Synthesis of spin-labeled analogues of drug molecules with potential action on neuroreceptors

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The syntheses of novel spin-labeled analogues of butaclamol, a spin-labeled phenylaziridinium and two amino derivatives of butaclamol, are presented. Preliminary results of in vitro activity of these compounds on dopaminergic, serotoninergic, and adrenergic receptors correlate with the importance of the previously proposed lipophilic accessory binding site in CNS dopamine receptor.

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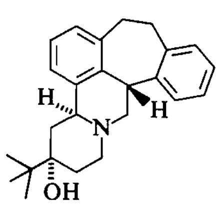
L'article présente la synthèse de nouveaux analogues marqueurs de spin de butaclamol, d'un marqueur de spin du type phénylaziridinium et de deux dérivés aminés du butaclamol. Les résultats préliminaires d'activité in vitro de ces produits sur les récepteurs dopaminergique, sérotoninergique et adrénergique sont en accord avec l'hypothèse d'un site auxiliaire lipophilique pour le récepteur de dopamine au système nerveux central.

Introduction

Spin-labeled (SL) drugs have become in the past ten years important molecular probes in studies of drug mechanisms at a molecular level. For example, a large number of drug analogues containing the nitroxide moiety have been synthesized and used to study the topography of specific binding sites in receptor macromolecules. The most representative efforts toward this goal include the preparation of SL-analogues of acetylcholine (1–3), SL-quaternary ammonium ligands (4, 5), SL-propranolol (6, 7), SL-narcotics (8), SL-steroids (9–11), SL-phenytoin (12), SL-penicillin (13), and a SL-analogue of *N,N*-dimethyl-2-phenylaziridinium (14).

The antipsychotic agent butaclamol **1** and related compounds are rigid dopamine antagonists and may thus be very helpful probes in receptor mapping. Furthermore, the fact that the dopamine receptor is highly stereoselective for the (+)-isomer of butaclamol derivatives (15–17), in both in vivo and in vitro experiments, renders the preparation of SL-analogues even more attractive as molecular monitors for the mapping of the dopamine receptor.



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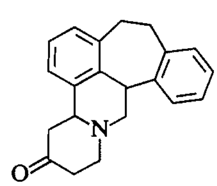

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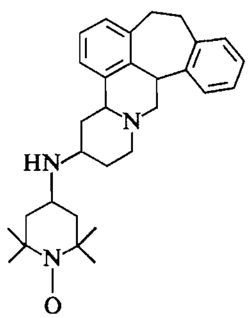

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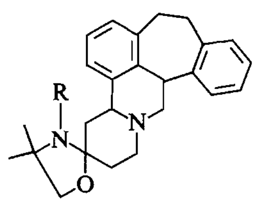


4 *a* R = H
b R = O

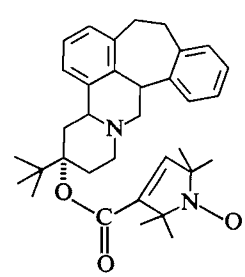
In this report, we describe for the first time the synthesis of such SL-analogues, a new SL-analogue of phenylaziridinium as well as two amino derivatives of butaclamol. Preliminary biochemical tests on these compounds are also disclosed.

Results and discussion

Chemistry

The ketone precursor **2** (15) was treated with 4-amino-2,2,6,6,-tetramethylpiperidinyl-1-oxyl in the presence of $NaBH_3CN$ to give **3** in 53% yield. In the presence of 2-methyl-2-amino-1-propanol and *p*-TsOH, **2** was converted to the oxazolidine **4***a* in 65% yield. Unfortunately, all attempts to obtain the corresponding nitroxide **4***b* using the procedure of Waggoner *et al.* (18), or variations thereof, were unsuccessful. This is probably due to the presence in the molecule of another nitrogen atom which may also be partially oxidized during the process, resulting only in decomposition products.

