1-[1-(2-Benzo[*b*]thiopheneyl)cyclohexyl]piperidine Hydrochloride (BTCP) Yields Two Active Primary Metabolites in Vitro: Synthesis, Identification from Rat Liver Microsome Extracts, and Affinity for the Neuronal Dopamine Transporter

Carine Deleuze-Masquefa,[†] Martine Michaud,[†] Jacques Vignon,[‡] and Jean-Marc Kamenka*[,†]

CNRS ERS 155, INSERM U 249, and INSERM U 336, Ecole Nationale Supérieure de Chimie, 8, rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France

Received February 5, 1997[⊗]

1-[1-(2-Benzo[*b*]thiopheneyl)cyclohexyl]piperidine hydrochloride (BTCP, **1**) and cocaine bind to the neuronal dopamine transporter to inhibit dopamine (DA) reuptake. However, on chronic administration, cocaine produces sensitization, but **1** produces tolerance. Because metabolites of **1** might be responsible for some of its pharmacological properties, we have identified the primary metabolites of **1** produced by rat liver microsomes and determined their affinities for the DA transporter. Five monohydroxylated derivatives (**3**, **5**, **9**, **10**, **14**) and two degradation compounds (**15**, **16**) were identified as metabolites through comparison with synthetic standards in HPLC and GC systems. Standards were obtained utilizing synthetic schemes previously used for the synthesis of phencyclidine metabolites. In vitro, two compounds (**3**, **5**) showed a high affinity for the DA transporter. These active metabolites might be important in the pharmacology of **1**.

Introduction

1-[1-(2-Benzo[*b*]thiopheneyl)cyclohexyl]piperidine HCl (BTCP, **1**) is a phencyclidine (PCP) analogue evoking behavior related to the stimulation of dopaminergic transmission by a different route to that of dextroamphetamine.[1] In a drug discrimination paradigm, **1** generalizes in rats trained to discriminate cocaine from saline.[2] It, like cocaine, binds strongly to the neuronal dopamine (DA) transporter to inhibit neuronal DA reuptake.[3−5] **1** is highly selective for the DA reuptake complex when compared to PCP as it binds very poorly to the PCP receptor[3,6] inside the *N*-methyl-D-aspartate (NMDA)-gated Ca^{2+} channel. Therefore, cocaine and **1** are both potent indirect dopaminergic agonists.

DA uptake inhibition appears to be important in mediating the rewarding[7] and reinforcing[8,9] effects of cocaine, as evidenced by the highly significant correlation between the inhibition of DA uptake and self-administration.[10,11] It has been recognized that the DA neuronal transporter can bind dopamine and reuptake blockers on different binding sites which are not completely overlapping.[12−14] This observation led to the hypothesis that selective ligands could interfere with the mechanisms which make cocaine a drug of abuse[15] and might help to block cocaine dependency in pharmacotherapeutic interventions.[16] In rodents, **1** and cocaine have similar dose-dependent behavioral and neurochemical effects after acute administration. Chronically, however, in mice and rats cocaine induces a behavioral sensitization while **1** produces tolerance.[17,18] These behavioral differences may be accounted for by the different binding sites for both drugs on the DA uptake complex.[12−14,19] Alternatively, the metabolites of **1** may also be involved in its pharmacology. The enzymes responsible for the metabolism of **1** may be induced during chronic treatment, thus leading to an apparent tolerance. On the other hand, **1** may be very slowly degraded in mice and rats, leading to a relatively high circulating concentration. This would be similar to the situation with cocaine where continued infusion induces tolerance.[20,21] The role for active metabolites in chronic administration studies is not known. We therefore decided to study the biotransformations of **1** initially in vitro. This study was designed to identify the primary metabolites of **1** obtained from rat liver microsomes and to measure their affinity for the rat striatal DA transporter labeled with [^{3}H]BTCP. Putative hydroxylated metabolites of **1** were synthesized according to those already known in the PCP series and used for comparative identification (GC and HPLC) of microsomal metabolites of **1**.

Chemistry

On the basis of structural similarities, the in vitro metabolism of **1** should be similar to that of PCP. Accordingly, structures of the putative metabolites to be synthesized as probes for identification were chosen according to known primary PCP metabolites.[22,23] Thus, we prepared monohydroxylated derivatives of **1** bearing the hydroxyl substitution at either the β and γ position from the nitrogen atom in the piperidine ring and at either the β and γ position from the quaternary carbon atom in the cyclohexyl ring (Figure 1). In this last case *cis*- and *trans*-diastereomers are possible. Their structures were defined by the relative position of the hydroxyl group and the piperidine ring in reference to the plane of the cyclohexyl ring. (In some previous papers isomers were inappropriately defined relative to the aromatic moiety, thus *cis* and *trans* were reversed.) Finally, during identification we had to synthesize three more compounds to fully identify the primary metabolites produced: the isomeric alcohols **15** and **17** and the corresponding unsaturated compound **16** (Figure 1). In our previous synthesis in the arylcyclohexylamines series, we showed that two general synthetic paths could be used depending on the desired final structures: (i) structures unsubstituted at the cyclohexyl ring or

* To whom correspondence should be addressed.
[†] CNRS ERS 155 and INSERM U 249.
[‡] INSERM U 336.
[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1997.

S0022-2623(97)00078-2 CCC: $14.00

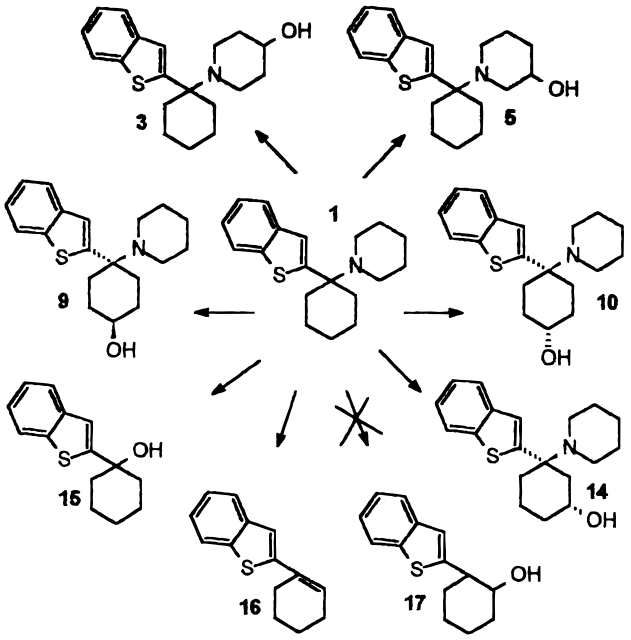
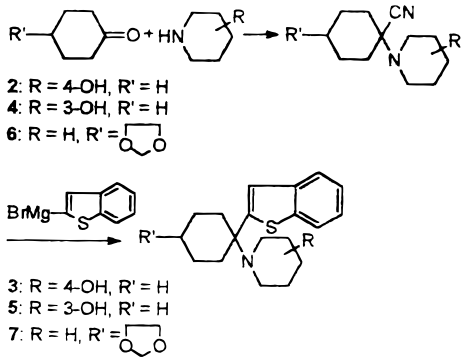


