A New Method to Prepare No-Carrier-Added Iodinated Spirodecanone Derivatives : Application to Dopaminergic and Serotoninergic Ligands.

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SUMMARY

Two new iodinated ligands for human brain exploration by SPECT were synthesized. For this purpose tosylethyl precursors were prepared in a one-pot synthesis from ethylene glycol di-p-toluene sulfonate and potassium salt of spiperone, a D_2 receptor antagonist, or spiroxatrine, a $5HT_{1A}$ receptor antagonist. Substitution of the precursor tosyl group by iodide or radioiodide yielded respectively iodoethylspiperone, iodoethylspiroxatrine and their radioiodinated analogs. *In vitro* displacement studies demonstrated that iodoethylspiperone and iodoethylspiroxatrine have specific binding properties for the D_2 and $5HT_{1A}$ receptors respectively. Thus this method of synthesis seemed suitable to prepare new potential agents to study receptors.

KEY WORDS : Spiperone, spiroxatrine, radioiodination, dopaminergic receptors, serotoninergic receptors

INTRODUCTION

Physiological and physiopathological processes studies in the living human brain are of great interest for research, diagnostic purposes and treatment evaluation of neurological and psychiatric diseases. These explorations can be performed by positron emission tomography (PET) (1) or by single photon emission computed tomography (SPECT) (2). For SPECT explorations it is essential to develop new

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ligands labelled with single photon emitting radionuclides (e.g. iodine 123), with high specific radioactivity.

For this aim we described a new method to prepare no-carrier-added iodoalkylated ligands. This method has been tested by preparing two new radioiodinated ligands derivated from spirodecanone series. The first one is iodoethylspiperone for dopamine receptor exploration and the second one is iodoethylspiroxatrine for serotonin receptor exploration.

To prepare these dopaminergic and serotoninergic ligands, a similar procedure was carried out. A dopaminergic ligand : iodoethylspiperone (3a) was prepared from spiperone (1a), a selective D_2 receptor antagonist. A serotoninergic ligand : iodoethylspiroxatrine (3b) was prepared from spiroxatrine (1b), a $5HT_{1A}$ receptor antagonist.

After confirming the chemical structure, *in vitro* experiments were performed with both of these iodinated derivatives.

DISCUSSION

Synthesis of iodoethylspiperone (3a) (Scheme 1) was realized in two steps from spiperone (1a). The precursor : tosylethylspiperone (2a) was first described by Kiesewetter et al. (3).In this report, we propose a new method to obtain this precursor by a one-pot synthesis. So, we used ethylene glycol di-paratoluene sulfonate opposing to an equimolar ratio of spiperone potassium salt. This proportion allows ethylene glycol di-paratoluene sulfonate to exchange a tosyl group by substitution with spiperone to obtain tosylethylspiperone (2a) as main product (60% yield for 20 min. at reflux). Iodoethylspiperone (3a) is obtained by substituting the tosyl group of the precursor by iodide. This reaction was performed with an excess of sodium iodide for 20 h at reflux of acetone and gave iodinated compound (3a) with a virtually quantitative yield. In comparison, exchange of iodide with brominated analog of (3a) prepared according to Kiesewetter's method supplied only to 30% yield of iodinated derivative of spiperone (our unpublished results).

The radiolabelled derivative (4a) was obtained from the precursor (2a) with a 2 h reflux of acetone (scheme 1). The radiolabelling yield is 80%.

The same method was used to obtain iodoethylspiroxatrine (3b) from spiroxatrine (1b) (scheme 1). The precursor : tosylethylspiroxatrine (2b) was prepared by a one-pot synthesis opposing ethylene glycol di-paratoluene sulfonate to an equimolar ratio of spiroxatrine potassium salt. Stable and radiolabelled

iodoethylspiroxatrine, (3b) and (4b), are obtained by substituting the tosyl group of the precursor by iodide, according to the described method for iodoethylspiperone.