The SL-ester **5** was obtained in 17% yield by heating (+)-butaclamol with the acid chloride of 2,2,5,5-tetramethylpyrrolidinyl-1-oxyl-3-carboxylic acid in the presence of AgCN (19). The methylamino compound **6***a* and the isobutylamino compound **6***b* were prepared by treatment of the ketone **2** with the corresponding amine in the presence of $NaBH_3CN$ and were obtained in 32% and 57% yield respectively.



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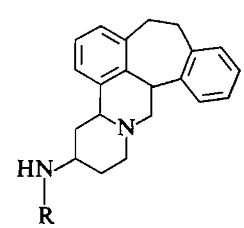


6
a R = CH_3
b R = CH_2—$CH(CH_3)_2$

The preparation of the 2-phenylaziridinium precursor **10***b* is illustrated in Scheme 1. Epoxystyrene **9** and *N*-methyl-4-amino-2,2,6,6-tetramethylpiperidinyl-1-oxyl **8**, prepared from tampone **7** according to the procedure of Rosen (20), were used as starting materials. The compounds were coupled in the pres-

TABLE 1. Receptor binding assays[a]

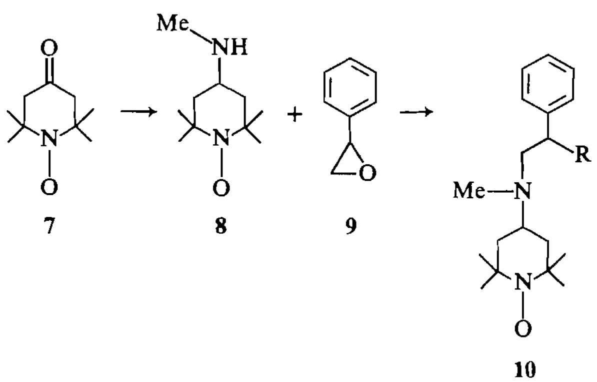
Compounds	^{3}H-Spiroperidol (dopamine)	^{3}H-LSD (serotonin)	^{3}H-WB4101 (α_1-adrenergic)
3	50%, 1.5×10^{-7} *M*[b]	—	—
4*a*	20%, 10^{-6} *M*	—	—
5[d]	21%, 10^{-6} *M*	—	—
6*a*	22%, 10^{-6} *M*	—	—
6*b*	64%, 10^{-6} *M*	—	—
10*b*	2%, 10^{-6} *M*	6%, 10^{-6} *M*	7%, 10^{-6} *M*
11	2%, 10^{-6} *M*	7%, 10^{-6} *M*	0%, 10^{-6} *M*
(±)-Butaclamol	IC_{50}, 5×10^{-9} *M*[c]		
Methysergide		IC_{50}, 1.6×10^{-8} *M*[c]	
Prazosin			IC_{50}, 2×10^{-9} *M*[c]

[a] The in vitro tests were performed on rat striatum (23) (dopamine), cortex (24) (serotonin), and forebrain (25) (α_1-adrenergic). Although at Ayerst spiroperidol is used routinely, Lazareno and Nahorski (26) have shown recently that ^{3}H-domperidone would be a much better ligand to label dopamine receptors.

[b] The concentration corresponds to the amount of drug-analogue used in the test and the percentage represents the amount of radioactive tracer displaced.

[c] These standards are included for comparative purposes.

[d] With the exception of **5**, all compounds are racemic mixtures.

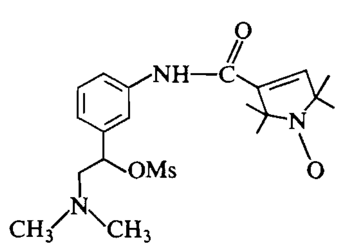


7 **8** **9** **10** *a* R = OH *b* R = OMs

SCHEME 1

ence of water to give **10***a* as the ring-opening product in 98% yield. Treatment of **10***a* with mesyl chloride in dry benzene followed by a trace of HCl gave the expected hydrochloride salt of the SL-mesyl amine **10***b* in 49% yield. The compound gives a typical three-line epr signal with a_N = 17.2 G in water. This salt is expected to cyclize instantaneously to the aziridinium chloride salt in aqueous buffer above pH 7 (21).

