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

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The following monohydroxy derivatives of 1-(1-phenylcyclohexyl)piperidine (phencyclidine, PCP) were synthesized: *o*-, *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 benzylate (QNB) in rat brain homogenates was measured for these compounds. Inhibition of PCP binding for selected compounds correlated with mouse rotarod 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 metaphenolic 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 430 times higher than that of PCP and only one order of magnitude lower than that of morphine itself.

1-(1-Phenylcyclohexyl)piperidine (phencyclidine), 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, phencyclidine is one of the most fascinating psychotropic drugs because the psychosis it elicits may provide the best available drug model of schizophrenia.[1,2]

Phencyclidine has been shown to bind to both muscarinic and opiate receptors[3–6] and to block the ionic channel coupled to the nicotinic receptor.[7] 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 phencyclidine.[8–10]

This paper describes the synthesis of five new monohydroxy derivatives of PCP: the *o*-, *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 synthesised by a route different from that recently published[11] 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[12] and 4-phenyl-4-(1-piperidinyl)cyclohexanol[11,12] which have already been identified as metabolites of PCP.

## Results

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

**Phenolic Compounds 1–3.** It was not possible to synthesize these three compounds from the amino derivatives obtained by hydrogenation of the nitro homologues as previously described by Kalir et al.[13] because of the almost exclusive metanitration of PCP.[14] The compound previously identified as 1 by Kalir et al.[13] is probably compound 2. The only way to obtain the phenols with a resonably good yield was to dealkylate the corresponding methoxy compounds by BBr₃. Thus, compounds 1–3 were isolated as their hydrobromide salts.

**Cyclohexanol Derivatives 4a,b and 5a,b.** Starting materials were the 4-hydroxy- and 3-hydroxycyclohexanones resulting from the oxidation of the diols[15,16] or

**Table I. Compounds Studied and Their Preferred Conformation in Salt Form (HCl or HBr)**



| compd | substituent[a] | Ph | cyclohexyl | piperidine |
|---|---|---|---|---|
| phencyclidine | | | | |
| 1 | OH | p | | |
| 2 | OH | m | | |
| 3 | OH | o | | |
| 4a | OH$_{eq}$ | | 1 | |
| 4b | OH$_{ax}$ | | 1 | |
| 5a | OH$_{eq}$ | | (1) | |
| 5b | OH$_{ax}$ | | (1) | |
| 6 | OH$_{eq}$ | | | [(1)] |

[a] eq = equatorial; ax = axial.

**Scheme I**
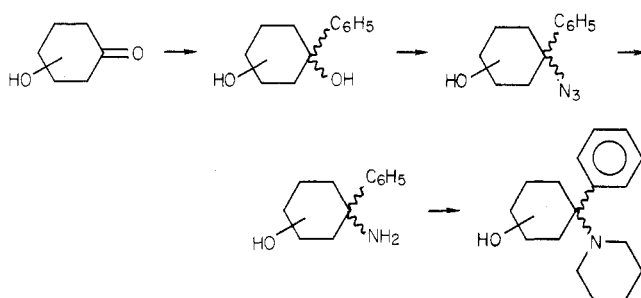


the selective hydrogenation of 1,4-cyclohexanedione[17] (Scheme I). The stereoisomeric pairs were prepared and

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Table II. Signals in $^1$H NMR at 60 MHz