After chemical characterization of iodoethylspiperone and iodoethylspiroxatrine, we studied the specificity of these compounds for dopaminergic and serotoninergic receptors. So, we perfomed in vitro displacement studies with tritiated ligands of dopaminergic D_2 (^{3}H-Spiperone), serotoninergic $5HT_2$ (^{3}H-Ketanserin), serotoninergic $5HT_{1A}$ (^{3}H-8OHDPAT), alpha-2 adrenergic (^{3}H-Rauwolscine) and alpha-1 adrenergic (^{3}H-Prazosin) receptors (Table 1). We measured inhibition constants (Ki) of iodoethylspiperone and iodoethylspiroxatrine with these ligands on sheep striatum or frontal cortex membrane preparations.

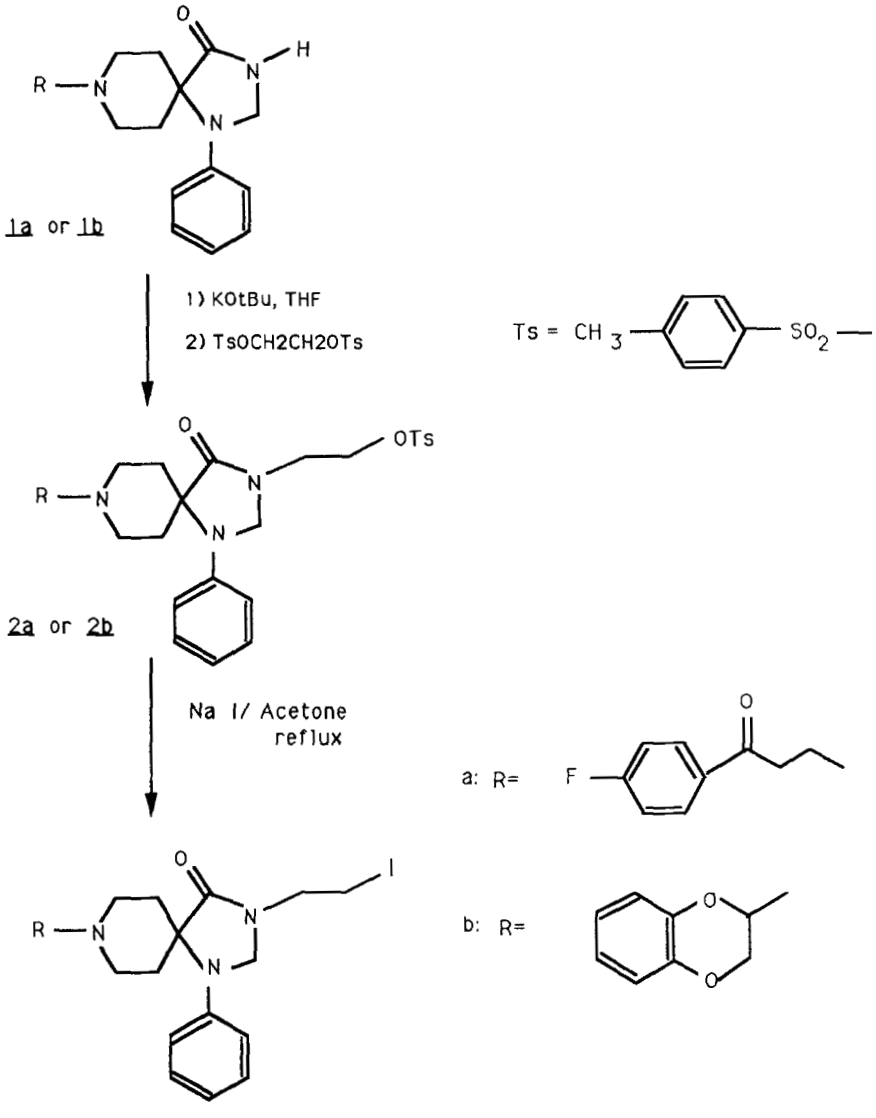
TABLE 1 : Inhibition of different ^{3}H-ligands binding by iodoethylspiperone and iodoethylspiroxatrine in sheep striatal or cortical membranes