The synthesis of a similar molecule **11**, but with the nitroxide function in a different position, has already been reported (14). Originally the synthesis of the SL-amine **11** was developed with the objective of probing the geometry of the anionic site of acetylcholinesterase since the corresponding *N*,*N*-dimethyl-2-phenylaziridinium chloride has been shown to be a potent anionic-site-directed irreversible inhibitor (21, 22).



11

However, the product turned out to be only a poor competitive inhibitor (K_i = 2.1×10^{-3} *M*) of bovine erythocyte acetylcholinesterase (14). The reason probably lies in the presence of a bulky spin label group on the aromatic ring. To overcome this problem, the synthesis of the *N*-SL analogue **10***b* was envisaged.

Biochemistry

The compounds cited in this article were tested on rat striatal membranes for dopaminergic activity (23), as well as rat cortex for serotoninergic (24) and rat forebrain for α_1-adrenergic (25) activities, for compounds **10***b* and **11**. The results are presented in Table 1. Generally, binding studies are not sufficient on their own to demonstrate the interaction of compounds with receptors, especially if only one concentration is used. Nevertheless, a general trend can be observed from these preliminary results even though the dopaminergic activity, represented in percentage of inhibition of ^{3}H-spiroperidol binding, is low. It seems that a bulky substituent at position 3 of butaclamol enhances the activity since the activity found is **6***b* ≈ **3** > **6***a*. On the other hand, the presence of a chemical group in the axial orientation reduces considerably the activity, as in **4***a* and **5**. This observation is in agreement with the hypothesis of a lipophilic accessory binding site for the *tert*-butyl group of butaclamol, as proposed previously by Humber and co-workers (16).

As for compounds **10***b* and **11**, both are inactive in all three assays and thus further investigations of the covalent binding of these compounds to receptor macromolecules, using the present spin-labeling approach, is unwarranted.

In conclusion, only the spin-label analogue **3** shows some promise for the study of its interaction with membrane systems in an attempt to ascertain the mode of action of this class of drugs. In particular, the molecule should allow monitoring of both dynamical and structural perturbations of the dopamine receptor macromolecules by neuroleptic agents at specific binding sites. Work is also in progress to bind covalently **6***a* and **6***b* to Sepharose gel for the eventual purification of solubilized dopaminergic D_2 receptor by affinity chromatography.

Experimental

Melting points were obtained with a Reichert micro hotstage apparatus and are uncorrected. Elemental analytical results were performed by H. Seguin of the National Research Council of Canada, Ottawa and at the Microanalysis Center, Université Louis Pasteur, Strasbourg. Infrared (ir) spectra were recorded on a Perkin–Elmer 710A spectrometer as films on NaCl or in solution in $CHCl_3$. The ^1H nmr spectra were recorded using a Varian Associates EM-360A or a Bruker-WH90 spectrometer in $CDCl_3$, using Me_4Si as the internal standard. Mass spectra (ms) were obtained using a Hitachi–Perkin–Elmer model RMU 6-D. For tlc, commercial plates of silica gel (Eastman or Mancherey–Nagel) were used. Nitroxide group gives white spots on a blue background with molybdenum (Zinzadze) reagent. Nitroxide starting materials were purchased from Aldrich and Frinton Laboratories.

*(4a,13b-*trans*)-2,3,4,4a,8,9,13b,14-Octahydro-1*H*-benzo[6,7]-cyclohepta[1,2,3,*d,e*]-pyrido[2,1,*a*]-isoquinolin-3-[4′-amino-2′,2′,6′,6′-tetramethylpiperidinyloxyl] (3)*

