Chemical Synthesis and Molecular Pharmacology of Hydroxylated 1-(1-Phenylcyclohexyl)piperidine Derivatives


Laboratoire de Chimie Organique Physique Appliquée, Ecole Nationale Supérieure de Chimie, 34075 Montpellier, and Centre de Biochimie du CNRS, Faculté des Sciences, Parc Valrose, 69034 Nice Cedex, France. Received July 28, 1981

The following monohydroxy derivatives of 1-(1-phenylcyclohexyl)piperidine (phenycyclidine, PCP) were synthesized: α-, m-, and p-phenols of PCP, 1-(1-phenylcyclohexyl)-4-piperidinol, and two stereoisomeric pairs of 3-phenyl-3-[(1-piperidinyl)cyclohexanol and 4-phenyl-4-(1-piperidinyl)cyclohexanol. Inhibition of specific binding of tritiated PCP, morphine, or quinuclidinyl benzilate (QNB) in rat brain homogenates was measured for these compounds. Inhibition of PCP binding for selected compounds correlated with mouse rotorod assay activity. The most characteristic effects of hydroxylation of PCP on the cyclohexyl, piperidine, or phenyl moieties are the following: (i) it generally decreases its activity in inhibiting [3H]PCP binding by a factor of 10 to 80; (ii) it does not produce a large variation in the affinity for the morphine receptor; (iii) it produces a considerable decrease of the affinity for the muscarinic receptor. An important exception to these general observations was the metapenthelic derivative of PCP. This PCP derivative has an affinity for the [3H]PCP binding sites that is 8 times higher than that of PCP itself; its affinity for the muscarinic receptor is only twice lower than that of PCP, but its affinity for the morphine receptor is 450 times higher than that of PCP and only one order of magnitude lower than that of morphine itself.

1-(1-Phenylcyclohexyl)piperidine (phenycyclidine), commonly known as PCP, is currently a major drug of abuse in the United States. In recent years, it has received widespread attention because of the violent, homicidal, and suicidal behavior of its users. Moreover, phenycyclidine is one of the most fascinating psychotropic drugs because the psychosis it elicits may provide the best available drug model of schizophrenia.  

Phenycyclidine has been shown to bind to both muscarinic and opiate receptors and to block the nicotinic channel coupled to the nicotinic receptor. However, probably the most powerful approach in characterizing PCP action stems from recent reports that have identified specific binding sites in the brain using tritiated phenycyclidine.

This paper describes the synthesis of five new monohydroxy derivatives of PCP: the α-, m-, and p-phenols of PCP and the stereoisomeric pair of 3-phenyl-3-(1-piperidinyl)cyclohexanol. Moreover, two other compounds, the stereoisomeric pair of 4-phenyl-4-(1-piperidinyl)cyclohexanol, were synthesized by a route different from that recently published while the present paper was being reviewed. All these derivatives and 1-(1-phenylcyclohexyl)-4-piperidinol were assayed for their binding properties to the [3H]PCP binding sites, to the opiate receptor sites, and to the muscarinic receptor sites in rat brain membranes. This series of hydroxylated compounds includes molecules like 1-(1-phenylcyclohexyl)-4-piperidinol and 4-phenyl-4-(1-piperidinyl)cyclohexanol which have already been identified as metabolites of PCP.

Results

Chemical Synthesis. The different molecules that have been synthesized are presented in Table I.

<table>
<thead>
<tr>
<th>compd</th>
<th>substituent</th>
<th>Ph</th>
<th>cyclohex</th>
<th>piperidine</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>OH</td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>OH</td>
<td>o</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>OH_e</td>
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<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>6</td>
<td>OH_e</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

α eq = equatorial; ax = axial.