LIGAND	IODOETHYLSPIPERONE		IODOETHYLSPIROXATRINE	
	Ki (nM)	nH	Ki (nM)	nH
^{3}H-Spiperone	12	0.95	45	0.98
^{3}H-8-OHDPAT	310	0.92	2	0.95
^{3}H-Ketanserin	460	0.80	1920	0.71
^{3}H-Prazosin	362	0.47	193	0.87
^{3}H-Rauwolscine	> 10000	ND	> 10000	ND

Ki values were calculated as follows : $Ki = IC_{50} / (1 + C/Kd)$ (4) where Ki = dissociation constant of the competing drug, IC_{50} = concentration of the drug inhibiting 50 % of the ^{3}H-ligand binding (obtained from inhibition curves), C = concentration of the ^{3}H-ligand and Kd = dissociation constant of the ^{3}H-ligand.
Each value is the mean of two determinations performed in triplicate.
nH (coefficient of Hill) were determined from hill plots.

For iodoethylspiperone, inhibition constants displayed the following rank of magnitude : ^{3}H-Rauwolscine (α_2) > ^{3}H-Ketanserin ($5HT_2$) > ^{3}H-Prazosin (α_1) > ^{3}H-8-OHDPAT ($5HT_{1A}$) > ^{3}H-Spiperone (D_2).

For iodoethylspiroxatrine, we obtained the following order : ^{3}H-Rauwolscine (α_2) > ^{3}H-Ketanserin ($5HT_2$) > ^{3}H-Prazosin (α_1) >



Scheme 1 : Synthesis of iodoethylspiperone and iodoethylspiroxatrine

^{3}H-Spiperone (D_2) > ^{3}H-8-OHDPAT ($5HT_{1A}$).

Iodoethylspiperone binds preferentially to dopaminergic receptors despite a relative affinity for serotoninergic and alpha-adrenergic sites. These results added with those obtained in vivo in the rat (5) showed that iodoethylspiperone can be considered as a marker for D_2 dopamine receptors. Iodoethylspiroxatrine has a maximal displacing effect on ^{3}H-8OHDPAT, a $5HT_{1A}$ receptor ligand ; however, a lower binding on D_2 receptor sites is also pointed out.

In conclusion, we demonstrated that iodoalkylation on 3-N position in the spirodecanone part of spiperone and spiroxatrine is obtained with a good chemical yield. This 3-N alkylation does not prevent in vitro binding of these ligands on dopaminergic and serotoninergic receptors. Moreover we showed that iodoethylspiperone is a suitable in vivo tracer of dopaminergic receptors (5); in the rat, the concentration ratio of this ligand in the striatum, rich in D_2 receptors, to the cerebellum, which represents non-specific binding, reached a high maximum value of ten. These results agree with recent data concerning 3-N fluoroalkylated derivatives of spiperone which kept binding properties on dopaminergic receptors in vivo in rodents (6).

Thus, we assume that our method of 3-N iodoalkylation can be applied to spirodecanone parts of different compounds to give ligands of central monoaminergic receptors.

EXPERIMENTAL PART

1- SYNTHESES

Potassium t-butoxide and spiperone were purchased from Janssen, ethylene glycol di-p-toluene sulfonate from Aldrich Chemical Company, spiroxatrine from RBI Bioblock Scientific and [^{125}I] sodium iodide from Amersham (37MBq in 10 µL NaOH 0.1N). All reagents were used without purification and all solvents were usually redistilled and dried.

Thin layer chromatographic analyses were conducted using silicagel 60F254 TLC plates (Merck) and compounds were revealed by U.V. detection. ^{1}H-NMR spectra were recorded at 200 MHz on a AM 300 Brüker NMR spectrometer using tetramethylsilane as standard. I.R.

spectra were recorded on a 1310 Perkin-Elmer spectrometer. Mass spectra were recorded on a ZAB 2 - SEQ mass spectrometer.

