1-[1-(2-thienyl)-2-methylcyclohexyl]-3-methylpiperidines and *cis*-(Pip/Me)-1-[1-(2-benzo[*b*]thiophenyl)-2-methylcyclohexyl]-3-methylpiperidines in homochiral forms have been prepared and their affinities for the PCP receptor ([^{3}H]TCP binding) and for the DA transporter ([^{3}H]BTCP binding) have been measured on rat brain and striatal membranes, respectively. None of the enantiomeric structures revealed affinities and/or selectivities in the same range as molecules bearing one or two asymmetric centres. In the TCP series the best compounds were in the same range as the parent compound. In the BTCP series, pure stereomers displayed lowered affinities and considerably reduced selectivities than the parent compound.

TCP/BTCP/PCP receptor / dopamine transporter / chirality / structure–activity relationships

Introduction

Derivatives of 1-(1-phenylcyclohexyl) piperidine (phencyclidine or PCP), a NMDA non-competitive antagonist, as well as other structures displaying the same property, have been designed for high affinity binding to the PCP receptor. Indeed, such compounds display a good potential to protect against neuronal injury: their binding to the PCP receptor in the NMDA gated channel blocks glutamate-induced excitotoxicity observed during various CNS pathologies like ischemia and brain or spinal cord trauma [1–6]. In a search for better affinity and/or selectivity, homochiral structures have been obtained and tested in vitro and in vivo in the PCP series [7, 8]. As anticipated, one enantiomer has revealed a better affinity than the other and a better affinity than the racemate. For our part, we have been mostly concerned with the 1-[1-(2-thienyl)-cyclohexyl]piperidine (TCP) and the 1-[1-(2-benzo[*b*]-thiophenyl)cyclohexyl]piperidine (BTCP) series bearing a 2-thienyl or a 2-benzo[*b*]thiophenyl heteroaromatic moiety, respectively, instead of a phenyl one (scheme 1). Indeed, these structural PCP-like molecules display interesting properties: TCP is a high affinity ligand of the PCP receptor and a good neuronal protector [6, 9, 10], BTCP is a potent inhibitor of dopamine (DA) reuptake and binds to the DA neuronal transporter [11–13]. By means of homochiral structures we have tried to evidence improved affinities and selectivities in both series. Thus, molecules bearing one asymmetric carbon atom in the piperidine ring demonstrated interesting properties: the homochiral TCP derivative (+)-*R*-GK230 (scheme 1) displayed a very high affinity (5.2 nM) and selectivity (> 900) for the PCP receptor. The homochiral BTCP derivative (–)-*S*-GK166, therefore of opposite configuration, displayed a very high affinity (3.5 nM) and selectivity (> 30 000) for the DA transporter [14]. Furthermore, homochiral molecules bearing two asymmetric carbon atoms in the cyclohexyl ring have also been obtained in the TCP series: (–)-1*S*,2*R*-GK11 (scheme 1) displayed a very high affinity (4.3 nM) and selectivity (> 900) for the PCP receptor and the highest efficacy for neuronal protection in comparison with (+)-1*R*,2*S*-GK11 [15].

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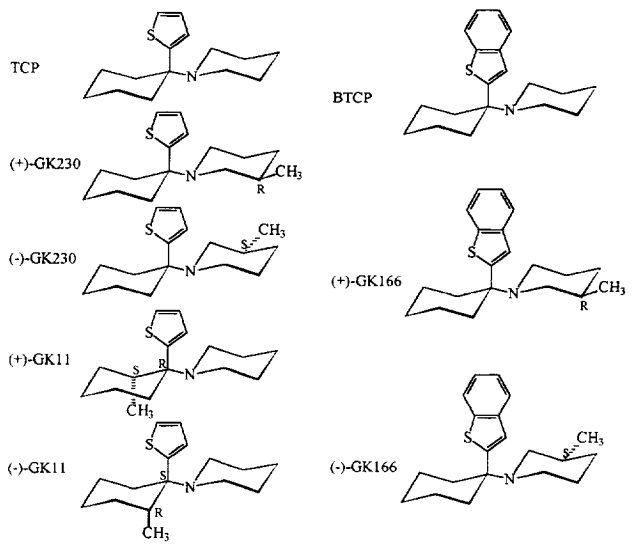


Scheme 1. Molecules bearing one or two asymmetric centres and their parent compounds.

It was thus interesting to consider the possible improvement in affinities and selectivities induced by homochiral structures bearing three asymmetric carbon atoms (two in the cyclohexyl and one in the piperidine moiety) in both TCP and BTCP series. The trans (pip/Me) GK11 diastereomer and related derivatives known for their low affinity and activity [8, 16, 17] will not be considered in the present work.

Chemistry

Synthesis

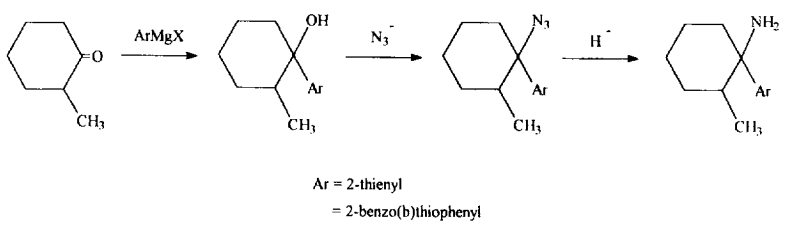
As stated above only compounds where the 2-methyl group has *cis* relationships to the piperidine ring have been considered. This reduces the number of isomers to four in each series. Three different strategies have been considered for their obtention.