| | $^1$H NMR (CDCl$_3$, Me$_4$Si), $\delta$ $(J, Hz)$ | | | | | |
|---|---|---|---|---|---|---|
| | bases | | | | hydrochlorides | |
| no. | Ph | He$_{3,5}$(2,4)$^f$ | H$_1$(H$_1$) | Ph | He$_{2,6}$(2,4) | H$_1$(H$_1$) |
| **4a** | 7.33 | 2.48$^a$ | 3.78 $(J_{aa} = 8; J_{ae} = 4)$ | 7.52 | 3.8$^b$ | 3.80$^b$ |
| **4b** | 7.33 | 2.54$^c$ | 3.75 $(\nu_{1/2} = 13)$ | 7.58 | 3.83$^b$ | 3.90$^b$ |
| **5a** | 7.38 | 2.98$^d$ $(J_1 = 13)$, 2.77$^d$ $(J_1 = 13)$ | 4.20 $(\nu_{1/2} = 11)$ | e | e | e |
| **5b** | 7.40 | 2.88$^d$ $(J_1 = 13)$, 2.65$^d$ $(J_1 = 13)$ | 4.20 $(J_{aa} = 10;^g J_{ae} = 4^g)$ | e | e | e |

$^a$ Signal partially masked by the piperidine. $^b$ 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$_2$. $^e$ Not obtained in CW. $^f$ Low-field part of the AB system given by equatorial and axial protons $\alpha$ to the quaternary carbon. $^g$ In the hypothesis of a predominant equatorial OH conformation in base.

Table III. Experimental and Calculated Spectra in $^{13}$C NMR of Hydrochlorides

| | | $^{13}$C NMR, $\delta$ | | | | |
|---|---|---|---|---|---|---|
| no. | | C$_3$(2) | C$_2$(1) | C$_1$(6) | C$_6$(5) | C$_5$(4) |
| **4a** | calcd$^a$ | 30.2 | 31.3 | 67.4 | 31.3 | 30.2 |
| | exptl | 29.1 | 31.3 | 68.6 | 31.3 | 29.1 |
| **4b** | calcd | 23.8 | 29.2 | 62.8 | 29.2 | 23.8 |
| | exptl | 24.8 | 29.5 | 63.2 | 29.5 | 24.8 |
| **5a** | calcd | 39.0 | 65.7 | 33.0 | 22.5 | 30.2 |
| | exptl | 39.2 | 66.8 | 33.2 | 22.3 | 30.5 |
| **5b** | calcd | 36.9 | 66.1 | 30.9 | 16.1 | 30.2 |
| | exptl | 36.2 | 66.6 | 31.9 | 17.3 | 30.1 |

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

separated according to a general method that previously has been described.[18,19] This method is applicable to various cyclohexyl substitutions.[20] The resolution of the stereoisomeric pair of **4a** and **4b** was recently achieved by a different method.[11]

**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 $\alpha$-amino-nitrile by a modified Strecker reaction in organic medium.[21] Compound 6 is identical with the one previously obtained from a very similar synthesis.[12,22]

**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.[23]

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

The axial phenyl conformations of phencyclidine are favored in both the base and the salt form ($-\Delta G° = 1$ and 4.4 kcal/mol, respectively[23]). Therefore, such conformations should be even more favored for **4a** because of the additional stabilization due to the equatorial OH ($-\Delta G°_{OH}$ = 0.5–0.9 kcal/mol,[24] ca. 93–96% of axial phenyl in the base form). Indeed, $^1$H NMR spectra of **4a** in the base and in the salt form do reflect the relative similarity of the major conformations (no change in the H$_1$ shifts, and $J_{aa}$ and $J_{ae}$ with nearly the same values found in conformationally homogeneous systems). Conversely, **4b** (base) should be more heterogeneous in conformation (30–45% of axial phenyl conformation) as shown by the signals coalescence (base) and the change in H$_1$ shift when protonation occurs. Such results are consistent with those obtained when OH is replaced by CH$_3$[20,23] ($-\Delta G°_{CH_3}$ = 1.7 kcal/mol).