The ketone **2** (Ayerst) (300 mg, 0.99 mmol) was dissolved in tetrahydrofuran (15 mL) and added to a solution of 4-amino-2,2,6,6-tetramethylpiperidinyloxyl (168 mg, 0.99 mmol) and sodium cyanoborohydride (Aldrich) (62 mg, 0.99 mmol) in methanol (30 mL). The reaction mixture was stirred at room temperature in the dark for 48 h. The solution was then evaporated to dryness under reduced pressure, dissolved in chloroform, and washed four times with cold water. The chloroformic solution was dried over magnesium sulfate and evaporated under reduced pressure to give a crude orange oil. The crude product was chromatographed on an alumina (activity I) column and the desired product was eluted with a mixture of benzene and acetone to give 167 mg (53%) of **3** as salmon colored crystalline needles; mp 75°–76°C; ir cm^{-1}: 3600–3400 (N—H), 2950–2850 (Boltzman's band), 1600, 1475 (aromatic); epr ($CHCl_3$) 3 lines, a_N = 14.5 G; ms *m/e*: 459 (M^+), 304, 287. *Anal.* calcd. for $C_{30}H_{40}N_3O$: C 78.57, H 8.89, N 9.16; found: C 77.42, H 8.81, N 8.35.

*(4a,13b-*trans*)-2,3,4,4a,8,9,13b,14-Octahydro-1*H*-benzo[6,7]-cyclohepta[1,2,3,*d,e*]-pyrido[2,1,*a*]-isoquinolin-3-[2′,2′-dimethyloxazolidine] (4*a*)*

Toluene (80 mL), *p*-toluenesulfonic acid (25 mg), and 2-methyl-2-amino-1-propanol (Aldrich) (724 mg, 8.1 mmol) were refluxed for 15 min with a Dean–Stark trap to remove the water. Then the ketone **2** (400 mg, 1.3 mmol) was added and the mixture left under reflux for 48 h in the dark. The solution was filtered, washed four times with a saturated solution of $NaHCO_3$ (15 mL), once with cold water, dried over $MgSO_4$, and evaporated to dryness to give 324 mg (65%) of the crystalline yellow product **4***a*, mp 61°–62°C; ir cm^{-1}: 3600–3400 (N—H), 2950–2850 (Boltzman's band), 1600, 1475 (aromatic); ^1H nmr ($CDCl_3$) δ: 1.35 (s, 6H), 3.70 (s, 2H), 4.5 (s, 1H), 7.4 to 6.9 (m, 7H). *Anal.* calcd. for $C_{24}H_{30}NO_2$: C 79.51, H 8.34, N 7.73; found: C 78.55, H 7.99, N 7.41.

*(4a,13b-*trans*)-2,3,4,4a,8,9,13b,14-Octahydro-1*H*-benzo[6,7]-cyclohepta[1,2,3,*d,e*]-pyrido[2,1,*a*]-isoquinolin-3-*tert*-butyl-3-[2′,2′,5′,5′-tetramethylpyrrolidinyloxyl-3′-carboxyl]ester (5)*

(+)-Butaclamol·HCl (Ayerst) was converted to the free base by passing a methanolic solution on an ion exchange resin AG1-X8, OH^- form (Bio-Rad). Butaclamol (200 mg, 0.552 mmol) was dissolved in 4 mL of anhydrous benzene and freshly prepared AgCN (19) (268 mg, 0.552 mmol) was added with vigorous stirring. To this was added the acid chloride (108 mg) of 2,2,5,5-tetramethylpyrrolidinyloxyl-3-carboxylic acid (from $SOCl_2$ in $CHCl_3$) and the mixture was refluxed for 18 h. The reaction mixture was diluted with 20 mL of pentane, filtered over Fuller's earth, washed with 10% $NaHCO_3$, water, then dried (Na_2SO_4) and evaporated to dryness to give 73 mg of a yellow oily crude product. Preparative tlc (Macherey–Nagel) silica gel G-100, UV_{254} with benzene–$CHCl_3$ (1:1) was used to purify the product and gave 42 mg (17%) of a yellow oil, homogenous on tlc; ir cm^{-1}: 1715 (ester), 1600 (aromatic), 1290 (C—O); epr ($CHCl_3$) 3 lines, a_N = 14.0 G. The ^1H nmr ($CDCl_3$) was taken on 7 mg of material in the presence of a microdroplet of phenylhydrazine to reduce the nitroxide function; δ: 0.9 (s, 9H, *tert*-butyl), 1.15 (d, 12H, tetramethyl), 6.8 to 7.3 (m, 7H, aromatic).

