(±)α-Phenyl-β-(3,4-dimethoxy)- and (±)α-Phenyl-β-(3,4-dihydroxy)phenethylamines: Potential Probes for Nicotinic Acetylcholine Receptor-Ion Channel Molecule from *Torpedo* Electric Organ

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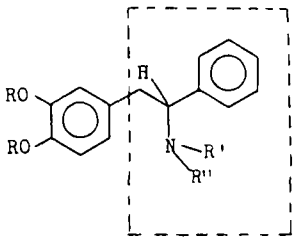
Received January 21, 1987, from the *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, the ‡Department of Biochemistry, Faculty of Medicine, University of Tanta, and the §Department of Plant Protection, Faculty of Agriculture, University of Alexandria, Egypt. Accepted for publication July 27, 1987.

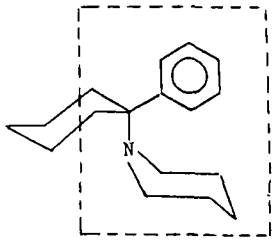
Abstract □ The synthesis of some *N*-methyl, *N*-alkyl derivatives of (±)α-phenyl-β-(3,4-dimethoxy)- and (±)α-phenyl-β-(3,4-dihydroxy)-phenethylamines was achieved. These compounds were shown to bear certain structural features of acetylcholine (ACh), as well as phencyclidine (PCP). The latter was reported to act as a specific probe for the nicotinic ACh receptor-ion channel molecule from *Torpedo* electric organ. Biochemical binding studies revealed that for the nicotinic ACh receptor, the 3,4-dimethoxy derivatives behaved as blockers for the binding interaction of [^{3}H]ACh, whereas the 3,4-dihydroxy analogues stimulated such binding. On the other hand, all of the tested phenethylamines exhibited potent blockade towards [^{3}H]PCP binding interactions. The results indicated that the tested compounds might be applied as potential probes for the ACh receptor-ion channel molecule.

In a previous report[1] we described the inhibitory effect exhibited by some newly synthesized (±)α-phenyl-β-(3,4-dimethoxy)phenethylamines [I: R = CH_3; R′ = H; R″ = H, CH_3, or $(CH_3)_2$] towards the choline acetyltransferase enzyme isolated from the electric organ of the fish *Torpedo ocellata*. Such unexpected activity was attributed to the apparent stereochemical resemblance between the most probable conformation of I (conformation A) and the preferential gauche disposition of the choline (Ch) substrate at physiological pH.

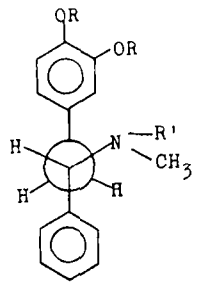
Owing to the facts that acetylcholine (ACh) could also exist in the same previous conformation[2] and that the presently synthesized I might also be shown to possess certain structural characteristics of phencyclidine (PCP), a well-recog-



I



PCP



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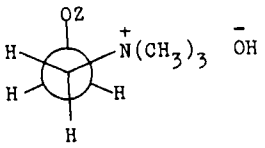
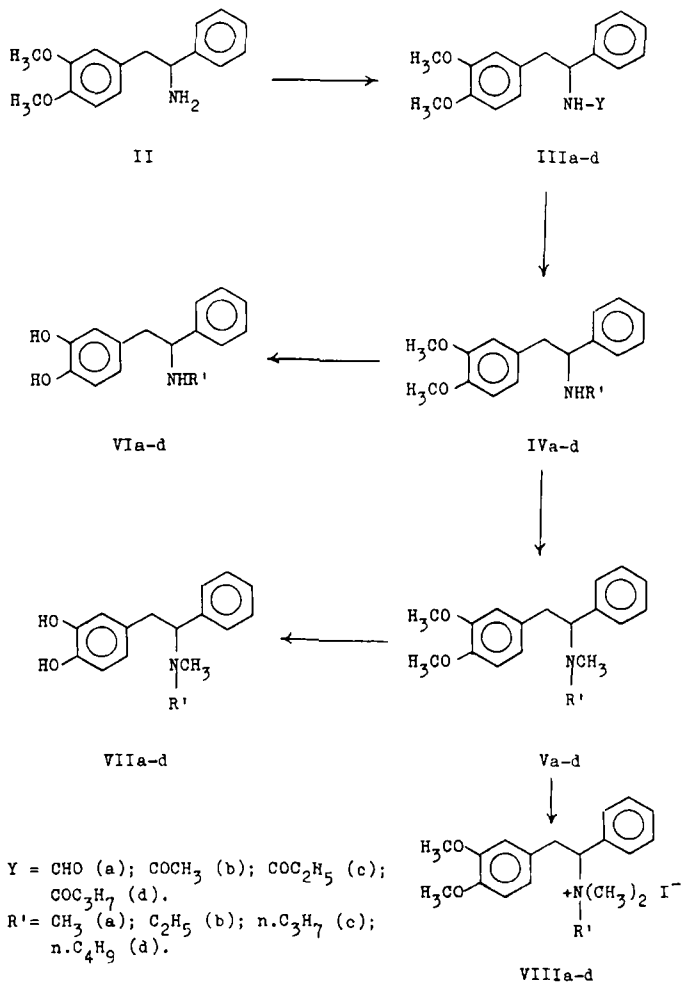