A possible intramolecular H bond in **5a** (base) may displace the conformation toward an axial OH form (equatorial phenyl), while in **5b** (base) the 1–3 diaxial interactions generated by an axial OH may force the conformation toward the equatorial OH form (equatorial phenyl). The observed signals (no coalescence) and coupling constants are consistent with such major conformations. The conformation as described for **5b** would also be consistent with the results obtained with its methylated homologues.[20,23]

$^{13}$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[25,26] with phencyclidine 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[20,23,27,28]) with a mostly axial OH for **4b** and **5b** (Table

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

| phencyclidines | rotarod: $ED_{50}$, mg/kg | phencyclidine receptor | | muscarinic receptor | | opiate receptor | |
|---|---|---|---|---|---|---|---|
| | | $K_{0.5}$,$^a$ $\mu$M | $n_H$ $^b$ | $K_{0.5}$,$^a$ $\mu$M | $n_H$ $^b$ | $K_{0.5}$,$^a$ $\mu$M | $n_H$ $^b$ |
| PCP | 4 | 0.25 | 1.0 | 30 | 0.8 | 26 | 0.9 |
| 1 | 28 | 20 | 1.0 | >>100 | | 35.4 | 0.9 |
| 2 | 2.2 | 0.03 | 0.9 | 70 | 1 | 0.06 | 0.9 |
| 3 | 18 | 0.75 | 1.0 | >100 | | 17 | 0.8 |
| 4a | NT$^c$ | 17 | 1.0 | >>100 | | 100 | 1.1 |
| 4b | NT$^c$ | 8.3 | 1.1 | >>100 | | 17.5 | 1.0 |
| 5a | 49.9 | 2.2 | 1.0 | >>100 | | 11.5 | 0.8 |
| 5b | NT$^c$ | 0.9 | 0.9 | >>100 | | 27.7 | 1.0 |
| 6 | 42 | 2.2 | 1.2 | >>100 | | 25 | 0.9 |

$^a$ $K_{0.5}$ is the concentration of unlabeled ligand that induces 50% dissociation of the tritiated ligand.   $^b$ $n_H$ is the Hill coefficient.   Values of $K_{0.5}$ and $n_H$ are computed values obtained as described under Experimental Section.   $^c$ Derivative not tested.
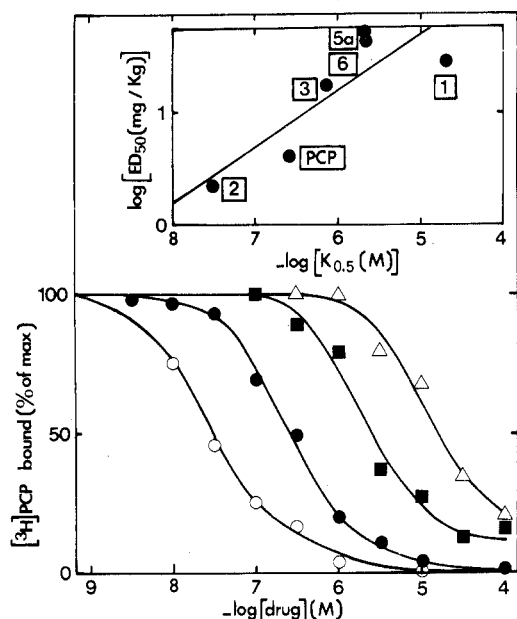
**Figure 1.** Competition for binding to the phencyclidine receptor between [³H]phencyclidine and PCP (●), 2 (○), 4a (△), and 5a (■). [³H]Phencyclidine (1 nM, 48 Ci/mmol) was incubated for 10 min at 25 °C with rat brain homogenate (1 mg of protein/mL) in 5 mL of a 50 mM Tris-HCl buffer, pH 7.7, with 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 2450) 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.[8,9] 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.[35] 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 ($y = -0.496x + 4.157$) 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.[11]