N-*methyl-(4a,13b*trans*)-2,3,4,4a,8,9,13b,14-octahydro-1*H*-benzo[6,7]cyclohepta[1,2,3,*d,e*]pyrido[2,1,*a*]isoquinolin-3-amine (6*a*)*

To a solution of the ketone **2** (300 mg, 0.99 mmol) in 15 mL anhydrous THF were added an excess of methylamine·HCl (Anachemia) (100 mg) and 61 mg (0.99 mmol) of $NaBH_3CN$ (Aldrich) dissolved in 40 mL MeOH.

The mixture was stirred at ambiant temperature for 48 h. The solution was evaporated and the residue redissolved in $CHCl_3$, washed four times with 10 mL water, dried (Na_2SO_4), filtered, and evaporated to dryness. The resulting oil was chromatographed on an alumina column (activity I) with $CHCl_3$ as eluant and 105 mg (32%) of a pale yellow crystalline material was obtained, mp 65°–66°C; ^1H nmr ($CDCl_3$) δ: 2.43 (s, 3H), 6.8 to 7.4 (m, 7H). For analysis, the compound was converted to its hydrochloride salt (HCl–Et_2O). *Anal.* calcd. for $C_{22}H_{28}N_2Cl_2$: C 67.51, H 7.21, N 7.16, Cl 18.12; found: C 67.98, H 7.46, N 7.45, Cl 17.11.

N-*isobutyl-(4a,13b*trans*)-2,3,4,4a,8,9,13b,14-octahydro-1*H*-benzo[6,7]cyclohepta[1,2,3,*d,e*]pyrido[2,1,*a*]isoquinolin-3-amine (6*b*)*

The methodology was the same as above. From 100 mg (0.33 mmol) of ketone **2**, 68 mg (57%) of the desired product was obtained, after preparative tlc (silica gel), as an oil; ^1H nmr ($CDCl_3$) δ: 0.8 (d, 6H), 2.0 to 3.5 (m, 2H), 6.7 to 7.4 (m, 7H).

N-*methyl-*N′*-4′-(2′,2′,6′,6′-tetramethylpiperidinyloxyl)-2-hydroxy-1-aminoethylbenzene (10*a*)*

N-methyl-4-amino-(2,2,6,6-tetramethylpiperidinyloxyl) **8** (20) (254 mg, 1.39 mmol) was dissolved in 3 mL of distilled water (33 % by weight) and epoxystyrene **3** (Eastman) (166 mg, 1.39 mmol) was added. The reaction mixture was left at room temperature in the dark for five days. At the end of this period, more than 95% of the products were coupled as judged by tlc. The solution was evaporated under reduced pressure to give 418 mg (98%) of an orange oil which was directly used for the next step; ir cm^{-1}: 3600–3200 (OH), 1240–1200 (C—O).

N-*methyl-*N′*-4′-(2′,2′,6′,6′-tetramethylpiperidinyloxyl)-2-methylsulfonyloxy-1-aminoethylbenzene hydrochloride salt (10*b*)*

The alcohol nitroxide **10***a* (418 mg, 1.38 mmol) was dissolved in 6 mL of dry benzene and mesyl chloride (157 mg, 1.38 mmol) was added dropwise over a period of 10 min at room temperature under argon atmosphere in the dark. After 2 h of stirring, 2 drops of concentrated HCl were added to precipitate the product in its hydrochloride form. The solvent was removed to yield 257 mg (49%) of the yellow crystalline compound **10***b* and 168 mg of an orange oily substance that can easily be separated. The compound is rather hygroscopic and melts at 145°–153°C; ^1H nmr ($CDCl_3$), in the presence of phenylhydrazine to reduce the nitroxide group, δ: 1.4 (s, 12H), 2.35 (s, 3H, N—CH_3), 3.0 (s, 3H, CH_3SO_2—), 7.5 (bs, 5H); ms *m/e*: 419. *Anal.* calcd. for $C_{19}H_{32}N_2O_4Cl\cdot 2H_2O$: C 50.48, H 8.02, N 6.21; found: C 50.48, H 8.22, N 6.84.

Acknowledgments

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