First, obtention via the azide synthesis [7, 15, 18] (scheme 2) of the *cis* primary amines followed by a cyclization with racemic 2-methyl-1,5-dibromopentane to yield the full isomeric mixtures **1** *cis*-(Pip/Me)-1-[1-(*R,S*)-(2-thienyl)-2-(*R,S*)-methylcyclohexyl]-3-(*R,S*)-methylpiperidine and **6** *cis*-(Pip/Me)-1-[1-(*R,S*)-(2-benzo[*b*]thiophenyl)-2-(*R,S*)-methylcyclohexyl]-3-(*R,S*)-methylpiperidine each containing four homochiral molecules (**2** + **3** + **4** + **5** on one side, **7** + **8** + **9** + **10** on the other) (scheme 3). After a separation of the diastereomeric pairs (**2** + **4**, **3** + **5** and **7** + **9**, **8** + **10**) an optical resolution of racemic materials obtained would give pure enantiomers.

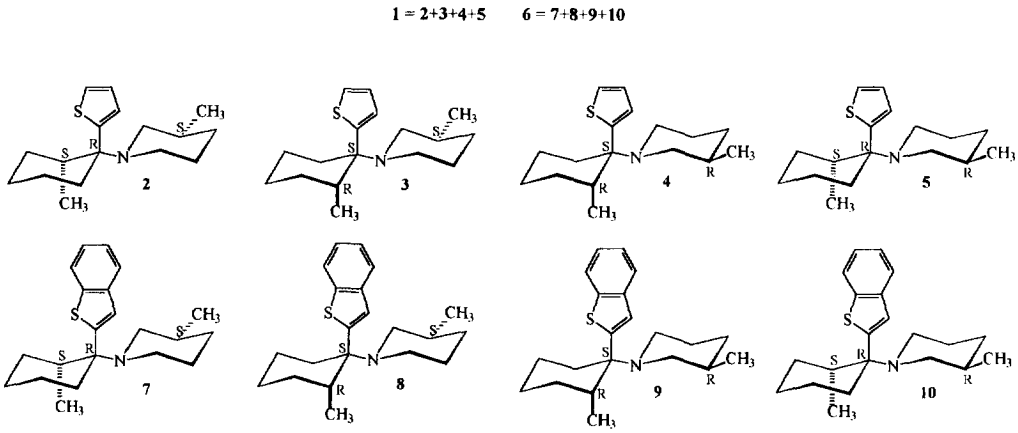
Second, obtention via scheme 2 of racemic *cis* primary amines followed by an optical resolution of these amines. Finally, a cyclization of an amine of known configuration with a molecule of 2-methyl-1,5-dibromopentane of known configuration would give homochiral molecules.

Third, preparation of racemic *cis* primary amines followed by a cyclization with a 2-methyl-1,5-dibromopentane of known configuration to yield stereomeric mixtures (**2** + **3**, **4** + **6** and **7** + **8**, **9** + **10**). Finally, a separation of diastereomers would give homochiral molecules.

Various attempts according to the first strategy were unable to yield quantitative separation of diastereomeric pairs like **2** + **4** and **3** + **5**. Similarly, we were unable to manage the second strategy: the optical resolution of racemic *cis* primary amines failed in both series. Finally, the last strategy (which is a combination of the two others) was successful. Thus, the racemic of *cis* primary amines was obtained from the *cis* + *trans* mixture resulting from scheme 2 with a low yield (30%). The Von Braun degradation of substituted piperidines is an interesting way to substitute 1,5-dibromopentanes [19]. Moreover, this degradation when applied to enantiomerically pure 3-methylpiperidines (obtained via a crystallization procedure with homochiral mandelic acids [20]) yields homochiral 2-methyl-1,5-dibromopentanes [21]. This procedure described in scheme 4 has been successfully used to prepare GK230 and GK166 enan-

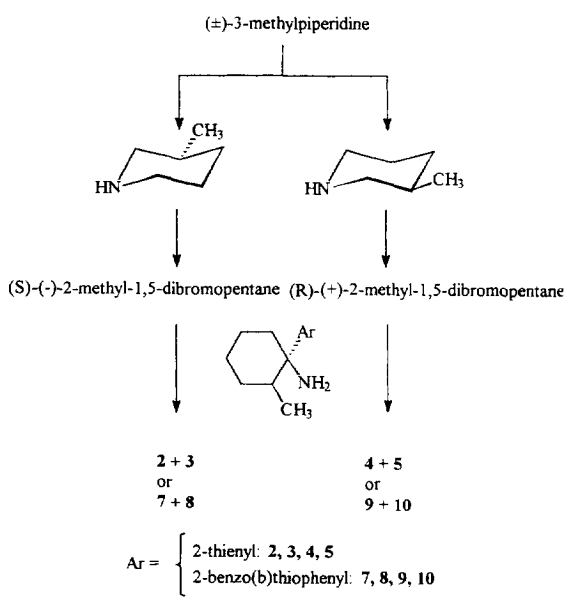


Scheme 2. Synthetic pathway for primary amines.