**Biological Activity of the Hydroxylated Derivatives of PCP.** Three types of competitive binding assays were
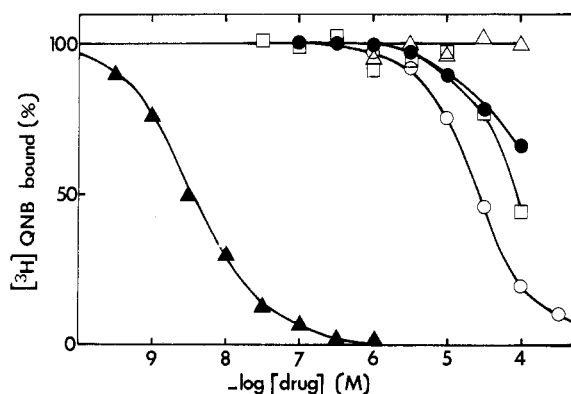


**Figure 2.** Inhibition of the specific [³H]QNB binding to the muscarinic cholinergic receptor of rat brain by atropine (▲), PCP (○), **6** (△), **2** (□), and **4b** (●). [³H]QNB (0.5 nM, 5 Ci/mmol) was incubated for 60 min at 25 °C with rat brain homogenate (0.27 mg of protein/mL) in 2 mL of a 50 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.[3,29] 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 $\mu$M atropine.

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,[3,29] (ii) inhibition of the specific binding of [³H]morphine,[3,30] and (iii) inhibition of the specific binding of [³H]phencyclidine.[8]

Figure 1 presents typical competition experiments between [³H]phencyclidine and PCP, 2, 4a, and 5a. The binding properties [dissociation constants ($K_{0.5}$) and Hill coefficients ($n_H$)] for different hydroxylated derivatives are presented in Table IV. There are large differences in binding affinities among all these derivatives, and one of them, **2**, has a binding activity which is nearly ten times higher than that of phencyclidine itself.

A satisfactory correlation is observed in this series of compounds between the binding activity ($K_{0.5}$) to the receptor structure that binds [³H]phencyclidine and the pharmacological parameter ($ED_{50}$) obtained from the rotarod assay (inset Figure 1). The correlation coefficient ($r$) is 0.848 ($p < 0.01$). It is probable that the correlation would also be satisfactory with another biological assay,

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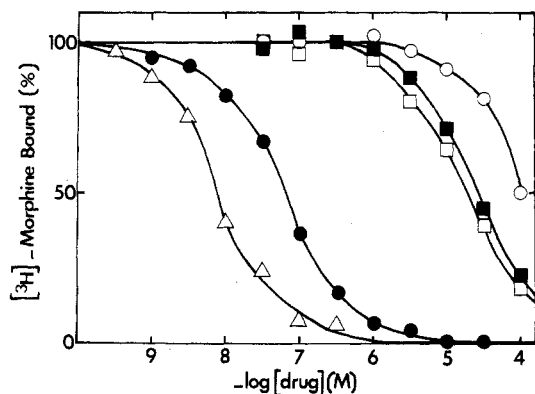(30) Pert, C. B.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2243.

**Figure 3.** Inhibition of the specific [3H]morphine binding to the opiate receptor of rat brain by morphine (△), 2 (●), 3 (□), 4a (○), and PCP (■). [3H]Morphine (1.2 nM, 30 Ci/mmol) was incubated for 2 h at 0 °C with rat brain homogenate (0.5 mg of protein/mL) in 2 mL of a 50 mM Tris-HCl buffer at pH 7.7 in the presence of the indicated concentrations of morphine or phencyclidines. Bound radioactivity was separated from free radioactivity by filtration on GF/B filters as previously described.[3,30] Specifically bound radioactivity is the difference between the radioactivity bound in the absence of unlabeled morphine and the radioactivity bound in the presence of 10 $\mu$M unlabeled morphine.

the rat discriminative stimulus test, which has been used by others to measure in vivo the pharmacological activity of PCP derivatives. A good correlation between binding activity to the [3H]PCP binding site and both the rotarod assay and the rat discriminative stimulus test has now been found in two different laboratories[10,31] for a series of nonhydroxylated derivatives of PCP.

Figures 2 and 3 present typical competition experiments between the hydoxylated derivatives of PCP and either [3H]QNB or [3H]morphine. Results for all the hydroxylated molecules that have been synthesized are summarized in Table IV.