Synthesis of tosylethylspiperone (2a) : 8-[4-(4-fluoro phenyl)-4-oxobutyl]-1-phenyl-3-[2-(4-toluenesulfonyloxy)ethyl]-1,3,8-triazaspiro[4,5]decan-4-one:

Spiperone (1a) : 8-[4-(4-fluorophenyl)-4-oxobutyl]-1 -phenyl-1,3,8-triazaspiro[4,5]decan-4-one (392 mg, 1 mmol) and potassium t-butoxide (160 mg, 1.42 mmol) were mixed in anhydrous tetrahydrofuran (50 mL) and heated at reflux for 20 min. Ethylene glycol di-p-toluene sulfonate (370 mg, 1 mmol) dissolved in anhydrous THF (10 mL) was added. The reaction mixture was maintained at reflux for 20 min. After evaporation of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (50 mL). The organic phase was washed with brine and dried with Na_2SO_4. Ethyl acetate was removed and the residue purified by flash chromatography on silicagel (230-400 Mesh) with 600 mL of eluent ($CHCl_3/CH_3OH$: 95/5).

Purification of tosylethylspiperone was performed on preparative silica gel TLC ($CHCl_3/CH_3OH$: 95/5 ; Rf = 0.30). The product (2a) (356 mg, 0.60 mmol) was obtained as a wax with 60% yield.

I.R. ($CHCl_3$) : 1690, 1640, 1595, 1495 cm^{-1}

Mass spectrum (EI) : m/z 552, 288, 270, 172, 165, 148, 138, 110, 93, 91, 42

^1H-NMR ($CDCl_3$) : ∂ = 1.52 (d, 2H, J = 13.5 Hz, CH_2) ; 1.93 (qn, 2H, J = 7 Hz, CH_2N) ; 2.34 (s, 3H, $ArCH_3$) ; 2.43 (m, 4H, $2CH_2$) ; 2.73 (m, 4H, CH_2NCH_2) ; 2.94 (t, 2H, J = 7 Hz, CH_2COAr) ; 3.61 (t, 2 H, J = 5 Hz, $N\underline{CH_2}CH_2OTs$) ; 4.16 (t, 2H, J = 5 Hz, $NCH_2\underline{CH_2}OTs$); 4.62 (s, 2H, NCH_2N) ; 6.81 (m, 3H arom.) ; 7.05 (t, 2H arom., J = 8.5 Hz) ; 7.15-7.29 (m, 4H arom.) ; 7.70 (d, 2H arom., J = 8Hz) ; 7.94 (dd, 2H arom., J = 9 Hz and 5.5 Hz).

Synthesis of iodoethylspiperone (3a) : 8-[4-(4-fluorophenyl) -4-oxobutyl]-3-(2-iodoethyl)-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one :

The tosylethylspiperone (2a) (9 mg, 15 µmol) and sodium iodide (22.5 mg, 150 µmol) were stirred in acetone (2.5 mL). The reaction mixture was heated at reflux for 20 h. After cooling, the

solvent was evaporated under reduced pressure. Cold dilute ammonium hydroxide (2 mL) was added and iodoethylspiperone (3a) was extracted with ethyl acetate (30 mL). Then, the organic layer was washed with brine and dried on Na_2SO_4. The product of the reaction was purified on silica gel preparative TLC ($CH_3COOC_2H_5/CH_3OH$: 75/25; Rf = 0.50) to give (3a) as a wax with 97% yield (80 mg, 14.5 µmol).

I.R.($CHCl_3$) : 1697, 1640, 1600, 1505 cm^{-1}

Mass spectrum (EI): m/z 421, 316, 165, 138, 57, 43, 41

^{1}H-NMR($CDCl_3$) : ∂ = 1.70 (d, 2H, J = 14 Hz, CH_2) ; 1.97 (m, 2H, CH_2N) ;2.25 (m, 4H, $2CH_2$) ; 2.63 (m, 4H, CH_2NCH_2) ;2.98 (t, 2H, J = 7 Hz, CH_2COAr) ; 3.31 (t, 2H, J = 6.5 Hz, $N\underline{CH_2}CH_2I$) ; 3.74 (t, 2H, J = 6.5 Hz, $NCH_2\underline{CH_2}I$) ; 4.72 (s, 2H, NCH_2N) ; 6.84 (m, 3H arom.) ; 7.05 (t, 2H arom., J = 8.5 Hz) ; 7.20 (t, 2H arom., J = 8Hz) ; 7.93 (dd, 2H arom., J = 9 Hz and 5.5 Hz).