Scheme I

the selective hydrogenation of 1,4-cyclohexanedione (Scheme I). The stereoisomeric pairs were prepared and

has been described. This method is applicable to stereoisomeric pair of lesions of two protons. Two AB systems of NMR of Hydrochlorides 

\[
\begin{array}{cccc}
\text{no.} & \text{Ph} & \text{He}_{\alpha,\beta}(2,4)^a & H_1(H_2) \\
4a & 7.33 & 2.48^a & 3.78 (J_{\text{ax}} = 8; J_{\text{eq}} = 4) \\
4b & 7.33 & 2.54^c & 3.75 (J_{\text{ax}} = 13) \\
5a & 7.38 & 2.95^d (J_{\text{ax}} = 13), 2.77^d (J_{\text{eq}} = 13) & 4.20 (J_{\text{ax}} = 11) \\
5b & 7.40 & 2.86^d (J_{\text{ax}} = 13), 2.65^d (J_{\text{eq}} = 13) & 4.20 (J_{\text{ax}} = 10^e J_{\text{eq}} = 4^e) \\
\end{array}
\]

a Signal partially masked by the piperdine. b Overlapping signals; the coupling constant cannot be determined. c Coalescence of two protons. d Two AB systems of 1 proton each; the downfield signal is attributed to H. e Not obtained in CW. Low-field part of the AB system given by equatorial and axial protons \(a\) to the quaternary carbon. f In the hypothesis of a predominant equatorial OH conformation in base.

Table III. Experimental and Calculated Spectra in 13C NMR of Hydrochlorides

<table>
<thead>
<tr>
<th>no.</th>
<th>(C_6(2))</th>
<th>(C_6(1))</th>
<th>(C_6(5))</th>
<th>(C_6(4))</th>
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</thead>
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<td>31.3</td>
<td>67.4</td>
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<td></td>
<td>exptl</td>
<td>31.3</td>
<td>31.3</td>
<td>68.6</td>
</tr>
<tr>
<td>4b</td>
<td>calc}^a</td>
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<td>29.2</td>
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<td>exptl</td>
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<td>33.2</td>
</tr>
<tr>
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<td>66.1</td>
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<td></td>
<td>exptl</td>
<td>36.2</td>
<td>66.6</td>
<td>31.9</td>
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</tbody>
</table>

a Calculated values supposing another conformation are not coherent with experimental results.

separated according to a general method that previously has been described. This method is applicable to various cyclohexyl substitutions. The resolution of the stereoisomeric pair of 4a and 4b was recently achieved by a different method.

Piperidinol Derivative 6. This PCP derivative was obtained via the classical Bruylants synthetic pathway using a Grignard reaction on the adequate \(\alpha\)-aminonitrile. Starting from the 4-piperidinol, we obtained the \(\alpha\)-aminonitrile by a modified Strecker reaction in organic medium. Compound 6 is identical with the one previously obtained from a very similar synthesis.

Structural Identification of 4a,b and 5a,b. The synthesis of 1–3 and 6 is unequivocal, and their NMR spectra are consistent with those previously described.

The identification of the cyclohexyl-hydroxylated stereoisomers has been made by \(^1\text{H}\) and \(^{13}\text{C}\) NMR (Tables II and II).

The axial phenyl conformations of phenylcyclidine are favored in both the base and the salt form \((-\Delta G^0 = 1.0 \text{ kcal/mole})\). Therefore, such conformations should be even more favored for 4a because of the additional stabilization due to the equatorial OH \((-\Delta G^0_{\text{OH}} = 0.5–0.9 \text{ kcal/mole})\). The observed signals (no coalescence) and coupling constants are consistent with those major conformations. The conformation as described for 5b would also be consistent with the results obtained with its methylated homologues.