It previously has been found that phencyclidine competes with [3H]QNB for the muscarinic receptor and that the corresponding dissociation constant is 30 $\mu$M.[3] All the hydoxylated derivatives used in this work are poor competitors of [3H]QNB (Table IV). The most potent compound was 2 with a $K_{0.5}$ = 70 $\mu$M.

All the hydroxylated compounds compete with [3H]morphine for binding to the opiate receptor. Their $K_{0.5}$ values ranged between 10 and 35 $\mu$M, except for 2 and 4a (Table IV). No statistically significant correlation was found between the binding activities of this series of compounds and the ED$_{50}$ parameters measured by the rotarod assay ($r$ = 0.67 and $p$ < 0.2).

## Discussion

Hydroxylation of the phenyl ring of PCP in the ortho and para positions decreases binding activity to the site identified using [3H]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 < 3 < 1, where relative potencies differ by a factor of 660 between the most and the least active derivatives.

Incorporation of a hydroxyl group on the cyclohexyl moiety of PCP also decreases its affinity for the receptor structure identified with [3H]PCP. Hydroxylation at C$_{(1)}$ gives isomers that are more active than those obtained by hydroxylation at C$_1$ (Table IV). At positions C$_{(1)}$ 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 an hydroxyl group in the piperidine moiety of PCP decreases the affinity for the brain binding sites indentified with [3H]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 [3H]morphine. $K_{0.5}$ 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 430, 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 [3H]PCP binding assay and is about 430 times as active as PCP in the opiate receptor binding assay. Other derivatives of PCP (nonhydroxylated) have been reported which are more active than PCP itself in inhibiting [3H]PCP binding,[8] however, none were found to bind with a high affinity to the opiate receptor. The derivative 1-[1-(2-thienyl)cyclohexyl]-piperidine, for example, has a $K_{0.5}$ of 0.026 $\mu$M for the PCP receptor[8] but a $K_{0.5}$ of only 11 $\mu$M for the opiate receptor.[3]

The hydroxylated derivatives that are known metabolites of PCP (4a,b and 6)[11,12] associate to the [3H]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,[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 metabolization of PCP.

## Experimental Section

**Phenols 1–3.** The previously prepared methoxy derivatives[23] were demethylated by BBr$_3$ according to a described method:[33]

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2.7 g (0.01 mol) of 1-(methoxyphenyl)cyclohexylpiperidine dissolved in 60 mL of $CH_2Cl_2$ was slowly added to an ice-cold solution of 3 mL of $BBr_3$ (0.03 mol) in 20 mL of $CH_2Cl_2$ while stirring continuously. Then the solution was stirred overnight at room temperature. With an efficient cooling, cold water was cautiously added until no more gas evolved (HBr) and a precipitate occurred. After 5 h in the cold (0–5 °C), the precipitate was collected and crystallized from alcohol to give the hydrobromide of the phenolic PCP derivative. In the case of 3, the hydrobromide is mostly concentrated in the organic layer where it precipitates slowly (48 h). 1: yield 1.7 g (50%); mp (HBr) 122–124 °C. Anal. ($C_{17}H_{26}NOBr$) C, H, N. 2: yield 1.5 g (45%); mp (HBr) 192–195 °C. Anal. ($C_{17}H_{26}NOBr$) C, H, N. 3: yield 1.9 g (55%); mp (HBr) = 154–157 °C. Anal. ($C_{17}H_{26}NOBr$) C, H, N.