Scheme 3. Stereomers obtained in TCP and BTCP series.

tiomers (scheme 1) [14]. Therefore, after cyclization of each enantiomerically pure 2-methyl-1,5-dibromopentane with the racemic primary amines (scheme 4) in the TCP and BTCP series successively, we obtained four diastereomeric mixtures: **2 + 3**, **4 + 5**, **7 + 8**, **9 + 10**. Chromatographic techniques failed to resolve quantitatively these mixtures. Thus, we decided to resolve them by a crystallization procedure by means of homochiral di-*O,O'*-4-toluoyltartaric acids as previously performed for the resolution of racemic GK11



Scheme 4. Synthetic strategy to yield binary mixtures of stereomers.

[15]. After crystallization procedures, some of which were very laborious, the eight desired isomers were isolated. It should be pointed out that HPLC analysis on a chiral column could detect no enantiomeric material in **2–5**, indicating a high level of enantiomeric purity. This result is in line with the synthetic strategy used. We have been unable to achieve such analysis on a chiral column for BTCP-like compounds. Nevertheless, given the similar technique of obtention as for TCP derivatives, the enantiomeric purity should be similar. In contrast, with the exception of **4**, all compounds were more or less contaminated with the companion stereomer arising from the synthetic strategy adopted. We were unable to achieve a very high purification for any of the eight molecules. Thus, stereochemical purities assessed by means of GC/MS analysis were in the range 92–100% (table I).

Configurations

Configurations were easily deduced both from the known configuration of the 2-methyl-1,5-dibromopentanes used for 3-methylpiperidine ring formation (scheme 4) and the following ^{13}C-NMR considerations. Indeed, the two methyl groups are either located on the same side (*cis* relationships) or on the two different sides of the molecules (*trans* relationships). These spatial arrangements can be solved by ^{13}C-NMR spectroscopy.

From previous spectroscopic studies of GK11 [15] and its benzothiophenyl analogue [18] we know that an axial 2- (or 6-) methyl group on the cyclohexyl moiety induces via a δ effect a downfield shift at the C2' (or C6') carbon located on the same side of the molecule in the piperidine moiety (see carbon

Table I. Rotatory power ($CHCl_3$), purities and melting points of the enantiomeric structures.

Compounds	*2*	*3*	*4*	*5*	*7*	*8*	*9*	*10*
α_D^{20}[a]	−26.5°	−12.5°	+26.7°	+14.2°	−34.0°	−7.8°	+34.4°	+8.5°
Purity (%)[b]	96.6[c]	96[c]	> 99[c]	91.9[c]	92.9[c]	93.3[c]	93.3[c]	95.2[c]
Mp (HCl) (°C)	162–164	155–157	161–164	150–152	125–132	128–132	130–135	138–145

[a]Determined on the base form; [b]GC/MS determination on the base form; [c]remaining material is the companion diastereomer.

numbering on scheme in table II). Consequently, in cyclohexyl methyl-substituted structures C2' (or C6') appears 2.16 or 2.00 ppm further downfield than C6' (or C2') in the TCP and the BTCP series, respectively. In the piperidine ring, the 3'- (or 5'-) methyl group induces (by combination of intracyclic β and δ effects) downfield shifts of 6.12 and 6.20 ppm at the C2' (or C6') carbon with regard to the C6' (or C2') carbon in the TCP and the BTCP series, respectively. In *cis* compounds the two downfield shifts are additive yielding shift differences between C2' and C6' in the two series of 8.28 and 8.20 ppm, respectively. Conversely, in *trans* compounds the two downfield shifts are subtractive yielding absolute differences between C2' and C6' of 3.96 and 4.20 ppm in both series, respectively. Table I shows that experimental chemical shift differences nicely follow the anticipated values. Thus, from the absolute configuration of the methyl substitution on the piperidine ring and the spatial relationships between the two methyl substitutions absolute configurations at C1 and C2(6) are straightforward (scheme 3).

Results and discussion

Even if stereomeric purities were low in some cases, the affinities of the eight compounds obtained for the PCP receptor ([^{3}H]TCP) and for the dopamine transporter ([^{3}H]BTCP) were measured on rat brain and striatal membranes, respectively (tables III and IV).

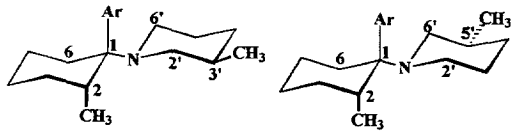
In the TCP series (table III) two stereomers, **2** and **5**, display high affinities for the PCP receptor, which are two times lower or in the same range, respectively, as TCP. Conversely, selectivities (*S*) are, respectively, three and two times better than for TCP. Nevertheless, affinities and selectivities remain lower than those measured for (+)-GK230 (IC_{50} = 5.2 nM, *S* = 923) and (−)-GK11(IC_{50} = 4.2 nM, *S* = 935) [14, 15] with one and two asymmetric centres, respectively. Enantiomeric pairs **2**-(−), **4**-(+) and 3-(−), **5**-(+) display variable affinity and selectivity differences. It appears that the PCP receptor discriminates very strongly enantiomers with *trans* relationships between methyl substitutions; **5**-(+) is 47 times as potent as **3**-(−). Conversely, the discrimination is lower for enantiomers having *cis* relationships; **2**-(−) is only twice as potent as **4**-(+). On the other hand, the DA transporter discriminates weakly the enantiomeric pairs with virtually no difference between structural *cis* and *trans* relationships. Surprisingly, the configurational combination of (+)-GK230 and (−)-GK11, the best ligands yet obtained in the TCP series [14, 15], yields **4**-(+) with three asymmetric centres but with a five times lower affinity and a three times lower selectivity compared to TCP.