Ch : Z = H
Ach: Z = $COCH_3$



Scheme I

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nized probe for the acetylcholine receptor-ion channel molecule,[3,4] it appeared interesting to investigate the possible interaction of I with both the nicotinic acetylcholine receptor and its associated ionic channel from *Torpedo* electric organ.

Two main primary binding sites have been acknowledged for the nicotinic ACh receptor,[5] and probably for PCP binding sites. Consequently, resolution of racemates of I might not be important since the required binding interaction would not involve three points of attachment.[6]

The synthetic route utilized is outlined in Scheme I. The starting compound was (±)α-phenyl-β-(3,4-dimethoxy)phenethylamine (II) which was obtained through the same procedure as that described by Shafik et al.;[1] that is, by reduction of 3,4-dimethoxy-α-nitrostilbene with $LiAlH_4$. Acylation of II, using either acetic anhydride, propionyl chloride-pyridine, or butyryl chloride-pyridine, yielded *N*-acetyl (IIIb), *N*-propionyl (IIIe), or *N*-butyryl (IIId) derivatives, respectively. Reduction with $LiAlH_4$ afforded the corresponding *N*-alkyl derivatives (IVb–d). The *N*-formyl (IIIa) and *N*-methyl (IVa) analogues were prepared using our previously reported procedures[1] from II via formylation with a mixture of formic acid and acetic anhydride, followed by $LiAlH_4$ reduction. The *N,N*-dimethyl (Va)[1] and the other *N*-alkyl analogues (Vb–d) were produced through a reductive alkylation process[7] using a mixture of formaldehyde and formic acid. Demethylation of IVa–d or Va–d was performed by heating with 48% hydrobromic acid to give the target (±)α-phenyl-β-(3,4-dihydroxy)-phenethylamines (VIa–d and VIIa–d). In addition, quaternarization of Va–d was done, using methyl iodide, to isolate the corresponding (±)*N,N*-dimethyl, *N*-alkyl-α-phenyl-β-(3,4-dimethoxy)phenethylammonium iodide salts (VIIIa–d).

Experimental Section

Melting points were taken in open glass capillaries and are uncorrected. The IR spectra were scanned, for KBr pellets, on a Beckman IR-4210 spectrophotometer. The ^1H NMR spectra were determined, for solutions in Me_2SO-d_6, on a Varian EM-390 NMR spectrometer and are reported in δ values (ppm) relative to an internal standard of $(CH_3)_4Si$. Analyses were performed by members of the Microanalytical Unit, Faculty of Science, University of Cairo, Egypt.

(±)*N*-Acetyl-α-phenyl-β-(3,4-dimethoxyphenethylamine (IIIb)—A mixture of 2.6 g (0.01 mol) of II, 6 mL of acetic anhydride, and 1.0 g (0.012 mol) of anhydrous sodium acetate was refluxed for 2 h. The reaction mixture was allowed to cool to room temperature, poured onto crushed ice, and then neutralized by gradual addition of powdered $NaHCO_3$. The product was filtered, washed with hot water, dried in a vacuum desiccator, and recrystallized from benzene:petroleum ether (bp 40–60 °C) to give 2.1 g (69.2% yield) of white crystals, mp 144–145 °C; IR: ν_{max} 3340(NH), 1640, 1550, and 1250 (amide bands) cm^{-1}.

Anal.—Calc. for $C_{18}H_{21}NO_3$: C, 72.2; H, 7.0; N, 4.7. Found: C, 71.8; H, 7.1; N, 4.3.

(±)*N*-Propionyl-α-phenyl-β-(3,4-dimethoxy)phenethylamine (IIIe)—A solution of 1.4 g (0.015 mol) of propionyl chloride in 10 mL of benzene was added in a dropwise manner to a cooled, stirred solution of 2.6 g (0.01 mol) of II in 10 mL of pyridine. Cooling and stirring were maintained for 2 h, and the reaction mixture was thereafter refluxed for 4 h and then poured onto an excess of 10% HCl. The benzene layer was separated, washed with water, dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was recrystallized from benzene:petroleum ether (bp 40–60 °C) to give 2.6 g (81.3% yield) of white crystals, mp 148–149 °C; IR ν_{max}: 3330 (NH), 1640, 1530, and 1340 (amide bands) cm^{-1}; ^1H NMR (Me_2SO-d_6): 1.0 (t, 3), 2.0 (q, 2), 2.9 (d, 2), 3.8 (S, 6), 5.0 (q, 1), 6.8 (m, 3, ArH), 7.3 (s, 5, ArH), and 8.3 (d, 1, NH; disappeared with D_2O) ppm.