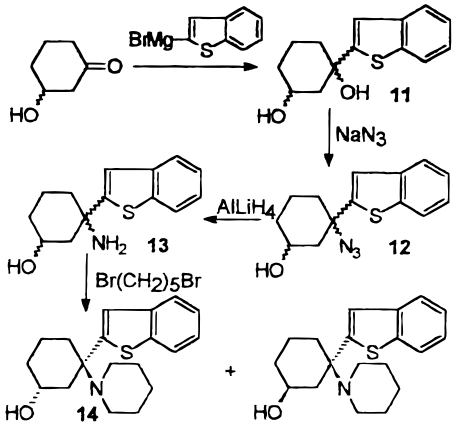
Figure 1. Metabolites obtained from oxidation of **1** by rat liver microsomes.

Scheme 1



obtained in the form of one diastereomer only and (ii) structures substituted at the cyclohexyl ring obtained as *cis*/*trans*-diastereomeric pairs. The first path is Bruylant's reaction from suitable α-amino nitriles and Grignard reagents (Scheme 1).[24,25] The stereospecificity of this reaction is well-known: it yields only one diastereomer whose configuration corresponds to an equatorial attack of the iminium intermediate by the Grignard reagent.[26−28] For example this path would yield only the *cis* compound **9** (Figure 1). The second path stems from the Schmidt and Curtius reactions adapted to yield diastereomeric mixtures when the cyclohexyl moiety is substituted (Scheme 2).[29−31] Moreover, depending on conditions (time and/or temperature), this azide synthesis may yield enriched mixtures of either of the anticipated diastereomers. Final chromatography is always needed to separate the isomers.[29,30] The common 2-benzo[*b*]thiopheneyl Grignard reagent necessary for both pathways was obtained by reacting $MgBr_2$ with 2-lithiobenzo[*b*]thiophene.[32] As anticipated, the piperidinol compounds **3** and **5** were easily obtained via the first path. However cyclohexanol derivatives were very difficult to obtain in a pure isomeric form. Two major problems were encountered: very low yields in the azide pathway and a great difficulty in resolving isomeric mixtures. Finally, the simplest method to obtain alcohols **9** and **10** was to

Scheme 2



prepare the ketal **7** via the first path (Scheme 1), regenerate the corresponding ketone, and reduce it by diborane or by potassium tri-*sec*-butylborohydride. This was done to isolate pure samples of *cis*-**9** or *trans*-**10** alcohols respectively after chromatography. The *trans*-alcohol **14** could have been obtained by either of the two paths. We used the azides route synthesis to obtain a mixture of *cis*- and *trans*-alcohols, but we were unable to achieve a complete separation of this mixture: only pure samples of the *trans*-alcohol **14** were isolated. Structures of isomers were determined by NMR measurements by comparison with those previously obtained for phenyl analogues in the PCP series.[33]

Results

Identification of in Vitro Metabolites. The biotransformation of **1** by rat liver microsomes was carried out at physiological pH in the presence of a NADPH-regenerating system. Figure 1 shows the molecules extracted from the microsomal system and identified by comparison with synthesized samples. Metabolites were identified both by means of a HPLC separation in heptane/2-propanol coupled to a UV or a MS detector and by means of a GC fitted with a capillary column (OV 17) coupled to a flame ionization detector (FID) or a MS detector. Each analysis contained an internal standard (**18**: 4-(2-benzo[*b*]thiopheneyl)-4-piperidinotetrahydropyran) prepared for this purpose and added to the microsomal medium before metabolite extraction. A HPLC analysis with a UV detection at 230 nm separated almost all the derivatives with the exception of **1** and **16** which were eluted at the same retention time. However, at a 289 nm wavelength where **1** absorbs less than at 230 nm, we were able to detect, but not quantify, compound **16**. A GC analysis did not separate all compounds and in particular did not allow for the quantification of **1** and **16** because **16** is also a thermolysis product of **1**. Nevertheless, it helped to confirm the presence of compounds difficult to detect by HPLC analysis. Metabolite identification was confirmed by HPLC and GC analysis coupled to a MS detector. The retention times measured by HPLC and GC analysis are reported in Table 1. They represent all peaks detected in the time window used. According to our hypothesis, most of the identified metabolites (**3**, **5**, **9**, **10**) were similar to those found for the PCP in vitro metabolism. As for PCP, no peak corresponding to a biotransformation of the aromatic ring was detected.[22,23] The *cis* diastereomer of **14** was

Table 1. Retention Times of Standards and Internal Standard **18**[a]

compd	HPLC (mn)	GC (mn)	compd	HPLC (mn)	GC (mn)
1	2.6	14.2	**14**	9.0	
3	14.8	23.9	**15**	5.9	12.8
5	7.4	22.5	**16**	3.8	9.8
9	12.0	23.9	**17**	6.8	
10	23.6	30.5	**18**	3.5	16.3

[a] 4-(2-Benzo[*b*]thiopheneyl)-4-piperidinotetrahydropyran.

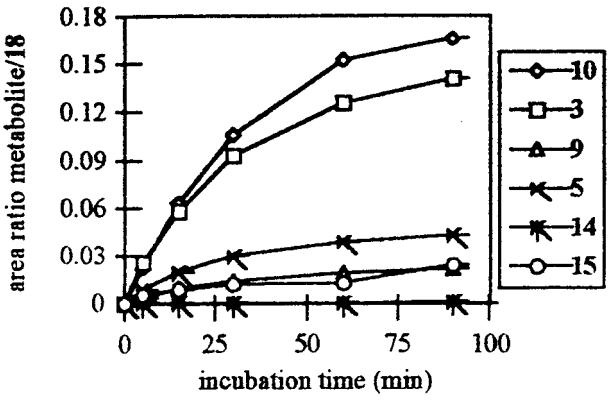


Figure 2. Effects of incubation time on metabolites production by rat liver microsomes. Each value is the mean (± SEM) of three independent determinations.

not detected although it is the sole diastereomer detected in the biotransformation of PCP.[22,23,34] Alcohol **15** apparently originates in the biotransformation of the unsaturated compound **16** since we detected **15** after incubation of **16** with rat liver microsomes under the same conditions as for **1**. It is interesting to note that the isomeric alcohol of **15**, compound **17**, was at no time detected under the conditions we used. This might be due to a regiospecific hydration of the double bond of **16** during incubation. The corresponding unsaturated compound has also been found in PCP-treated mouse liver microsomes and its production explained either by a thermolysis during treatment (GC analysis particularly) or by a Cope elimination following a N-oxidation.[35,36] In our case, **1** incubated without microsomes and treated as usual did not yield such an unsaturated structure (HPLC analysis). As for PCP[35] in vitro metabolization, at no time did we detect a peak which could be attributed to **1** *N*-oxide.