In the BTCP series (table IV), two diastereomers **8**-(−) and **9**-(+) display only a two and three times affinity decrease, respectively, when compared to BTCP. Their selectivity is more than fivefold reduced when compared to the parent compound. Thus, none of these compounds possesses properties in the same range as the best BTCP-like enantiomer yet obtained: (−)-GK166 (IC_{50} = 3.5 nM, *S* = 38, 140) [14]. Enantiomeric pairs **7**-(−), **9**-(+) and **8**-(−), **10**-(+) display important affinity but low selectivity differences. The DA transporter apparently discriminates equally well enantiomers with *cis* or *trans* spatial relationships between methyl groups; **9**-(+) is eight times as potent as **7**-(−), and **8**-(−) is ten times as potent as **10**-(+).

Finally, a third asymmetric carbon atom appears to affect mostly the ability of both receptor proteins to discriminate TCP and BTCP series causing a selectivity drop.

The stereomeric contamination of the compounds may partly be the reason of such results in some cases. Indeed, **2**-(−) (21 nM) contaminated with **3**-(−) (610 nM) displays very likely underestimated affinity and selectivity (table III). It is unlikely, however, that a correction for purity, if it would have been possible, would give a high level of selectivity to the six remaining compounds given the low affinity differences between molecule and contaminant (**4**-(+), **5**-(+)) or the very low selectivity of both (tables III and IV).

Table II. ^{13}C-NMR spectra of homochiral hydrochlorides in $CDCl_3$ at 50.323 MHz.



Carbon	*2*	*3*	*4*	*5*	*7*	*8*	*9*	*10*
1	73.19	73.11	72.93	73.06	73.49	73.44	73.42	73.52
2	26.78	35.74	35.70	26.85	26.66	35.00	35.74	26.77
3	*22.81*	*31.27*	*31.11*	*22.78*	*22.56*	*30.43*	*30.38*	*22.93*
4	18.03	18.04	17.91	17.98	17.89	17.94	17.82	17.92
5	*30.47*	*22.84*	*22.67*	*31.19*	*30.45*	*22.93*	*22.43*	*30.44*
6	35.95	26.87	22.65	35.64	36.00	26.77	26.64	35.66
2'	46.38	48.37	54.51	52.50	47.04	48.83	54.91	53.12
3'	*22.40*	*21.98*	27.51	28.42	*22.90*	*22.08*	27.61	28.57
4'	*31.27*	*30.39*	*30.26*	*30.32*	*31.15*	*31.19*	*31.08*	*31.17*
5'	27.54	28.45	*22.30*	*21.94*	27.72	28.60	*22.81*	*22.05*
6'	54.76	52.56	46.21	48.40	54.98	52.99	46.80	48.84
2(6)-CH_3	16.12	16.12	15.90	16.02	16.06	16.12	16.00	16.06
3'(5')-CH_3	19.27	19.46	19.17	19.39	19.25	19.48	19.15	19.45
|C2'-C6'|	8.38	4.19	8.30	4.10	7.94	4.16	8.11	4.28
C_{Ar}	137.55–127.30	137.49–127.34	137.27–127.22	137.36–127.31	139.65–122.19	139.61–122.19	139.57–122.09	139.62–122.17

Chemical shifts (δ ppm) from TMS (italicized chemical shifts may be exchanged).

We know from previous work that one asymmetric or two asymmetric carbon atoms introduced in the parent TCP and BTCP structures improve only slightly the affinity (two times at mean) but considerably the selectivity (six to 50 times) [14, 15]. When introducing three asymmetric carbon atoms, affinities and selectivities are simultaneously lowered even when a priori favourable configurations are combined as in **4**-(+). Such an observation is particularly true for the BTCP derivatives displaying considerably lowered selectivities. Both binding sites remain able to discriminate stereomeric and/or enantiomeric structures. Thus, it can be hypothesized that simultaneous substitution with two methyl groups exceeds more or less the acceptable steric interactions inside both receptor proteins. This can result in a reflex distortion of proteins to accommodate ligands making active configurations unpredictable. If this was true, a possible consequence could be a loss in the heteroaromatic moiety discriminating ability resulting in a loss of selectivity. Indeed, it has been demonstrated that differential discrimination of aromatic moieties is the main factor governing selectivity for the PCP receptor and the DA transporter in the PCP, TCP and BTCP molecules [24]. Thus, when generating more than two chirality centres by means of methyl substitutions, the chirality recognition is not much affected whereas the heteroaromatic ring recognition, closely related to the selectivity, could be affected inside the ligand–protein complexes.

Table III. TCP derivatives: inhibition constants[a] (IC_{50}, nM ± SEM), Hill's number (n_H), and selectivity (S) of the binding of [^{3}H]TCP and [^{3}H]BTCP on rat brain and striatum, respectively.