\(^{13}\text{C}\) NMR of hydrochlorides presented in Table III gives more structural information. Theoretical calculations of the chemical shifts were made according to Beierbeck and Saunders with phenylcyclidine hydrochloride as reference for the axial phenyl structure. Experimental and calculated spectra are very similar; they indicate that most of the hydrochlorides have an axial phenyl group (as usual) with a mostly axial OH for 4b and 5b (Table

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Hydroxylated Phencyclidine Derivatives

Table IV. Competition between Phencyclidine Derivatives and [3H]Phencyclidine, [3H]QNB, and [3H]Morphine on the Phencyclidine, Muscarinic, and Opiate Receptors

<table>
<thead>
<tr>
<th>Phencyclidines</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; mg/kg</th>
<th>Phencyclidine receptor</th>
<th>Muscarinic receptor</th>
<th>Opiate receptor</th>
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<tbody>
<tr>
<td>PCP</td>
<td>4</td>
<td>0.25</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>20</td>
<td>1.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>0.03</td>
<td>0.9</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.75</td>
<td>&gt;100</td>
<td>1</td>
</tr>
<tr>
<td>4a</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4b</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>5a</td>
<td>49.9</td>
<td>2.2</td>
<td>&gt;100</td>
<td>11.5</td>
</tr>
<tr>
<td>5b</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.9</td>
<td>&gt;100</td>
<td>27.7</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>2.2</td>
<td>&gt;100</td>
<td>25</td>
</tr>
</tbody>
</table>

<sup>a</sup> <sub>K<sub>d</sub></sub> is the concentration of unlabeled ligand that induces 50% dissociation of the tritiated ligand. <sup>b</sup> <sub>nH</sub> is the Hill coefficient. Values of <sub>K<sub>d</sub></sub> and <sub>nH</sub> are computed values obtained as described under Experimental Section. <sup>c</sup> Derivative not tested.

Figure 1. Competition for binding to the phencyclidine receptor between [3H]phencyclidine and PCP (1), 2 (2), 4a (3), and 5a (6). [3H]Phencyclidine (1 nM, 48 Ci/mmol) was incubated for 10 min at 25 °C with rat brain homogenate (1 mg of protein/mL) in 5 mL of a 50 mM Tris-HCl buffer, pH 7.7, with the indicated concentration of phencyclidine. Bound [3H]phencyclidine was separated from free [3H]phencyclidine by filtration on a GF/B glass-fiber filter (Whatman). The radioactivity retained on the filter was measured by liquid scintillation spectrometry (Packard Tri-Carb 2400) in 8 mL of Biofluor (New England Nuclear) at a counting efficiency of 45-50%. The radioactivity that is specifically bound to the rat brain homogenate was measured as previously described. Results are expressed as a percent of the maximal specific binding in the absence of unlabeled phencyclidine. Data was fitted to the Hill equation with a Wang 2200 calculator according to Atkins. Inset: Correlation between the inhibition of [3H]phencyclidine binding and the activity in the rotarod test for phencyclidine and its derivatives. The equation of the straight line in the figure (γ = -0.496x +4.157) was obtained by the least-squares method. The correlation coefficient and the statistical significance are r = 0.848 and p < 0.01, respectively. Phencyclidine derivatives are designated by their abbreviation as given in Table I.

I. NMR results for compounds 4a,b are consistent with those found by others. Biological Activity of the Hydroxylated Derivatives of PCP. Three types of competitive binding assays were used to measure the relative potencies of the monohydroxy derivatives of phencyclidine: (i) inhibition of the specific binding of [3H]quinuclidinyl benzylate (QNB, a labeled muscarinic antagonist) to the muscarinic receptor, (ii) inhibition of the specific binding of [3H]morphine, and (iii) inhibition of the specific binding of [3H]phencyclidine.

Figure 2. Inhibition of the specific [3H]QNB binding to the muscarinic cholinergic receptor of rat brain by atropine (A), PCP (C), 2 (Q), and 4b (O). [3H]QNB (0.5 nM, 5 Ci/mmol) was incubated for 60 min at 25 °C with rat brain homogenate (0.27 mg of protein/mL) in 2 mL of a 50 mM phosphate buffer at pH 7.4 in the presence of indicated concentrations of atropine or phencyclidines. Bound radioactivity was separated from free radioactivity by filtration on GF/B filters as previously described. Specifically bound radioactivity is defined as the difference between the radioactivity bound in the absence of atropine and the radioactivity bound in the presence of 10 μM atropine.

