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SYNTHESIS OF 3-[^{125}I]-IODO-PHENCYCLIDINE FOR BIOLOGICAL STUDIES

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SUMMARY

After verification of the biological activity of 3-iodo-PCP by binding studies to the N-methyl D-aspartate gated channel, 3-[^{125}I]iodo-PCP was prepared by reaction of sodium iodide with a triazene precursor. The radiochemical yield was optimized and after HPLC purification, 3-[^{125}I]iodo-PCP was obtained with a high radiochemical purity.

Key words: *Sodium iodide, Iodine-125, 3-iodo-PCP or N-[1-(3-iodophenyl)cyclohexyl]piperidine, PCP/NMDA receptors*

INTRODUCTION

Several labelled compounds have been synthesized in order to study phencyclidine (PCP) receptors (1,2,3). Recently, labelling with fluorine-18 has been used for in vivo studies (4). These compounds are necessary tools for testing and estimating a large number of PCP analogs (5,6,7). Moreover, in vitro experiments showed us that the group on the meta position of the phenyl ring was very important and induced affinity for different receptors (8). It appeared very interesting to study the influence of an iodine atom in the meta position on the activity of PCP on its receptors. In the case of affinity of this iodinated compound, it would be interesting to obtain ^{125}I-labelled PCP to study the distribution of PCP receptors in rat brain by autoradiography.

CHEMISTRY

The iodinated compound **3** was synthesized in two steps from the 3-amino derivative **1** (9) via the stable triazene **2** (scheme 1) (10,11). The

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latter was obtained in good yield (72%) by subsequent reaction of 3-amino PCP with sodium nitrite and pyrrolidine and purified by HPLC. The displacement of the triazene group by iodine led to the crude iodinated PCP 3 in high yield (80%). The final yield after purification by HPLC was rather low (10.8%) which may be due to the absorption of 3 on the phase of the column. The purification by HPLC had to be performed very carefully to eliminate the impurities having a biological activity and a retention time similar to that of 3-iodo-PCP 3, i.e. N[1-phenylcyclohexyl]piperidine (10%) 6 and N[1-(3-hydroxyphenyl) cyclohexyl]piperidine (10%) 5. In some experiments, the displacement reaction of triazene 2 stopped before completion, probably due to the heterogeneity of the reaction mixture. In such cases, it was necessary to evaporate the mixture under vacuum and to add again the reagents.
The synthesis of the radioactive iodinated derivative 4 was performed with slight modifications and a good radioactive yield (about 10%) after purification by HPLC was obtained. The yield of the radioiodination was studied in correlation with the quantity of triazene 2 (TABLE I). The best result (about 10%) was obtained with 39.2 equivalents of 2. As for the cold iodinated compound 3, major part of the radioactive product 4 was lost by adsorption onto the HPLC column.

TABLE I : Yields of 3-[^{125}I]iodo-PCP 4
(for each experiment, 1.04 µmol of trifluoroacetic acid was used)

Assay		1	2	3	4	5	6
PCP	nmol	1.84	4.77	9.33	19.08	27.76	37.31
Triazene 1	eq	2.8	5.2	7.6	18.17	28	39.2
[^{125}I]-NaI	mCi	1.32	1.82	2.46	2.1	1.98	1.9
	nmol	0.66	0.91	1.23	1.05	0.99	0.95
[^{125}I]-3-iodo-PCP 4	yield %	3.5	2.5	5	8.5	7	9.4

The cold material 3 was characterized by ^{1}H-NMR, UV and mass spectrometry. Due to its high specific radioactivity, the labelled compound 4 was only characterized by comparison with 3 by HPLC. Affinity of the unlabelled compound 3 for the NMDA gated ion channel (PCP receptor) has been measured by in vitro competitive binding assays performed on rat brain membranes as previously described (1,2), using [^{3}H]TCP as ligand. Affinity of 3-iodo-PCP is as high as that of PCP but lower than that of 3-hydroxy-PCP or TCP (TABLE II). Saturation experiments of the labelled compound 4 gave the same affinity as compound 3 for all radioactive batches tested. Detailed studies will be published later (12). They confirm that 3-[^{125}I]iodo-PCP may be used as a probe for the study of PCP/NMDA receptors. Thus, 3-[^{125}I]-3-iodo-PCP 4 was easily prepared in approximately 10% radiochemical yield.