Compounds	*[^{3}H]TCP*	n_H	*[^{3}H]BTCP*	n_H	S[b]
TCP	9.3	1.00	1330	0.82	143
(+)-GK230[c]	5.2	1.04	4800	1.04	923
2-(–)	21.0 ± 0.2	0.94	9700 ± 1050	1.02	462
3-(–)	610.7 ± 128.2	1.03	8800 ± 600	1.11	14
4-(+)	47.0 ± 4.8	1.04	1900 ± 636	1.05	40
5-(+)	13.4 ± 3.6	1.00	3800 ± 778	1.33	284

[a]Each value is the mean of three to four determinations performed in triplicate. [b]S = IC_{50} ([^{3}H]BTCP)/IC_{50} ([^{3}H]TCP). Since compounds were tested in similar conditions with radioligand concentrations lower than their respective K_d values, IC_{50}s are close to K_i values. Furthermore, because of close structural similarities, one can assume a competitive inhibition of both bindings like for TCP and BTCP [11]. Thus, S is assumed to reflect the in vitro selectivity. [c]From reference [14].

Table IV. BTCP derivatives: inhibition constants[a] (IC_{50}, nM ± SEM), Hill's number (n_H), and selectivity (S) of the binding of [^{3}H]TCP and [^{3}H]BTCP on rat brain and striatum, respectively.

Compounds	*[^{3}H]TCP*	n_H	*[^{3}H]BTCP*	n_H	S[b]
BTCP	6000	1.0	8.0	1.02	750
(–)-GK166[c]	133 500	0.92	3.5	0.82	38 140
7-(–)	15 700 ± 1000	1.06	170.4 ± 25	1.05	92
8-(–)	820 ± 80	1.10	16.7 ± 0.3	0.71	49
9-(+)	4000 ± 600	1.02	23.1 ± 1.2	0.81	173
10-(+)	13 800 ± 1000	0.93	171 ± 13	1.27	81

[a]Each value is the mean of three to four determinations performed in triplicate. [b]S = IC_{50} ([^{3}H]TCP)/IC_{50} ([^{3}H]BTCP). Since compounds were tested in similar conditions with radioligand concentrations lower than their respective K_d values, IC_{50}s are close to K_i values. Furthermore, because of close structural similarities, one can assume a competitive inhibition of both bindings like for TCP and BTCP [11]. Thus, S is assumed to reflect the in vitro selectivity. [c]From reference [14].

Experimental protocols

Generalities

Melting points were determined with a Büchi–Tottoli apparatus and are uncorrected. Elemental analysis was performed at the CNRS Microanalytical Section in Montpellier on the hydrochloride salts and were within ±0.4% of theoretical values. Optical rotations were obtained in $CHCl_3$ with a Perkin-Elmer 241 polarimeter in a 1 dm microcell at 20 °C. GC/MS analyses were performed on a Hewlett-Packard 5890 instrument equipped with a 9825B computer through a 25 m OV-1 capillary column. HPLC analysis were performed on a Shimadzu HPLC equipment (LC-1O AD pump, SPD-6A UV spectrophotometer), computer-controlled by the Class LC-10 program. Analyses were made on a Chiralcel-OD column (10 µm, 4.6 x 250 mm) (Daicel Chemical Industries). UV-detection was performed at 240 nm. Typical injection volumes were 5 µL of a 30 µM solution of base compound in heptane. ^{13}C-NMR spectra were obtained on a Brucker AC 200 spectrometer at

50.323 MHz in 5 mm sample tubes in the FT mode. For signal assignments, a spin-echo sequence (Jmod) was used. Chemical shifts are reported in (δ) ppm downfield from TMS. Some purifications were made on a preparative HPLC (Modulprep Jobin et Yvon) with a refractometric detection (Iota, Chromatofield). All compounds were isolated as their hydrosoluble hydrochloride salts used for the in vitro experiments; salts were precipitated by bubbling a dry stream of HCl in an etheral solution of bases. After filtration, the solids collected were dried in vacuo.

Binding assays

[^{3}H]TCP binding to the PCP receptor was performed as previously described [22]. Briefly, the rat brain (minus the cerebellum) was removed and homogenized with an Ultraturax (Ika Werke, maximum setting) in a 50 mM Tris/HCl, pH 7.7 buffer for 20 s at 4 °C. The homogenate was then centrifuged at 49 000 *g* for 20 min. The pellet was resuspended in the same buffer and the homogenization-centrifugation steps performed a second time. The final pellet was resuspended in 10 volumes of a 50 mM Tris/Hepes, pH 7.7 buffer and used without further purification.

The homogenate (0.5–0.8 mg protein/mL) was incubated with [^{3}H]TCP (1 nM) (Amersham, 48 Ci/mmol) in a 5 mM Tris/Hepes, pH 7.7 buffer in the absence (total binding) and in the presence of the competing drug for 30 min at 25 °C in a volume of 0.5 mL. The incubation was terminated by filtration over GF/B (Whatman) glass fiber filters presoaked in 0.05% polyethyleneimine (PEI, Aldrich) with an MR24 Brandel cell harvester. The filters were rinsed twice with 5 mL of a 50 mM NaCl, 10 mM Tris/HCl, pH 7.7 buffer and the radioactivity retained counted in 3.5 mL ACS (Amersham) with an Excel 1410 (LKB) liquid scintillation spectrophotometer. The non-specific binding was determined in parallel experiments in the presence of 100 µM unlabelled TCP.