Discussion

Hydroxylation of the phenyl ring of PCP in the ortho and para positions decreases binding activity to the site identified using \(^{3}H\)PCP by a factor of 3 to 80. Hydroxylation in the meta position increases activity, the significance of which will be discussed later. The order of affinity for the phenolic derivatives relative to PCP is \(2 < PCP < 8 < 1\), where relative potencies differ by a factor of 660 between the most and the least active derivatives.

The previously prepared methoxy derivatives\(^\text{23}\) have isomers that are more active than those obtained by hydroxylation at C\(_1\) (Table IV). At positions C\(_{11}\) and C\(_{1}\), axial hydroxy compounds are not very significantly more active than equatorial ones, since the difference in affinity observed between axial and equatorial compounds is only 2- to 2.5-fold.

Incorporation of a hydroxyl group in the piperidine moiety of PCP decreases the affinity for the brain binding sites identified with \(^{3}H\)PCP by a factor of about 10. The affinity of this derivative, \(6\), is nearly identical with that of one of the molecules hydroxylated on the cyclohexyl ring, \(5a\). The ED\(_{50}\) of these two compounds measured with the rotarod assay are also very similar.

A characteristic feature of the hydroxylation of PCP is that it considerably decreases the affinity of the molecule for the muscarinic receptor (Table IV).

For all but two exceptions, \(2\) and \(4a\), hydroxylation of PCP does not produce dramatic variations in its affinity for the opiate receptor as measured by competition with \(^{3}H\)morphine. \(K_{D}\) values found for hydroxylated PCP derivatives are all in the range between 10 and 35 \(\mu M\) (Table IV). Hydroxylation on the meta position of the phenyl ring increases the affinity of PCP for the opiate receptor by a factor of 400, whereas equatorial hydroxylation on the cyclohexyl ring in \(4a\) decreases the affinity by a factor of about 4. A change of the hydroxyl group at this position of the cyclohexyl ring from the equatorial to the axial position increases the affinity by a factor of about 5 relative to the equatorial isomer.

Differences between equatorial and axial positions are much less important when hydroxylation of the cyclohexyl ring is as in \(5a\) and \(5b\) (Table IV). In this case, the equatorial compound is only slightly more active than the axial one.

Of special interest is \(2\) in which the hydroxyl group is in the meta position of the phenyl ring. This compound exhibits low activity in the muscarinic binding assay, but it is 10 times as active as PCP in the \(^{3}H\)PCP binding assay and is about 430 times as active as PCP in the opiate receptor binding assay. Other derivatives of PCP (non-hydroxylated) have been reported which are more active than PCP itself in inhibiting \(^{3}H\)PCP binding; however, none were found to bind with a high affinity to the opiate receptor. The derivative 1-[2-(thienyl)cyclohexyl]-piperidine, for example, has a \(K_{D}\) of 0.026 \(\mu M\) for the PCP receptor\(^\text{2}\) but a \(K_{D}\) of only 11 \(\mu M\) for the opiate receptor.\(^\text{3}\)

The hydroxylated derivatives that are known metabolites of PCP (\(4a,b\) and \(6\))\(^\text{11,12}\) associate to the \(^{3}H\)PCP binding sites with much less activity than PCP itself. They are not very different from PCP in their binding properties to the opiate receptor and they present no significant affinity for the muscarinic receptor. Although the existence of hydroxylated metabolites on the phenyl ring of PCP has been suggested,\(^\text{32}\) it has not yet been clearly demonstrated, and it is not known whether the most interesting compound in the series we have studied, \(2\), is formed by metabolism of PCP.

Experimental Section

Chemical Synthesis. Melting points were measured in capillary tubes and are uncorrected. Analytical results from the Centre CNRS of ENSC Montpellier were within ±0.4% of the theoretical values. \(^1H\) NMR spectra were recorded on a Varian EM 360; \(^13C\) NMR spectra were recorded on a Bruker HX 90, equipped with a Nicolet calculator working in the PPT mode (Sherbrooke University, Canada). In both cases, the solvent was CDCl\(_3\), and Me\(_4\)Si was the internal reference. IR spectra were recorded on a Perkin-Elmer 197 in CHCl\(_3\).