**4-Phenyl-4-(1-piperidinyl)cyclohexanols 4a,b.** Phenylmagnesium bromide was added to 4-hydroxycyclohexanone to obtain 5.1 g of 1-phenyl-4-hydroxycyclohexanol. The substitution of the tertiary OH by $N_3^-$ was made using an already published procedure[18,19] with slight modifications: 5.1 g (0.027 mol) of the diol was added at 0 °C to a vigorously stirred suspension of 3.51 g (0.054 mol) of $NaN_3$ and 4.59 (0.027 mol) of $CCl_3COOH$ in 30 mL of $CHCl_3$. After a 5-h reaction, neutralization with $NH_4OH$, and extraction with $CHCl_3$, the organic layer was dried on $Na_2CO_3$. After evaporation in vacuo, the residue containing the two isomeric azides weighed 5.48 g. These crude azides were refluxed in ether overnight with 1.5 g of $LiAlH_4$. $CHCl_3$ (20 mL) was added after decomposition by $H_2O$, and the complex was filtered. The chloroform filtrate was extracted with 10% HCl; then the acidic solution, after neutralization with $NH_4OH$, extraction by $CHCl_3$, drying ($Na_2SO_4$), and evaporation in vacuo, gave a residue of primary amines (2.3 g). The crude amines in a solution of 1 equiv of 1,5-dibromopentane in 20 mL of anhydrous acetone were refluxed for 48 h. Then, 1 equiv of $K_2CO_3$ was added, and the reflux was maintained for an additional 48 h. After filtration, evaporation, dissolution in 10% HCl, and ether extraction, the resulting aqueous phase was neutralized ($NH_4OH$) and extracted ($CHCl_3$). Drying and evaporation of the solvent gave a crude residue in a yield of 2.2 g. Column chromatography on silica gel gave 0.8 g of **4b**, eluted with 15% MeOH in ether, and 1.1 g of **4a**, eluted with 20% MeOH in ether. **4a**: mp (base) 152–153 °C; mp (HCl) 190 °C dec (lit.[11] 200–201 °C). **4b**: mp (base) 170–171 °C; mp (HCl) 205 °C dec (lit.[11] 201–202 °C). Anal. ($C_{17}H_{26}NOCl$) C, H, N.

**3-Phenyl-3-(1-piperidinyl)cyclohexanols 5a,b.** 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. ($C_{17}H_{25}NO$) C, H, N.

**1-(1-Phenylcyclohexyl)-4-piperidinol (6).** The α-aminonitrile was prepared in an organic medium according to a published method[21] from cyclohexanone, 4-piperidinol, and KCN with a yield of 77% from crude material. The Bruylants reaction was performed as usual[12,22] on 2.1 g of the α-aminonitrile 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.[12,19,21] 116–118 °C); mp (HCl) 223–224 °C. Anal. ($C_{17}H_{26}NOCl$) C, H, N.

**Binding Assays.** Brain tissue preparation and binding experiments were carried out as described by Vincent et al.[8,9] ([$^3H$]phencyclidine binding), Yamamura and Snyder[29] (muscarinic cholinergic receptor), and Pert and Snyder[30] (opiate receptor).

Radioactively labeled compounds were obtained as follows: [$^3H$]phencyclidine (48 Ci/mmol) from New England Nuclear; [$^3H$]quinuclidinyl benzylate (QNB; 5 Ci/mmol) and [$^3H$]morphine (30 Ci/mmol) from Amersham.

Dissociation constants ($K_{0.5}$) and Hill coefficients ($n_H$) were computed using a Wang 2200 calculator as previously described.[8]

**Rotarod Test.** This test, involving the ability of mice to remain on a rotating rod, was carried out as previously described.[34]

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# A Comparison of the Inhibitory Action of 5-(Substituted-benzyl)-2,4-diaminopyrimidines on Dihydrofolate Reductase from Chicken Liver with That from Bovine Liver[†]

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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-NHCOCH$_3$, 3-OC$_4$H$_9$, 3-I, 3-CF$_3$-4-OCH$_3$, and 3,4,5-(OCH$_3$)$_3$. The inhibition constants have been used to formulate quantitative structure–activity relationships for comparative purposes.

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-(OCH$_3$)$_3$] developed by Roth et al.[1,2] of the