Synthesis of [^{125}I]Iodoethylspiperone (4a) : 8-[4-(4-fluorophenyl)-4-oxobutyl]-3-(2-[^{125}I]-iodoethyl)-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one :

To tosylethylspiperone (2a) (0.1 mg, 0.17 µmol) in acetone (500 µL) were added 37 MBq of [^{125}I] sodium iodide (in 10 µL NaOH 0.1 N). The mixture was heated at reflux for 2 h. Then, the labelled compound (4a) was extracted with ethyl acetate (1 mL) and controlled by TLC in the same conditions as the unlabelled compound (3a) (80% yield ; about 30 MBq with a radiochemical purity of 98%).

Synthesis of tosylethylspiroxatrine (2b) : (±)-8-[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]-3-[2-(4-toluenesulfonyloxy)ethyl]-1-phenyl-1,3,8-tiazaspiro[4,5] decan-4-one :

To spiroxatrine(1b):(±)-8-[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one (19 mg, 50 µmol) was added potassium t-butoxide (7.85 mg, 70µmol) in anhydrous THF (2.5 mL) and the temperature was maintained at reflux for 20 min. Then, ethylene di-p-toluene sulfonate (18.5 mg, 50 µmol) was added and the temperature was maintained at reflux for a further 20 min. After cooling, the THF was removed under reduced pressure and the residue dissolved in ethyl acetate (10 mL), washed with brine and dried with Na_2SO_4.

Purification was performed by preparative TLC ($CH_3COOC_2H_5/CH_3OH$: 99/1; Rf = 0.49) and the product (2b) (17.3 mg, 30 µmol) obtained as a wax with 60% yield.

I.R. ($CHCl_3$) : 1700, 1595, 1493 cm^{-1}

Mass spectrum (EI) : m/z 463, 288, 236, 172, 164, 149, 123, 91, 39

^1H-NMR ($CDCl_3$) : ∂ = 2.34 (s, 3H, $ArCH_3$) ; 2.50-2.85 (m, 8H, $CH_2CH_2NCH_2CH_2$) ; 3.62 (t,2H, J = 5 Hz, $N\underline{CH_2}CH_2OTs$) ; 3.90-4.30 (m, 7H, OCH_2CHCH_2N, $NCH_2\underline{CH_2}OTs$) ;4.63 (s, 2H, NCH_2N) ; 6.78 (m, 7H arom.) ; 7.25 (m, 4H arom.) ; 7.71 (d, 2H arom., J = 8 Hz).

Synthesis of iodoethylspiroxatrine (3b) : (±)-8-[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]-3-(2-iodoethyl)-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one :

The tosylethylspiroxatrine (2b) (10 mg, 17.3 µmol) and sodium iodide (25.95 mg, 173 µmol) were stirred in acetone (2.5 mL) and the reaction mixture was heated at reflux for 20h. Cold dilute ammonium hydroxide (2 mL) was added and iodoethylspiroxatrine (3b) was extracted with ethyl acetate (30 mL).The organic layer was washed with brine and dried on Na_2SO_4.

Purification of iodoethylspiroxatrine was performed on silica gel preparative TLC ($CHCl_3/CH_3OH$: 90/10 ; Rf = 0.63) to give (3b) as a wax with 95% yield (8.8 mg, 16.5 µmol).