Quantification of metabolites obtained in Vitro. To identify of all the putative metabolites and to obtain good quantification, the dependency of the biotransformation of **1** on the pH, time, protein content, and NADPH concentrations was studied in preliminary experiments. Optimal conditions, i.e., a maximum number of detectable and measurable metabolites without inhibition of the microsomal system, were found to be 1 mg/mL microsomal protein, pH = 7.4, 500 μM **1**, and a NADPH-regenerating system constituted with 10 mM glucose 6-phosphate (G6P), 0.5 mM NADP, 1 unit of glucose-6-phosphate dehydrogenase (G6PDH). A 500 μM concentration of **1** probably does not comply with physiological conditions, but at lower concentrations detection and/or quantification failed and thus linearity could not be verified. Interestingly, the concentration we used here is the same as that previously used to analyze PCP metabolites (0.25 μmol in 0.5 mL).[23] Evolution of the biotransformation was checked after various incubation times (5, 15, 30, 60, 90 min). The consumption of **1** was not quantifiable because it could not be completely distinguished from **16** after HPLC separation; in GC it yields in part compound **16** by thermolysis. Metabolite production is represented by nonlinear monophasic curves which reach a plateau after about 90 min (Figure 2). This nonlinear process probably reflects the formation of secondary metabolites which may possibly be dihydroxylated derivatives of **1**. These were observable at much longer retention times and have not yet been identified (not shown). A degradation of metabolizing enzymes, however, cannot be excluded. After a 90 min incubation time, two major metabolites, **10** and **3**, were produced, three metabolites, **5**, **9**, and **15**, were present but were at least 4 times less abundant, and a very low amount of **14** was detected (Table 2).

Table 2. Amount[a] of Metabolites in the Microsomal Medium after a 90 min Incubation of **1** (500 μM)

metabolite	amount (pmol)	metabolite	amount (pmol)
10	765 ± 11	**15**	141 ± 19
3	615 ± 11	**9**	91 ± 2
5	147 ± 2	**14**	4 ± 1.4

[a] Mean of three independent determinations (± SEM).

Table 3. Affinities (IC_{50},[a] nM) for the Dopamine Uptake Complex and Hill Numbers (n_H) of **1** and Metabolites

compd	IC_{50} ± SEM	n_H ± SEM
1	8.4	1.01
3	15.9 ± 4	1.03 ± 0.1
5	1.75 ± 0.1	0.92 ± 0.08
9	53.4 ± 9.9	0.92 ± 0.06
10	113 ± 20.5	0.97 ± 0.09

[a] IC_{50}: concentration of unlabeled drug that inhibited 50% of specific [^{3}H]BTCP binding to rat striatal membranes. Each value is the mean of at least three independent determinations.

Biological Activity of the Hydroxylated Derivatives of 1. The affinities of the alcoholic compounds identified as in vitro metabolites to bind for the DA transporter were measured by inhibition of the specific binding of [^{3}H]BTCP to rat striatum membranes. Due to their insolubility in an aqueous medium, the affinities of compounds **15**, **16**, and **17** for the dopamine transporter were not measured. Nevertheless, such molecules are very different from **1**. Particularly the lack of a nitrogen atom in their structure may be assumed to considerably reduce their affinities for [^{3}H]BTCP binding sites. Measured affinities are reported in Table 3. Relatively large differences were observed in comparison with **1**: **3**, **9,** and **10** displayed affinities respectively 2, 6, and 13 times lower, but **5** displayed a 4 times higher affinity.

Discussion

Eight compounds were prepared, seven of which were identified among the primary metabolites yielded by incubation of **1** with rat liver microsome preparations. We were able to quantify five compounds whose structures correspond to the monooxidation of cyclohexyl or piperidine moieties of **1**. Another compound, 1-(2-benzo[*b*]thiopheneyl)cyclohexanol **15,** was quantified which might be formed by regioselective hydration of 1-(2-benzo[*b*]thiopheneyl)cyclohexene (**16**). Compound **16** yielded **15**, but not **17** after incubation of 500 μM **16** with microsomes. It is unlikely that the unsaturated or the hydroxylated compound would result from a

degradation of **1** during incubation, extraction, or HPLC separation since when **1** was incubated in the usual fashion but without microsomes, **15** and **16** were not detected by the HPLC analysis of extracts. Given the experimental results, the hypothesis that hydration is a metabolic step is an attractive concept that requires additional study.

An analogue to compound **14** *trans*- (pip/OH) 1-[1-(2-benzo[*b*]thiopheneyl)-3-hydroxycyclohexyl]piperidine was not detected at any time in PCP-treated rat liver microsomes whereas mouse liver microsomes yielded the corresponding *cis* structure.[22,23,34]

Hydroxylation at the 3- or 4-position of the piperidine ring of **1** yields high-affinity ligands for the DA transporter. Conversely, hydroxylation at the 3- or 4-position of the cyclohexyl ring decreases the affinity. Indeed, **3** and **5** are high-affinity ligands for the neuronal dopamine transporter, which is different from the situation with PCP metabolites: all monohydroxylated compounds are low- or very low-affinity ligands for the PCP receptor, and consequently none of them has been considered as an active metabolite.[33] Experiments in vivo are needed to evaluate the potential contribution of **3** and **5** to the pharmacology of **1**.

Conclusion

The identification of two high-affinity ligands for the DA transporter after metabolism of **1** by rat liver microsomes leads to the hypothesis that in vivo metabolism could produce the same metabolites. Thus, considering the very important role which could be played by highly active metabolites during chronic administrations, in vivo biotransformations of **1** are currently being studied in our laboratories.

Experimental Section

Melting points were determined with a Büchi-Tottoli apparatus and remain uncorrected. Elemental analyses were performed at the CNRS Microanalytical Section in Montpellier on the hydrochloride salts and were within ±0.4% of theoretical values. ^{13}C NMR spectra were obtained on a Brucker AC 200 spectrometer at 50.323 MHz (Brucker Spectrospin, Wissembourg, France) in 5 mm sample tubes in the FT mode. A spin−echo sequence (Jmod) was used for signal assignments. HPLC/MS data given for synthetic compounds was obtained from a Shimadzu (Touzart et Matignon, Les Ulis, France) LC10AT HPLC coupled to a Fisons Trio-1000 mass spectrometer (Fisons Instruments, Les Ulis, France) coupled to a Fisons Particle Beam Interface LINC; data was treated by Fisons MassLab system software. For in vitro experiments, compounds were isolated in their hydrosoluble hydrochloride salt form. The salts were precipitated by bubbling a dry stream of HCl in an ethereal solution of bases. After filtration, the solids collected were dried in vacuum.