Phenols 1-5. The previously prepared methoxy derivatives\(^\text{23}\) were demethylated by BB\(_3\) according to a described method.\(^\text{33}\)
A Comparison of the Inhibitory Action of 5-(Substituted-benzyl)-2,4-diaminopyrimidines on Dihydrofolate Reductase from Chicken Liver with That from Bovine Liver

Ren-li Li,1 Corwin Hansch,*1 and Bernard T. Kaufman1

Department of Chemistry, Pomona College, Claremont, California 91711, and National Institute of Arthritis, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20214. Received September 14, 1981

Forty-four 5-(substituted-benzyl)-2,4-diaminopyrimidines have been tested as inhibitors of chicken and bovine liver dihydrofolate reductase. The chicken enzyme is, on the average, about 10 times less easily inhibited than bovine enzyme. Substituents which show the greatest selectivity are 4-NHCOCH3, 3-OCH3, 3-OC4H9, 3-1, 3-CF3-4-OCH3, and 3,4,5-(OCHJ3. Compounds 5a and 5b were prepared as described for the 4-hydroxy derivatives: 2.1 g of the crude isomeric mixture was obtained from 8.3 g of 1-phenyl-3-hydroxycyclohexanol. Column chromatography on silica gel gave 1 g of 5b, eluted with 30% of petroleum ether in ether, and 0.7 g of 5a, eluted with 1% MeOH in ether. 5a: base, oily; HCl salt, hygroscopic; 5b: mp (base) 117–121 °C; mp (HCl) 175 °C dec. Anal. (C17H17NO) C, H, N.

1-(1-Phenylcyclohexyl)-4-piperidinol (6). The o-amino-nitrite was prepared in an organic medium according to a published method31 from cyclohexanone, 4-piperidinol, and KCN with a yield of 77% from crude material. The Brunel reaction was performed as usual12,20 on 2.1 g of the o-amino-nitrite crystallized in petroleum ether and gave 2.2 g of crude material. After a chromatography on aluminum oxide in pure ether, we obtained 1.5 g (58%) yield of 6: mp (base) 116–117 °C (lit.12,23 116–118 °C); mp (HCl) 223–224 °C. Anal. (C17H16NO) C, H, N.

Binding Assays. Brain tissue preparation and binding experiments were carried out as described by Vincent et al.13 ([3H]phencyclidine binding), Yamamura and Snyder13 (muscarinic cholinergic receptor), and Pert and Snyder20 (opiate receptor). Radioactively labeled compounds were obtained as follows: [3H]phencyclidine (48Ci/mol) from New England Nuclear; [3H]hydromazindine benzylate (QNBCi/mol) and [3H]morphine (30Ci/mol) from Amersham. Dissociation constants (Kd) and Hill coefficients (nH) were computed using a Wang 2200 calculator as previously described.

Rotarod Test. This test, involving the ability of mice to remain on a rotating rod, was carried out as previously described.

Acknowledgment. The authors are grateful to Dr. G. Trouriller for the determination of the ED50 of different phencyclidine derivatives in the rotarod test, to Ellen Van Oeverghen-Schilling for a very careful reading of the manuscript, and to Martine Valetti for skilful technical assistance. This work was supported by the Institut National de la Santé et de la Recherche Medicale (Grant A.T.P. no. 58.78.90) and the Centre National de la Recherche Scientifique (A.T.P. "Pharmacologie des Récepteurs des Neuromédiateurs") and D.E.R.T. (80/011 and 81/1166).

One approach to the development of new drugs, when the biochemistry is known, is to find inhibitors that are selective for a crucial enzyme from a pathogen that is relatively nontoxic to the enzyme from the host. When the enzymes can be readily obtained, this allows one to establish an intrinsic therapeutic index before one commences the study of the inhibitors under extremely complex conditions in animals. An outstanding success story based on such a concept is the antibacterial trimethoprim [I, X = 3,4,5-(OCH3)3] developed by Roth et al.12,13 of the...