TABLE II : IC_{50} values of PCP and some derivatives determined by the inhibition of 1 nM [^{3}H] TCP binding performed with rat brain homogenates in 5 mM Hepes-Tris buffer (pH 7.6) for 30 minutes at 25°C.

Ligand		IC_{50}(nM)	nH
PCP	**1**	37	0.92
3-iodo PCP	**3**, **4**	25	0.74
3-hydroxy PCP	**5**	6.3	1.01
TCP		9.3	0.96

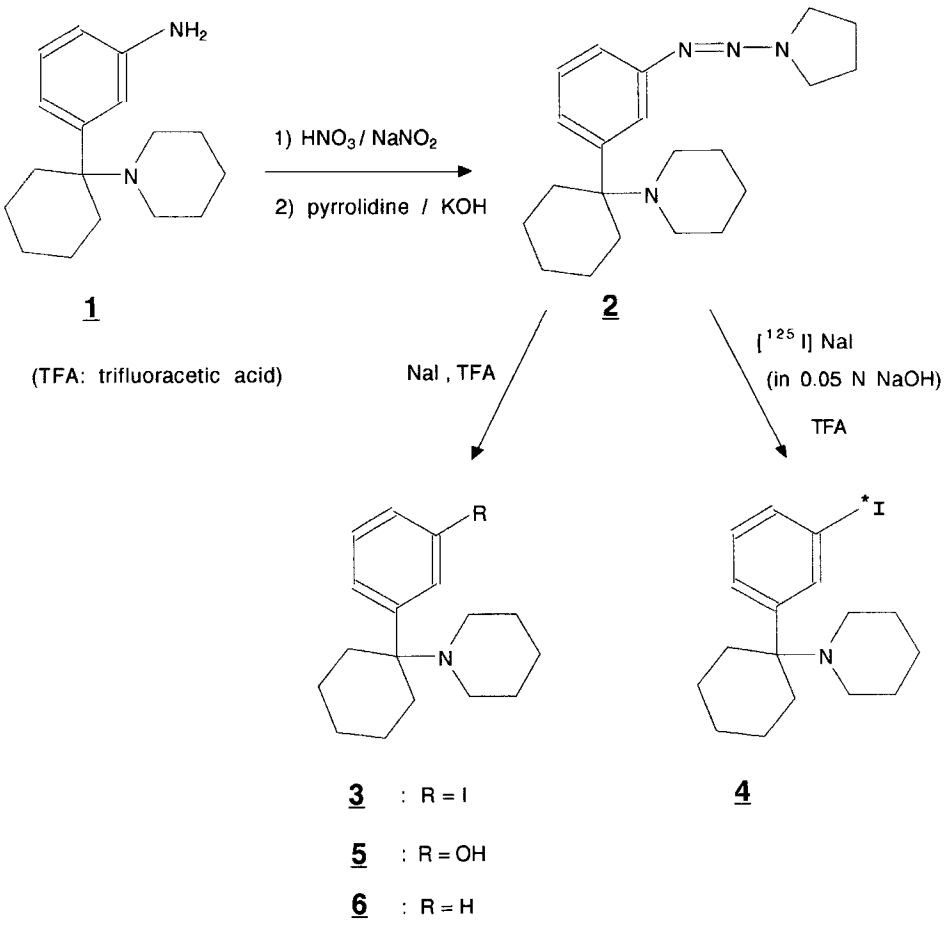
3-hydroxy-PCP : N[1-(3-hydroxyphenyl) cyclohexyl] piperidine
TCP : N[1-(2-thienyl) cyclohexyl] piperidine

Its biological activity has shown that it is a high affinity new tool for the study of potent PCP receptors and for their easy localization in rat brain by autoradiography.