[^{3}H]BTCP binding to the DA uptake complex was performed using the method described by Vignon et al [11]. Rat striata were dissected on ice and homogenized with an ultra turax in a 320 mM sucrose, 10 mM Tris/HCl pH 7.4 buffer and centrifuged at 1000 *g* for 10 min. The supernatant was then centrifuged at 49 000 *g* for 20 min. The resulting pellet (synaptosomal homogenate) was resuspended in the same buffer (1 mL per striatum). The homogenate (0.05–0.1 mg protein/mL) was incubated with [^{3}H]BTCP (0.2–0.5 nM) (CEA, Service des molécules marquées, 55 Ci/mmol) in the absence or the presence of the competing drug in a 50 mM Na_2HPO_4 pH 7.4 buffer in a volume of 2 mL for 90 min at 4 °C. The incubation was terminated in the same manner as for the [^{3}H]TCP with the exception that the filter was presoaked in 0.5% PEI. The non-specific binding was determined in the presence of 10 µM unlabelled BTCP. These conditions are those that allow the measurement of [^{3}H]BTCP binding to the dopamine transporter with a pharmacological specificity identical to that of [^{3}H]GBR 12935 [25].

Chemistry

Synthesis of (–)-(S)-2-methyl-1,5-dibromopentane and (+)-(R)-2-methyl-1,5-dibromopentane

The procedure was essentially the same as that described for the obtention of (–)-(*S*) and (+)-*R*-1-[1-(2-thienyl)cyclohexyl]-3-methylpiperidine (GK230) and (–)-(*S*) and (+)-*R*-1-[1-(2-benzo[*b*]thiophenyl)cyclohexyl]-3-methylpiperidine (GK166) [14] (scheme 1) and based on references [20, 21].

Preparation of racemic cis-1-(2-thienyl)-2-methylcyclohexylamine and cis-1-(2-benzo[b]thiophenyl)-2-methylcyclohexylamine

The pathway described in scheme 4 for obtention of primary amines has been described previously particularly in references [14, 18, 23]. We describe only the last step of the preparation and the separation of diastereomers.

cis-1-(2-Thienyl)-2-methylcyclohexylamine

Crude 1-azido-1-(2-thienyl)cyclohexane (*cis* + *trans* mixture) (10 g) was dissolved in isopropanol (100 mL) and heated to 60 °C for 30 min. Raney Ni was added in portions until the gas evolution stopped. A vigorous stirring at 60 °C was maintained for an additional period of 30 min. After cooling to room temperature, the mixture was filtered on celite, the precipitate rinsed with a solution of NH_4OH 5% (100 mL) and CH_2Cl_2. Solvents were evaporated under reduced pressure. The remaining aqueous solution was then extracted successively with ether and CH_2Cl_2. The combined organic layers were washed with water until neutrality, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield primary amines separated on a chromatography column (silica gel chromagel ACC 60–200 µ (SDS)). A mixture of petroleum ether/ether (95:5 v/v) eluted the pure oily *cis* amine (2.9 g) (GC/MS: 70–250 °C (10 °C/min), R_t = 13.68 min, *m/e* = 195.15).

cis-1-(2-Benzo[b]thiophenyl)-2-methylcyclohexylamine

Crude 1-azido-1-(2-benzo[*b*]thiophenyl)cyclohexane (*cis* + *trans* mixture) (10 g) treated as above yielded crude primary amines which were submitted to a preparative HPLC separation (silica gel Merck 15–40 µm). A mixture of dichloromethane/ethanol (99.3:0.7 v/v) eluted the pure *cis* amine (2 g) (GC/MS: 70–250 °C (10 °C/min), R_t = 20.11 min, *m/e* = 245.10).

cis-1-[1-(R,S)-(2-Thienyl)-2-(R,S)-methylcyclohexyl]-3-(R,S)-methylpiperidine 1

(±)-2-Methyl-1,5-dibromopentane (1.87 g, 7.68 mmol) was added to a suspension of *cis*-1-(2-thienyl)-2-methylcyclohexylamine (1 g, 5.12 mmol), K_2CO_3 (1.4 g, 10.2 mmol) and freshly distilled hexamethylphosphoramide (HMPA, Aldrich) (16 mL). The mixture was stirred for 72 h at 60 °C. (±)-2-Methyl-1,5-dibromopentane (0.6 g) and K_2CO_3 (0.35 g) were then added and stirred at 60 °C for 72 h to complete the cyclization. After cooling at room temperature the solution was poured onto water and yielded a crude oil after usual workup. Chromatography on a silica-gel column (Lobar) in petroleum ether/ethyl acetate (98:2, v/v) yielded pure oily **1** (0.34 g, 24%) (GC/MS: 70–250 °C (10 °C/min), R_t = 19.21, 19.35 min, *m/e* = 277.20; HPLC: heptane, 0.5 mL/min, λ = 240 nm, R_t = 502, 553, 584, 619 s).