1-(4-Hydroxypiperidino)cyclohexanecarbonitrile (2). Acetone cyanohydrin (10.71 g, 0.126 mol) was added to a stirred mixture of cyclohexanone (12.35 g, 0.126 mol), anhydrous magnesium sulfate (75 g, 0.63 mol), dimethylacetamide (16.63 g, 0.189 mol), and 4-hydroxypiperidine (18.9 g, 0.189 mol). The pastelike mixture obtained was heated at 45 °C for 48 h, cooled to room temperature, poured onto ice, and vigorously stirred for 30 min. The aqueous mixture was extracted with ether (3 × 200 mL), and the organic phases were washed with water until neutrality was achieved. The dried (Na_2SO_4) organic layer was concentrated to dryness under reduced pressure to yield a white solid (22.2 g, 85%) which was used without further purification.

1-[1-(2-Benzo[*b*]thiopheneyl)cyclohexyl]piperidin-4-ol (3). A $MgBr_2$ solution was prepared by the gradual addition of 1,2-dibromoethane (43 mL, 0.33 mol) to Mg turnings (8.4 g, 0.35 mol) covered with 100 mL of anhydrous ether. Simultaneously, a solution of 2-lithiobenzo[*b*]thiophene was prepared at −20 °C, in a nitrogen atmosphere, by the careful addition of a *n*-butyllithium solution (1.6 M in hexane) (200 mL, 0.32 mol) to a solution of benzo[*b*]thiophene (43.2 g, 0.32 mol) in ether. The resulting solution was refluxed for 2 h, cooled to room temperature and added under nitrogen to the $MgBr_2$ solution. This, after stirring at room temperature for 30 min, yielded 2-MgBr-benzo[*b*]thiophene. **2** (22.2 g, 0.106 mol) in ether was added dropwise to the Grignard reagent solution, and the resulting mixture was stirred and refluxed for 16 h. After being cooled to room temperature, the yielded solution was poured onto an ice-cold saturated solution of NH_4Cl in water, stirred for 30 min, and treated as follows: decantation, extraction with ether (2 × 200 mL) and CH_2Cl_2 (200 mL), washing of the pooled organic phases with 10% HCl (3 × 200 mL), neutralization of the aqueous phase with 20% NH_4OH, extraction with ether (2 × 200 mL) and CH_2Cl_2 (200 mL), and washing of the pooled organic phases with water until neutrality was achieved. The dried (Na_2SO_4) organic layer was evaporated to dryness under reduced pressure to yield a solid material purified by crystallization (CH_2Cl_2) in the form of a white solid (15.3 g, 46%): mp (HCl) 185−6 °C; ^{13}C NMR ($CDCl_3$) δ 25.35, 26.31, 32.13, 33.82, 35.77, 48.28, 67.55, 72.68, 124.93, 127.23, 127.67, 128.65, 131.27, 138.79, 141.67, 142.48; MS 315 (M^+, 16), 272 (20), 214 (52), 185 (42), 173 (25), 147 (100), 134 (25), 115 (32). Anal. ($C_{19}H_{26}NOSCl$) C, H, N.

1-(3-Hydroxypiperidino)cyclohexanecarbonitrile (4). A mixture of cyclohexanone (4.9 g, 0.05 mol), anhydrous magnesium sulfate (42 g, 0.35 mol), dimethylacetamide (7 g, 0.8 mol), piperidin-3-ol (8 g, 0.082 mol), and acetone cyanhydrin (4.7 g, 0.055 mol), treated as described for **2**, yielded a yellow oil (6.75 g, 54%) used without further purification.

1-[1-(2-Benzo[*b*]thiopheneyl)cyclohexyl]piperidin-3-ol (5). A Grignard reagent obtained as described for **3** from Mg turnings (4.8 g, 0.2 mol), 1,2-dibromoethane (26 mL, 0.2 mol), and a 1.6 M solution of *n*-butyllithium in hexane (125 mL, 0.2 mol) and benzo[*b*]thiophene (27 g, 0.2 mol) was made to react with the α-amino nitrile **4** (12.5 g, 0.06 mol) to yield an oily residue. Purification on a column (aluminoxide Merck II-III) yielded (CH_2Cl_2) a white solid (3.8 g, 37%): mp (HCl) 165 °C; ^{13}C NMR ($CDCl_3$) δ 20.55, 23.12, 23.91, 31.37, 32.95, 33.38, 46.63, 51.77, 63.85, 70.28, 122.17, 124.50, 125.03, 125.86, 127.75, 136.25, 138.99, 139.97; MS 315 (M^+, 18), 272 (21), 215 (54), 214 (43), 185 (30), 173 (21), 147 (100), 134 (18), 115 (24). Anal. ($C_{19}H_{26}NOSCl$) C, H, N.

1-(1-Piperidino)-4,4-(ethylenedioxy)cyclohexanecarbonitrile (6). A mixture of 1,4-hexanedione-monoethylene ketal (20 g, 0.138 mol), anhydrous magnesium sulfate (76 g, 0.638 mol), dimethylacetamide (11.35 g, 0.129 mol), piperidine (11 g, 0.129 mol), and acetone cyanhydrin (10.71 g, 0.126 mol), treated as described for **2**, gave a white solid (20.2 g, 63%) which was used without further purification.

1-[1-(2-Benzo[*b*]thiopheneyl)-4,4-(ethylenedioxy)cyclohexyl]piperidine (7). A Grignard reagent obtained as described for **3** from Mg turnings (8.4 g, 0.35 mol), 1,2-dibromoethane (43 mL, 0.33 mol), a 1.6 M solution of *n*-butyllithium in hexane (200 mL, 0.32 mol), and benzo[*b*]-thiophene (43.2 g, 0.32 mol) was made to react with the α-amino nitrile **6** (22.2 g, 0.106 mol) to yield an oily residue purified by precipitation in petroleum ether (21.2 g, 53%).