EXPERIMENTAL

All commercial chemicals and solvents were of reagent grade and used without purification. 3-Amino-PCP was a gift from Dr KAMENKA J.M., (Ecole Nationale Supérieure de Chimie de Montpellier). Sodium [^{125}I] iodide (carrier free, in aqueous 0.05 N sodium hydroxide) was purchased from International C.I.S. In vitro competitive binding assays were performed in the laboratory of Pr CHICHEPORTICHE R. in Montpellier (FRANCE). The chemical reactions were monitored either by TLC (Whatman KC 18 Thus, [^{125}I]-3-iodo-PCP **4** was easily prepared in approximately 10% radiochemical yield. Thus, [^{125}I]-3-iodo-PCP **4** was easily prepared in approximately 10% radiochemical yield. plates) or by HPLC on a reversed phase column (C18-ODS Prolabo, 30 cm, 4.6 mm O.D). Purifications were performed on the same type of column but with 25 mm O.D. Peaks were detected by U.V at 235 nm (Water-associates Lamda Max 481). The flow rate of the mobile phase (90% : methanol, 10% : water, 0,05% : triethylamine) was 1.35 ml/mn for analytical determinations and 14 ml/mn for preparative chromatography. Mass spectra were obtained on a quadripole Finnigan Mat 4600, I.R spectra on a Beckman 4250 and U.V spectra on KONTRON (UVIKON 860 type). Radioactive detection was performed on a Berthold detector(sodium iodide crystal with LB 2040 electronic). Mass spectrometry, I.R and U.V analyses were in agreement with the assigned structures.

SCHEME I: Synthetic pathway of 3-[^{125}I] iodo-PCP



N[1-(3-(N-pyrrolidinoazo)phenyl)cyclohexyl]piperidine : PCP-triazene 2

0.32 mmol (83.5 mg) of 3-amino PCP 1 and 0.68 mmol (57 µl) of 12 N HCl were stirred at 0±5 °C. A cold sodium nitrite solution (0.33 mmol, 22.5 mg in 1.2 ml water) was added and the mixture stirred for 5 min. After addition of 0.53 mmol pyrrolidine (37.8 mg) in 5 ml aqueous potassium hydroxide (4.4 mmol), a precipitate was formed, filtered off on a Millex 5 µm filter, washed with water and purified by HPLC to give 77.7 mg (72%) of a yellow stable oil corresponding to the pure triazene derivative 2.

HPLC analysis: retention time 27 min(1.35 ml/mn)
MS(EI): m/e(%) = 341.8 (55.5%)
I.R (KBr) 1340 mµ (triazene)
U.V (MeOH): λ max 1 = 289 nm (ε = 12054)
λ max 2 = 318 nm (ε = 11717)
λ min 1 = 247 nm (ε = 2523)
λ min 2 = 306 nm (ε = 11212).

N[1-(3-iodophenyl)cyclohexyl]piperidine : 3-iodo-PCP **3**

A cold solution of 0.19 mmol sodium iodide (30 mg) in 514 µl water was added dropwise to a mixture of 0.19 mmol (66 mg) of triazene **2** in a cold mixture of 0.5 ml of methanol and 3 ml of water. After acidification with 0.4 mmol of trifluoroacetic acid (46.2 mg) in 31 ml of water, the solution was stirred for 20 hours at room temperature and concentrated under vacuum. HPLC purification gave 7.6 mg (10.8 %) of a yellow oil corresponding to the iodinated derivative **3**, isolated as the free base.

HPLC analysis : retention time : 15.6 min (1.35 ml/min)
MS(EI):m/e(%) = 369.4 (19%)
U.V.(MeOH): λ max 1 = 207.5 nm (ε = 15313)
λ max 2 = 227 nm (ε = 12425)
λ min 1 = 218 nm (ε = 10746).

N[1-(3-[^{125}I]iodophenyl)cyclohexyl]piperidine :
3-[^{125}I] iodo-PCP **4**

All batches of radioactive material **4** were obtained using the same experimental conditions (see TABLE I). A mixture of 37.31 nmol of triazene (12.7 µg) in 43 µl methanol solution, 0.95 nmol [^{125}I] sodium iodide (1900 µCi) and 1.04 µmol of trifluoroacetic acid (119 µg) in 8 µl water was stirred for one hour at room temperature in a "reacti-vial". Evaporation of the reaction mixture to dryness was carried out under a stream of nitrogen.. After HPLC purification and counting, 180 µCi (9.4% radiochemical yield) of pure 3-[^{125}I] iodo-PCP **4** were obtained. The radiochemical purity (99.5%) was checked by reverse phase HPLC : Zorbax C8 column, 30 cm, 4.6 mm O.D (80% : methanol, 20% : water, 0.05% triethylamine) and **4** was identified by co-elution with the cold compound.

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