I.R. ($CHCl_3$) : 1698, 1598, 1583, 1495 cm^{-1}

Mass spectrum (EI) : m/z 405, 305, 149, 57, 43, 41

^1H-NMR ($CDCl_3$) : ∂ = 2.50-2.75 (m,8H, $CH_2CH_2NCH_2CH_2$) ; 3.31 (t, 2H, J = 6.7 Hz, $N\underline{CH_2}CH_2I$) ; 3.74 (t, 2H, J = 6.7 Hz, $NCH_2\underline{CH_2}I$) 3.95-4.30 (m, 5H,OCH_2CHCH_2N) ; 4.72 (s, 2H, NCH_2N) ; 6.79 (m, 7H arom.) ; 7.25 (d, 2H arom., J = 4.5 Hz).

Synthesis of [^{125}I]Iodoethylspiroxatrine (4b) : (±)-8-[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]-3-(2-[^{125}I]-iodo ethyl)-1- phenyl-1,3,8-triazaspiro[4,5]decan-4-one : :

To tosylethylspiroxatrine (2b) (0.1 mg, 0.17 µmol) in acetone (500 µL) were added 37 MBq of [^{125}I] sodium iodide (in 10 µL NaOH 0.1 N). The mixture was heated at reflux for 2 h. Then, the labelled compound (4b) was extracted with ethyl acetate (1 mL) and controlled by TLC in the same conditions as the unlabelled compound (3b).

2- BINDING EXPERIMENTS

Preparation of sheep cerebral membranes

Sheep brains were rapidly removed . Striatum and frontal cortex were dissected on ice and homogenized in 10 vol of ice-cold 50 mM Tris-HCl pH 7.4 buffer containing 0.25 M sucrose, using a teflon-glass homogenizer (POTTER S BRAUN). After a first centrifugation (1000 g, 15 min at 4°C), pellets were rehomogenized and centrifuged in same conditions. Both supernatants were centrifuged at 50000 g for 30 min at 4°C. The final pellet was resuspended in 50 mM Tris-HCl pH 7.4 buffer, rapidly frozen and stored at -80°C until used. Protein content of tissue homogenates was determined by a modified Lowry procedure (7) using bovine serum albumin as the standard.

Binding assays

Aliquots of tissue suspension were incubated with tritiated ligands and different concentrations of iodoethylspiperone or iodoethylspiroxatrine. Binding assays were carried out as described in Table 2. Incubations were stopped by a rapid filtration under vacuum through glass fiber filters (Whatman) which were washed 3 times with 4 ml of ice-cold buffer. Filters were suspended in 7.5 ml of premixed scintillation cocktail (Pico-fluor™ 15 Packard). Radioactivity was measured in a counter (LKB WALLAC 1217) at 37% efficiency. Specific binding was defined as the excess over blanks containing unlabeled appropriate ligand (Table 2).

TABLE 2 : Characteristics of binding assays for different receptors

RECEPTORS	D2 dopaminergic	5HT1A serotoninergic	5HT2 serotoninergic	α1 adrenergic	α2 adrenergic
TISSUE	Striatum	Cortex	Cortex	Cortex	Cortex
mg prot	0.15	0.8	0.8	1	1
^{3}H-LIGAND	Spiperone	8-OHDPAT	Ketanserin	Prazosin	Rauwolscine
Ci /mmol	95	183	73.3	83	91
Supplier	Amersham	Amersham	N E N	Amersham	Amersham
Conc. (M)	0.2 10^{-9}	2 10^{-9}	0.8 10^{-9}	0.4 10^{-9}	2 10^{-9}
UNSPECIFIC BINDING Ligand	Butaclamol	Serotonin	Methysergide	WB 4101	Guanfacine
Conc. (M)	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-5}
INCUBATION Volume (ml)	1	2	1	2	2
Buffer	Tris-HCl* 50 mM	Tris-HCl 50 mM	Tris-HCl 50 mM	Tris-HCl 50 mM	Tris-HCl 50 mM
pH	7.4	7.4	7.4	7.6	7.6
Temperature	37°C	37°C	37°C	25°C	25°C
Time (mn)	30	30	15	25	25
FILTERS	GF/B	GF/B	GF/B	GF/C	GF/C
REFERENCE	8	9	10	11	12

* : containing NaCl 120 mM, KCl 5 mM, $CaCl_2$ 1 mM and $MgCl_2$ 1 mM

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