1-[1-(2-Benzo[*b*]thiopheneyl)-4-oxo-cyclohexyl]piperidine (8). Compound **7** (10 g, 0.028 mol) was dissolved in 5% H_2SO_4 (100 mL) and stirred for 16 h at room temperature. The reaction was halted by neutralization with 20% NH_4OH, and the mixture was treated as follows: extraction with CH_2Cl_2 (3 × 50 mL), washing of the pooled organic phases with 10% HCl (3 × 50 mL), neutralization of the aqueous phase with 20% NH_4OH, extraction with CH_2Cl_2 (3 × 50 mL), and washing of the pooled organic phases with water until neutrality was achieved. The dried (Na_2SO_4) organic layers were evaporated under reduced pressure to give an oily residue. This residue dissolved in a minimum of CH_2Cl_2 was precipitated by petroleum ether at 4 °C to produce **8** in the form of a solid (5.5 g, 58%).

***cis*- and *trans*- (pip/OH) 4-(2-Benzo[*b*]thiopheneyl)-4-piperidinocyclohexanols (9 and 10).** A 1 M diborane solution in THF (18.2 mL, 0.018 mol) was added to **8** (2.6 g, 0.0083 mol) dissolved in anhydrous THF (20 mL) in a nitrogen atmosphere. The solution was subsequently stirred at room temperature for 1 h, then diluted with ethanol and dissolved in CH_2Cl_2, and treated as described for **8** to produce a 60/40 mixture eluted on a silica gel chromatography column (silicium dioxide, SDS, 70−200 μm). This yielded 1.5 g of the isomeric mixture and 900 mg of the pure cis isomer **9**: mp (HCl) 176 °C; ^{13}C NMR ($CDCl_3$) δ 22.38, 23.16, 27.11, 29.67, 47.66, 63.20, 69.41, 122.23, 124.53, 125.11, 125.95, 127.79, 136.35, 138.98, 139.97; MS 315 (M^+, 21), 256 (100), 230 (32), 213 (66), 185 (31), 173 (25), 147 (70), 134 (23), 115 (34). Anal. ($C_{19}H_{26}$NOSCl) C, H, N.

A 0.5 M solution of potassium tri-*sec*-butyl borohydride in THF (165 mL), diluted with anhydrous THF (66 mL), was added dropwise at −78 °C to **8** (1.1 g, 0.0035 mol) dissolved in anhydrous THF (16.5 mL) in a nitrogen atmosphere. The solution was stirred for 5 h at −78 °C and then diluted with water (11 mL) and ethanol (33 mL), and 6 N NaOH (22 mL) and 30% H_2O_2 (22 mL) were added. The resulting aqueous solution was extracted with CH_2Cl_2 (2 × 50 mL), and the pooled organic phases were treated as described for **8** to give a 20/80 mixture of *cis*/*trans* isomers (1 g, 90%). In the same conditions as above a chromatography on a silica gel column (silicium dioxide, SDS, 70−200 μm) yielded 600 mg of the isomeric mixture and 400 mg of the pure trans isomer **10**: mp (HCl) 182 °C; ^{13}C NMR ($CDCl_3$) δ 22.22, 23.05, 30.95, 31.49, 31.61, 48.02, 67.96, 68.72, 122.28, 124.45, 125.22, 126.07, 127.68, 136.14, 139.01, 140.07; MS 315 (M^+, 11), 256 (73), 230 (37), 213 (73), 185 (44), 173 (34), 147 (100), 134 (36), 115 (50). Anal. ($C_{19}H_{26}$NOSCl) C, H, N.

1-(2-Benzo[*b*]thiopheneyl)-1,3-cyclohexanediols (11). A Grignard reagent obtained as described for **3** from Mg turnings (6.21 g, 0.25 mol), 1,2-dibromoethane (31.8 mL, 0.26 mol), a 1.6 M solution of *n*-butyllithium in hexane (160 mL, 0.26 mol), and benzo[*b*]thiophene (35 g, 0.26 mol) was made to react with 3-hydroxycyclohexanone (7.38 g, 0.065 mol) to yield 16.25 g of an oily residue containing two isomeric diols.

3-Azido-3-(2-benzo[*b*]thiopheneyl)cyclohexanols (12). Trichloroacetic acid (10.8 g, 0.065 mol) dissolved in $CHCl_3$ (10 mL) was added dropwise to a stirred suspension of sodium azide (850 mg, 0.013 mol) covered with $CHCl_3$. The solution was cooled to −20 °C and well stirred until the medium resembled a jelly; **11** (16.25 g, 0.065 mol) was then dissolved in $CHCl_3$ (10 mL) and added dropwise, after which the medium was maintained at −20 °C. It was then vigorously stirred for 3 h and Na_2CO_3 was added until gas evolvement ceased. The mixture was filtered, and the solvent was evaporated under reduced pressure to give 9 g of crude isomeric alcohols in the form of an oily residue.

***cis*- and *trans*-3-Amino-3-(2-benzo[*b*]thiopheneyl)cyclohexanols (13).** The crude oily azide mixture (3 g, 0.011 mol) dissolved in THF (30 mL) was added dropwise to a stirred suspension of $AlLiH_4$ (450 mg, 0.011 mol) in THF (50 mL) in a nitrogen atmosphere. The solution was stirred for 16 h at room temperature, and then a 5% NaOH (5 mL) solution was carefully added. The mixture was stirred for 15 min and filtered on Celite. After evaporation to dryness under reduced pressure, the residue was dissolved in 10% HCl and rinsed with ether. The acidic phase was neutralized with 20% NH_4OH and extracted with CH_2Cl_2 (3 × 50 mL). The dried ($MgSO_4$) organic phases were evaporated under reduced pressure to give a brownish oil (500 mg) containing the crude diastereoisomeric primary amines mixture **13**.

***trans*- (pip/OH) 1-[2-(Benzo[*b*]thiopheneyl)-3-hydroxycyclohexyl]piperidine (14).** The crude amines mixture **13** (500 mg, 0.002 mol) in a solution of 1,5-dibromopentane (466 mg, 0.002 mol) and K_2CO_3 (550 mg, 0.004 mol) in acetonitrile (10 mL) was refluxed for 48 h. Then hexamethylphosphoramide (HMPT) (9 mL) was added and the reflux maintained for an additional 48 h. After filtration, dissolution in 10% HCl, and ether extraction (3 × 15 mL), the resulting aqueous phase was neutralized by 20% NH_4OH and extracted by CH_2Cl_2 (3 × 15 mL). Drying ($MgSO_4$) and solvent evaporation gave a crude residue (180 mg). Chromatography on a silica gel column (silicium dioxide, SDS, 70−200 μm) in ether/methanol (98:2) yielded the pure *trans* isomer **14** (40 mg): mp (HCl) 169 °C; ^{13}C NMR ($CDCl_3$) δ 17.33, 22.32, 22.97, 31.16, 32.87, 38.42, 47.21, 47.52, 65.95, 68.36, 122.12, 124.57, 125.03, 125.87, 128.04, 137.54, 138.76, 139.70; MS 315 (M^+, 8), 231 (36), 230 (47), 213 (88), 187 (45), 174 (26), 147 (100), 134 (41), 115 (53). Anal. ($C_{19}H_{26}$NOSCl) C, H, N.