Mixture of 1-[1-(R)-(2-thienyl)-2-(S)-methylcyclohexyl]-3-(S)-methylpiperidine 2 and 1-[1-(S)-(2-thienyl)-2-(R)-methylcyclohexyl]-3-(S)-methylpiperidine 3

(–)-(*S*)-2-Methyl-1,5-dibromopentane (6.2 g, 25.49 mmol), *cis*-1-(2-thienyl)-2-methylcyclohexylamine (3.31 g, 16.95 mmol), K_2CO_3 (5.38 g, 38.98 mmol) and HMPA (60 mL) were reacted as above. After stirring for 5 days at 60 °C and same treatment as above a crude oil was obtained. A preparative HPLC (silica gel Merck 15–40 µm) in petroleum ether/ether (99:1, v/v) yielded a pure oily mixture of **2** + **3** (1.33 g, 28.3%) ($[\alpha_D^{20}]_{base}$ = –24.19 ° (*c* 0.62, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 19.17, 19.30 min, *m/e* = 277.20; HPLC: heptane 0.5 mL/min, *t* = 30 °C, λ = 240 nm, R_t = 739, 870 s).

*Mixture of 1-[1-(S)-(2-thienyl)-2-(R)-methylcyclohexyl]-3-(R)-methylpiperidine **4** and 1-[1-(R)-(2-thienyl)-2-(S)-methylcyclohexyl]-3-(R)-methylpiperidine **5***

(+)-(*R*)-2-Methyl-1,5-dibromopentane (6.31 g, 25.88 mmol), *cis*-1-(2-thienyl)-2-methylcyclohexylamine (3.37 g, 17.25 mmol), K_2CO_3 (5.48 g, 39.68 mmol), and HMPA (60 mL) were reacted as above. After stirring for 5 days at 60 °C and same treatment as above a crude oil was otained. A preparative HPLC (silica gel Merck 15–40 μm) in petroleum ether/ether (99:1, v/v) yielded a pure oily mixture of **4** + **5** (1.27 g, 26.5%) ($[\alpha_D^{20}]_{base}$ = + 20.42° (*c* 0.705, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 19.230, 19.35 min, *m/e* = 277.20; HPLC: heptane 0.5 mL/min, *t* = 30 °C, λ = 240 nm; R_t = 774, 816 s).

*cis-1-[1-(R,S)-(2-Benzo[b]thiophenyl)-2-(R,S)-methylcyclohexyl]-3-(R,S)-methylpiperidine **6***

(±)-2-Methyl-1,5-dibromopentane (1.45 g, 5.93 mmol) was added to a suspension of *cis*-1-(2-benzo[*b*]thiophenyl)-2-methylcyclohexylamine (0.97 g, 3.95 mmol), K_2CO_3 (1.26 g, 9.09 mmol) and freshly distilled hexamethylphosphoramide (HMPA, Aldrich) (20 mL). The mixture was stirred for 7 days at 60 °C. After cooling to room temperature, the solution was poured onto water and gave a crude oil after the usual workup. Purification on a chromatography column (aluminium oxide 90, 2–3 Merck) in hexane/ether (98.5:1.5, v/v) yielded a pure mixture (0.3 g, 23%) (GC/MS: 90–250 °C (10 °C/min), R_t = 24.42, 24.62 min, *m/e* = 327.25).

*Mixture of 1-[1-(R)-(2-benzo[b]thiophenyl)-2-(S)-methylcyclohexyl]-3-(S)-methyl-piperidine **7** and 1-[1-(S)-(2-benzo[b]thiophenyl)-2-(R)-methylcyclohexyl]-3-(S)-methyl-piperidine **8***

The same protocol as for **2** and **3** was used with (–)-(*S*)-2-methyl-1,5-dibromopentane (1.56 g, 6.18 mmol), *cis*-1-(2-benzo-[*b*]thiophenyl)-2-methylcyclohexylamine (1.05 g, 4.28 mmol), K_2CO_3 (1.36 g, 9.84 mmol) and HMPA (20 mL). The mixture was stirred for 96 h at 60 °C. After the usual workup, the crude mixture was purified on a chromatography column (aluminium oxide 90, 2–3 Merck) in hexane/ether (98:2, v/v) and yielded a pure mixture of **7** + **8** (0.39 g, 27.8%) (GC/MS: 70–250 °C (10 °C/min), R_t = 25.48 and 25.64 min, *m/e* = 327.25).

*Mixture of 1-[1-(S)-(2-benzo[b]thiophenyl)-2-(R)-methylcyclohexyl]-3-(R)-methyl-piperidine **9** and 1-[1-(R)-(2-benzo[b]-thiophenyl)-2-(S)-methylcyclohexyl]-3-(R)-methyl-piperidine **10***

The same protocol as above was applied to (+)-(*R*)-2-methyl-1,5-dibromopentane (1.58 g, 6.78 mmol) and *cis*-1-(2-benzo[*b*]-thiophenyl)-2-methylcyclohexylamine (1.06 g, 4.32 mmol), K_2CO_3 (1.37 g, 9.35 mmol) and HMPA (20 mL). The mixture was stirred for 120 h at 60 °C. After usual workup, the crude mixture was purified on a chromatography column (aluminium oxide 90, 2–3 Merck) in hexane/ether (98.5:1.5, v/v) and yielded a pure mixture of **9** + **10** (0.31 g, 22%) (GC/MS: 70–250 °C (10 °C/min), R_t = 25.42, 25.63 min, *m/e* = 327.25).