1-(2-Benzo[*b*]thiopheneyl)cyclohexanol (15). A Grignard reagent obtained as described for **3** from Mg turnings (4.8 g, 0.2 mol), 1,2-dibromoethane (25 mL, 0.2 mol), a 1.6 M solution of *n*-butyllithium in hexane (125 mL, 0.2 mol), and benzo[*b*]thiophene (27 g, 0.2 mol) was made to react with cyclohexanone (10.5 mL, 0.1 mol) to yield a solid residue (43.4 g) purified on silica gel in dichloromethane: mp 88 °C; ^{13}C NMR ($CDCl_3$) δ 22.15, 25.28, 39.53, 72.34, 118.37, 122.23, 123.27, 123.82, 124.03, 138.94, 139.77, 155.15; MS 232 (M^+, 40), 189 (82), 176 (33),161 (42), 147 (35), 134 (100). Anal. ($C_{14}H_{16}$OS) C, H.

1-(2-Benzo[*b*]thiopheneyl)cyclohexene (16). A mixture (3 mL) of H_2SO_4/acetic acid (20:80) was added to fused **15** (3.48 g, 0.0015 mol). After 30 s of stirring and immediate cooling, ether (9 mL) and water (15 mL) were added. The organic phase was washed with a Na_2CO_3 solution and water until neutrality was reached, dried (Na_2SO_4), and evaporated under reduced pressure to yield **16** as a white solid (3.23 g, 100%): mp 90 °C; ^{13}C NMR ($CDCl_3$) δ 22.03, 22.58, 25.78, 26.91, 117.68, 121.91, 123.09, 123.98, 124.06, 126.98, 131.50, 138.24, 140.47, 146.72; MS 214 (M^+, 100), 199 (23), 186 (53), 185 (69), 171 (20), 147 (21), 134 (20), 115 (14). Anal. ($C_{14}H_{14}$S) C, H.

2-(2-Benzo[*b*]thiopheneyl)cyclohexanol (17). A 0.77 M solution of diborane in THF (13 mL, 0.01 mol) was added dropwise under a nitrogen atmosphere to **16** (2.14 g, 0.01 mol) dissolved in anhydrous THF. The solution was then stirred at room temperature for 2 h and the reaction stopped by addition of 15% H_2O_2 (2 mL). Separation of the two isomeric alcohols was achieved on a chromatography column (silicium dioxide, SDS, 70−200 μm) in a petroleum ether/ether mixture (70:30) to yield pure **17** (0.92 g, 40%): mp 122 °C; ^{13}C NMR ($CDCl_3$) δ 24.73, 25.77, 33.79, 34.13, 49.04, 74.79, 121.17, 122.29, 122.99, 123.77, 124.25, 138.90, 139.73, 148.27; MS 232 (M^+, 45), 173 (15),161 (42), 148 (48); 147 (100), 134 (27).

Microsomal Preparation. Rats deprived from food for 24 h were killed by decapitation. Their livers were perfused by a 10 mM Na_2HPO_4, 9% NaCl, 1 mM EDTA (pH 7.4) buffer, removed, washed, and cut up finely with scissors. The liver tissue was homogenized with a Elvehjem potter (10 strokes, 700 rpm at 4 °C) in three volumes of a 0.25 M sucrose, 10 mM EDTA, 0.1 mM DTT, 50 mM Na_2HPO_4/NaH_2PO_4 (pH 7.4) buffer. The homogenate was centrifuged at 1000*g* for 10 min, the supernatant was centrifuged at 10000*g* for 20 min, and the resulting supernatant was centrifuged at 105000*g* for 60 min. All centrifugation stages were performed at 4 °C. The pellet was suspended in three volumes of a 100 mM K_2HPO_4/KH_2PO_4, 1 mM DTT, 1 mM EDTA, and 20% glycerol (pH 7.4) buffer and centrifuged at 105000*g* for 60 min. The final pellet was suspended in one volume of the same buffer, the protein concentration was determined with the Pierce's reagent[37] using BSA as standard, and the microsomes were stored at −80 °C until used.

Incubation and Extraction. A typical incubation medium contained 1 mg of microsomal protein, 100 mM potassium phosphate (pH 7.4) buffer, 10 mM $MgCl_2$, and a NADPH-generating system (10 mM G6P, 0.5 mM NADP and 1 unit G6PDH) in a final volume of 1 mL. Incubation mixtures were preincubated in the presence of a NADPH-generating system at 37 °C for 15 min. The reaction was initiated by the addition of 500 μM of **1**. Incubations were performed in air at 37 °C in a shaking bath. Reactions were stopped by adding 1 mL of 0.2 N NaOH and calibrated by adding 100 nmol of **18** as internal standard in each reaction tube. Extraction of **1**, **18**, and metabolites was performed as follows and repeated three times: addition of 2 mL of ethyl acetate, vortexing for 1 min, centrifugation at 4000*g* for 10 min at 4 °C. The organic layers were pooled, filtered, and evaporated under nitrogen for 12 h

at 60 °C. The dried residue was solubilized in heptane before analysis (50 μL for GC analysis, 400 μL for HPLC analysis).

Identification, Quantification, and Extraction yield of 1, Metabolites, Synthetic Compounds, and Internal Standard. Compounds from rat liver microsomal preparations were recovered by extraction with ethyl acetate. **1**, internal standard **18**, and metabolites were generally recovered with a very good yield with the exception of **3**. Standard calibration curves were determined from peak surface analysis of standards solutions in heptane of increasing amounts of metabolites (0.3 nmol to >300 nmol) and a fixed amount of **18** (100 nmol). Linear regression lines were obtained with correlation coefficients varying from 0.991 to 0.996. The metabolites were identified and quantified through reference to calibration standards (retention time and peak area). To determine the extraction yield, 500 nmol of **1** and 100 nmol of each compound were added to the microsomal preparation previously stopped by 1 mL of 0.2 N NaOH and then extracted as described above and analyzed by HPLC separation. The areas were compared to those obtained after the separation of 500 nmol of **1** and 100 nmol of each compound in its base form. The extraction yield did not vary regardless of the amount of **1** used or regardless of the relative proportion of **1** and synthetic compounds. Finally, the recovery yields (% ± SEM) of synthetic compounds after extraction with ethyl acetate from microsomal medium were as follows: **1**, 100 (0.4); **3**, 87 (1); **5**, 94 (1.1); **9**, 92 (1.2); **10**, 98 (1); **14**, 91 (1.4); **15**, 98 (1.6); **16**, 99 (1.5); **17**, 96 (1.7); **18**, 100 (1.4).

Analysis Conditions. HPLC system: Shimadzu LC-10AD pump (Touzart et Matignon, Les Ulis, France); Lichrocart Lichrospher 100 NH_2 column (5 μm, 250 × 4 mm, 40 °C) (Merck-Clévenot, Nogent/Marne, France); Shimadzu SPD-6A UV spectrophotometric detector (at 230 or 289 nm); heptane/2-propanol (98/2, v/v) as mobile phase (1 mL/min); 5 μL injection volumes.

GC system: Shimadzu gas chromatograph/FID GC-14B; Quadrex 007-OV-1701 fused silica capillary column (25 m × 0.25 mm × 0.1 μm) with a methyl 7% phenyl 7% cyanopropylsilicone bonded phase (Touzart et Matignon, Les Ulis, France); injector and detector temperatures: 240 °C and 270 °C respectively; splitless mode injection for 0.5 min; oven temperature, 60 °C for 0.5 min increasing linearly by 40 °C/min to 130 °C, 10 °C/min to 205 °C, 1 °C/min to 220 °C, then 10 °C/ min to 250 °C for 6 min to purge the column; carrier gas (H_2), 115 Kpa, split vent 1 mL/min. For HPLC and GC data acquisition and treatment: Shimadzu Class-LC 10 system. Comparative mass spectra between standards and metabolites were obtained from Hewlett-Packard equipments (Les Ulis, France): a 1050 HPLC with a particle beam interface HP 59980 B, a HP 5890 gas chromatograph, and an Engine mass spectrometer HP 5989 A. Columns and elution programs were as described above.

In Vitro Binding Experiments. [^{3}H]-BTCP binding to the DA uptake complex: the method used was the same as described by Vignon et al.[3] Rat striata were dissected on ice and homogenized with an ultraturax 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 49000*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 of protein/mL) was incubated with [^{3}H]BTCP (0.2−0.5 nM) (CEA, Service des Molécules Marquées, Saclay, France, 55 Ci/mmol) in the absence or in 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 by filtration over GF/B Whatman glass fiber filters (Labover, Montpellier, France) presoaked in 0.5% polyethylenimine (Aldrich, Saint-Quentin Fallavier, France) with a MR24 Brandel cell harvester (Beckman, Gagny, France). The filters were rinsed three times with 5 mL of a 50 mM NaCl, Tris/HCl 10 mM (pH 7.7) buffer, and the radioactivity retained was counted in 3.5 mL of ACS (Amersham) with an Excel 1410 (LKB, Orsay, France) liquid scintillation spectrophotometer. The nonspecific binding was determined in parallel incubations in the presence of 10 μM unlabeled **1**.

Acknowledgment. This work was supported by CNRS, INSERM, and grant MESR 94V0255 (to J.V.). We would like to thank Patrick Graffin for his assistance in obtaining the HPLC/MS spectra and Brian Bolger and Daniel Fisher for their careful reading of the manuscript.

References

(1) Duterte-Boucher, D.; Kamenka, J. M.; Costentin, J. Comparison of the effects of 3 indirect dopamine agonists, GK13 (BTCP), GBR 12783 and dexamphetamine on behavioral tests involving central catecholaminergic transmissions. *Psychopharmacology* **1990**, *101*, 344−353.

(2) Koek, W.; Colpaert, F. C.; Woods, J. H.; Kamenka, J. M. The phencyclidine (PCP) analog N-(1-(2-benzo[*b*]thiopheneyl)cyclohexyl)piperidine shares cocaine-like but not other characteristic behavioral effects with PCP, ketamine and MK-801. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 1019−1027.

(3) Vignon, J.; Pinet, V.; Cerruti, C.; Kamenka, J. M.; Chicheportiche, R. [^{3}H]N-[1-(benzo[b]thienyl)cyclohexyl]piperidine [[^{3}H]-BTCP]: a new phencyclidine analog selective for the dopamine uptake complex. *Eur. J. Pharmacol.* **1988**, *148*, 427−436.

(4) Cerruti, C.; Drian, M. J.; Kamenka, J. M.; Privat, A. Localization of dopamine carriers by BTCP, a dopamine uptake inhibitor, on nigral cells cultured *in vitro*. *Brain Res.* **1991**, *55*, 51−57.

(5) Maurice, T.; Vignon, J.; Kamenka, J. M.; Chicheportiche, R. Differential interaction of phencyclidine-like drugs with the dopamine uptake complex in vivo. *J. Neurochem.* **1991**, *56*, 553−559.

(6) Ilagouma, A. T.; Maurice, T.; Duterte-Boucher, D.; Coderc, E.; Vignon, J.; Costentin, J.; Kamenka, J. M. Arylcyclohexylamines derived from BTCP are potent indirect catecholamines agonists. *Eur. J. Pharmacol.* **1993**, *28*, 377−385.

(7) Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **1987**, *237*, 1219−1223.

(8) Kuhar, M. J.; Ritz, M. C.; Boja, J. W. The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci.* **1991**, *14*, 299−302.

(9) Kleven, M. S.; Anthony, S. W.; Woolverton, W. L. Pharmacological characterization of the discriminative stimulus effect of cocaine in rhesus monkey. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 312−317.

(10) Spealman, R. D.; Madras, B. K.; Bergman, J. Effect of cocaine and related drugs in nonhuman primates. II. Stimulant effect on schedule controlled behavior. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 142−149.

(11) Bergman, J.; Madras, B. K.; Johnson, S. E.; Spealman, R. D. Effects of cocaine and related drugs in nonhuman primates. III. Self-administration by squirrel monkeys. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 150−155.

(12) Reith, M. E. A.; de Costa, B.; Rice, K. C.; Jacobson, A. E. Evidence for mutually exclusive binding of cocaine, BTCP, GBR12935, and dopamine to the dopamine transporter. *Eur. J. Pharmacol.* **1992**, *227*, 417−425.

(13) Akunne, H. C.; Dersch, C. M.; Cadet, J. L.; Baumann, M. H.; Char, G. U.; Partilla, J. S.; de Costa, B. R.; Rice, K. C.; Ivy Carroll, F.; Rothman, R. B. Studies of the biogenic amine transporter. III. Demonstration of two binding sites for [3H]-GBR12935 and [3H]BTCP in rat caudate membranes. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1462−1475.

(14) Rothman, R. B.; Cadet, J. L.; Akunne, H. C.; Silverthorn, M. L.; Baumann, M. H.; Ivy Carroll, F.; Rice, K. C.; de Costa, B. R.; Partilla, J. S.; Wang, J. B.; Uhl, G.; Glowa, J. R.; Dersch, C. M. Studies on the biogenic amines transporter. IV. Demonstration of a multiplicity of binding sites in rat caudate membranes for the cocaine analog [125I]RTI-55. *J. Pharmacol. Exp. Ther.* **1994**, *270*, 296−309.