Resolution of diastereomeric pairs

General protocol of one crystallization procedure cycle (the procedure is stopped when no significant improvement occurs): solubilization of bases in hot 2-propanol; addition of homochiral di-*O,O'*-4-toluoyltartaric acid dissolved in hot 2-propanol; stirring at room temperature, then at 7 °C for 16 h (or until crystallization); collection of the salt rinsed with 2-propanol or hexane; regeneration of the base form (treatment of the salt with Na_2CO_4 10%, extraction with Et_2O and $CHCl_3$, washing with H_2O until neutrality, drying over Na_2SO_4, filtration and evaporation under reduced pressure); and verification of the resolution progress by means of GC/MS analysis.

*Separation of diastereomers **2** and **3***

The above protocol was applied to a primary **2** + **3** mixture (1.16 mg) in 2-propanol (26 mL), 0.5 equiv (+)-di-*O,O'*-4-toluoyltartaric acid in 2-propanol (8.1 mL). Two cycles yielded **2** (70 mg) (**2/3** = 96.6:3.4). (Mp_{HCl} = 162–164 °C; $[\alpha_D^{20}]_{base}$ = –26.5 ° (*c* 1, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 18.39 min, *m/e* = 277.15).

The material recovered from above filtrates (680 mg) was treated with 0.5 equiv (–)-di-*O,O'*-4-toluoyltartaric acid. Two cycles yielded **3** (40 mg) (**3/2** = 96:4). (Mp_{HCl} = 155–157 °C; $[\alpha_D^{20}]_{base}$ = –12.5 ° (*c* 0.415, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 18.24 min, *m/e* = 277.15).

*Separation of diastereomers **4** and **5***

A **4** + **5** mixture (200 mg) in 2-propanol (4.5 mL) treated with 0.5 equiv (+)-di-*O,O'*-4-toluoyltartaric acid in 2-propanol (1.4 mL) yielded pure **4** (70 mg) (**4/5** > 99%). (Mp_{HCl} = 161–164 °C); $[\alpha_D^{20}]_{base}$ = + 26.67 ° (*c* 0.525, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 18.35 min, *m/e* = 277.15).

The material recovered from the above filtrate (100 mg) was treated with 0.5 equiv (–)-di-*O,O'*-4-toluoyltartaric acid. Two cycles yielded **5** (40 mg) (**5/4** = 91.9:8.1). (Mp_{HCl} = 150–152 °C; $[\alpha_D^{20}]_{base}$ = + 14.16° (*c* 0.445, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 18.33 min, *m/e* = 277.15).

*Separation of diastereomers **7** and **8***

The above protocol was applied to a primary **7** + **8** mixture (390 mg) in 2-propanol (7 mL) with 0.5 equiv (+)-di-*O,O'*-4-toluoyltartaric acid in 2-propanol (2.2 mL). Two cycles yielded **7** (50 mg) (**7/8** = 92.9:7.1). (Mp_{HCl} = 118 °C and 125–132 °C); $[\alpha_D^{20}]_{base}$ = –34.03° (*c* 0.955, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 23.80 min, *m/e* = 327.25).

The material recovered from above filtrates (220 mg) was treated with 0.5 equiv (–)-di-*O,O'*-4-toluoyltartaric acid. Two cycles yielded **8** (38.2 mg) (**8/7** = 93.3:6.7) (Mp_{HCl} = 118 and 128–132 °C); $[\alpha_D^{20}]_{base}$ = –7.81° (*c* 0.205, $CHCl_3$); GC/ MS: 70–250 °C (10 °C/min), R_t = 23.69 min, *m/e* = 327.25).

*Separation of diastereomers **9** and **10***

The above protocol was applied to a primary **9** + **10** mixture (310 mg) in 2-propanol (6 mL) and 0.5 equiv (+)-di-*O,O'*-4-toluoyltartaric acid in 2-propanol (2 mL) to yield **9** (40 mg) (**9/10** = 93.3:6.7) after one cycle. (Mp_{HCl} = 118 and 130–135 °C; $[\alpha_D^{20}]_{base}$ = + 34.4° (*c* 0.515, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 23.77 min, *m/e* = 327.25).

The material recovered from the above filtrate (270 mg) treated with 0.5 equiv (–)-di-*O,O'*-4-toluoyltartaric acid yielded after one cycle a new crop of **9** (110 mg) (**9/10** = 94.4:5.6). Treatment of the material recovered from the last filtrate (168.3 mg) with 0.5 equiv (+)-di-*O,O'*-4-toluoyltartaric acid yielded after one cycle a new mixture (**10/9** = 86.2:13.8). One cycle applied to the filtrate recovered material with 0.5 equiv (–)-di-*O,O'*-4-toluoyltartaric acid yielded **10** (20.9 mg) (**10/9** = 95.2:4.8) (Mp_{HCl} = 110 and 138–145 °C; $[\alpha_D^{20}]_{base}$ = + 8.45° (*c* 0.13, $CHCl_3$); GC/MS: 70–250 °C (10 °C/min), R_t = 23.63 min, *m/e* = 327.25).

Acknowledgments

We thank M Michaud for excellent technical assistance and the Direction des recherches et études techniques for financial support (grant DRET 92/050).

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