(15) Baumann, M. H.; Char, G. U.; de Costa, B. R.; Rice, K. C.; Rothman, R. B. GBR12909 attenuates cocaine-induced activation of mesolimbic dopamine neurons in rat. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1216−1222.

(16) Elmer, G. I.; Brockington, A.; Gorelick, D. A.; Carrol, F. I.; Rice, K. C.; Matecka, D.; Goldberg, S. R.; Rothman, R. B. Cocaine cross-sensitization to dopamine uptake inhibitors: unique effects of GBR12909. *Pharmacol. Biochem. Behav.* **1996**, *53*, 911−918.

(17) Martin-Fardon, R.; Arnaud, M.; Rousseau, E.; Kamenka, J. M.; Privat, A.; Vignon, J. N-[1-benzo[*b*]thiopheneyl)cyclohexyl]-piperidine (BTCP) and cocaine induce similar effects on striatal dopamine: a microdialysis study in freely moving rats. *Neurosci. Lett.* **1996**, *211*, 179−182.

(18) Prinssen, E. P. M.; Kleven, M. S.; Vignon, J.; Kamenka, J. M.; Koek, W. Effects of repeated administration of N-[1-(2-benzo-(b)-thiophenyl)cyclohexyl]piperidine and cocaine on locomotor activity in C57BL/6 mice. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 904−911.

(19) Maurice, T.; Barbanel, G.; Kamenka, J. M.; Vignon, J. Modulation by dopamine of [^{3}H]-N-(1-(2-benzo[*b*]thiopheneyl)cyclohexyl)piperidine ([^{3}H]BTCP, a phencyclidine derivative) to the dopamine uptake complex. *Neuropharmacology* **1991**, *30*, 591−598.
(20) Reith, M. E. A., Benuck, M., Lajtha, A. Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 281−287.
(21) Woolverton, W. L., Johnson, K. M. Neurobiology of cocaine abuse. *Trends Pharmacol. Sci.* **1992**, *13*, 193−200.
(22) Holsztynska, E. J.; Domino, E. F. Quantification of phencyclidine, its metabolites, and derivatives by gas chromatography with nitrogen−phosphorus detection: Application for in vivo and in vitro biotransformation studies. *J. Anal. Toxicol.* **1986**, *10*, 107−115.
(23) Gole, D. J.; Pirat, J. L.; Kamenka, J. M.; Domino, E. F. Hydroxy metabolites of phencyclidine: Identification and quantification of two novel metabolites. *Drug. Metab. Dispos.* **1987**, *16* (3), 386−391.
(24) Geneste, P.; Kamenka, J. M.; Dessapt, P. Approche de la synthèse d'α-aminonitriles en milieu organique anhydre. *Bull. Soc. Chim. Fr.* **1980**, 3−4, II, 186−191.
(25) Ilagouma, A. T.; Dornand, J.; Liu, C. F.; Zenone, F.; Mani, J. C.; Kamenka, J. M. Preparation and study of derivatives and analogs of the phencyclidine molecule possessing immunosuppressive properties in vitro. *Eur. J. Med. Chem.* **1990**, *25*, 609−615.
(26) Sicsic, S.; Welvart, Z. Effet de la gem-substitution dans la série cyclohexanique. I.-Relation de la structure et de la basicité des benzylamines α-substituées. *Bull. Soc. Chim. Fr.* **1967**, *2*, 575−582.
(27) Mousseron, M.; Bessiere, J. M.; Geneste, P.; Kamenka, J. M.; Marty, C. Etude structurale par RMN dans la série des phényl-1 cyclohexylamines. *Bull. Soc. Chim. Fr.* **1968**, *9*, 2803−2807.
(28) Mousseron, M.; Kamenka, J. M.; Darvich, M. R. Obtention des dérivés de la phényl-1 cyclohexylamine comportant un groupe phényl en axial. *Bull. Soc. Chim. Fr.* **1970**, *4*, 1435−1439.
(29) Geneste, P.; Herrmann, P.; Kamenka, J. M.; Pons, A. Nouvelle voie d'accès aux isomères des phényl-1 cyclohexylamines substituées au cyclohexane. *Bull. Soc. Chim. Fr.* **1975**, *7−8*, 1619−1626.
(30) Geneste, P.; Kamenka, J. M.; Herrmann, P.; Moreau, J. Isomérisation d'azides intermédiaires dans la synthèse de phényl-1 cyclohexylamines substituées au cyclohexane. *Recl. Trav. Chim. Pays-Bas* **1976**, *95*, 116−120.
(31) Brine, G. A.; Boldt, F. G.; Coleman, M. L.; Williams, E. E.; Krcelic, T. M.; Caroll, F. I. Synthesis and carbon-13 nuclear magnetic resonance analysis of arylcyclohexylamines related to phencyclidine. In *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications*; Kamenka, J. M., Domino, E. F., Geneste, P., Eds.; NPP Books: Ann Arbor, 1983; pp 13−34.
(32) Minato, A.; Tamao, K.; Hayashi, T.; Suzuki, K.; Kumada, M. Palladium-phosphine complex catalysed cross-coupling reaction of 1-methyl-2-pyrrolyl-magnesium bromide and zinc chloride with organic halides. *Tetrahedron Lett.* **1981**, *52*, 5319−5322.
(33) Kamenka, J. M.; Chiche, B.; Goudal, R.; Geneste, P.; Vignon, J.; Vincent, J. P.; Lazdunski, M. Chemical synthesis and molecular pharmacology of hydroxylated 1-(1-phenylcyclohexyl) piperidine derivatives. *J. Med. Chem.* **1982**, *25*, 431−435.
(34) Gole, D. J.; Pirat, J. L.; Domino, E. F. New aspects of phencyclidine (PCP) metabolism. In *Sigma and Phencyclidine-Like Compounds as Molecular Probes in Biology*; Domino, E. F., Kamenka, J. M., Eds.; NPP Books: Ann Arbor, 1988; pp 625−641.
(35) Martin, B. R.; Bailey, B. B.; Awaya, H.; May, E. L.; Narasimhachari, N. In vitro metabolism of 1-phenyl-1-cyclohexene, a pyrolysis product of phencyclidine. *Drug Metab. Dispos.* **1982**, *10*, 685−689.
(36) Holsztynska, E. J.; Domino, E. F. Overview of phencyclidine biotransformation. In *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications*; Kamenka, J. M., Domino, E. F., Geneste, P., Eds.; NPP Books: Ann Arbor, 1983; pp 157−194.
(37) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gocke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **1985**, *150*, 76−85.

JM970078Z