Anal.—Calc. for $C_{19}H_{23}NO_3$: C, 72.8; H, 7.4; N, 4.5. Found: C, 73.0; H, 7.3; N, 4.4.

(±)*N*-Butyryl-α-phenyl-β-(3,4-dimethoxy)phenethylamine (IIId)—A solution of 1.6 g (0.015 mol) of butyryl chloride in 10 mL of benzene was added in a dropwise manner to a cooled, stirred solution of 2.6 g (0.01 mol) of II in 10 mL of pyridine. The reaction mixture was manipulated as described for IIIe. The product was recrystallized from benzene:petroleum ether (bp 60–80 °C) to give 1.9 g (58.4% yield) of white crystals, mp 143 °C; IR ν_{max}: 3335 (NH), 1670, 1550, and 1240 (amide bands) cm^{-1}.

Anal.—Calc. for $C_{20}H_{25}NO_3$: C, 73.4; H, 7.7; N, 4.3. Found: C, 73.7; H, 7.3; N, 3.9.

(±)*N*-Alkyl-α-phenyl-β-(3,4-dimethoxy)phenethylamines Hydrochloride (IVa–d; Table I)—To a stirred mixture of 1.9 g (0.05 mol) of $LiAlH_4$ and 200 mL of tetrahydrofuran was added 0.01 mol of the appropriate III. The mixture was heated at reflux for 15 h, and the solvent was removed under reduced pressure. The residue was chilled, and the excess $LiAlH_4$ was destroyed with crushed ice and 20% aqueous NaOH, respectively. The product was isolated by ether extraction and was purified in the usual manner[7] by alternative pH adjustments to give the corresponding IV. The hydrochloride salt was obtained by passing dry hydrogen chloride into the ethereal solution. The separated salt was collected and recrystallized from methanol:ether. The IR ν_{max} for IVa–d were: splitted band centered at the range 2610–2440 (NH_2^+), and band at 1040–1025 (Ar—O—C) cm^{-1}.

(±) *N* - Methyl, *N* - Alkyl - α - phenyl - β - (3,4 - dimethoxy)phenethylamines Hydrochloride (Va–d; Table I)—A mixture of 0.01 mol of the hydrochloride salt of the appropriate IV, 2.3 g (0.05 mol) of 98% formic acid, and 0.66 g (0.022 mol) of 38% formaldehyde was refluxed for 12 h. The mixture was transferred to an evaporating dish and heated on a water bath until most of the unreacted formic acid and formaldehyde were removed. The syrupy residue was treated with 0.6 mL of 98% formic acid and 0.2 mL of 38% formaldehyde and allowed to evaporate on the water bath until dryness. The last traces of water were removed by azeotropic distillation with absolute ethanol and benzene. The residual hydrochloride salt of the corresponding V was recrystallized from methanol ether. The IR ν_{max} for Va–d were: splitted band centered at the range 2610–2410 (NH^+), and band at 1020–1000 (Ar—O—C) cm^{-1}.

(±)*N*-Alkyl-α-phenyl-β-(3,4-dihydroxy)phenethylamines Hydrochloride (VIa–d), and *N*-methyl, *N*-Alkyl-α-phenyl-β-(3,4-dihydroxy)phenethylamines Hydrochloride (VIIa–d; Table I)—A mixture of 0.001 mol of the appropriate IV or V and 6 mL of 48% hydrobromic acid was heated at 120–125 °C, under nitrogen atmosphere, for 3 h. The excess acid was then removed under reduced pressure and the residual hydrochloride salt of the corresponding VI or VII was recrystallized from methanol:ether. The IR ν_{max} for VIa–d were: a band at 3340 (OH), and split band centered at the range 2630–2510 (NH_2^+) cm^{-1}. The IR ν_{max} for VIIa–d were: a band at 3300–3120 (OH), and split band centered at 2670 (NH^+) cm^{-1}.

(±)*N,N* - Dimethyl, *N*-alkyl-α-phenyl-β-(3,4 - dimethoxy)phenethylammonium Iodides (VIIIa–d)—To a solution of 0.005 mol of the appropriate V (free base) in 100 mL of dry ether was added 1.4 g (0.01 mol) of methyl iodide. The reaction mixture was kept at room temperature for 72 h, and the corresponding VIII was precipitated, collected, and recrystallized from methanol:ether.

Biochemical Assays—*Membrane Preparations*—Frozen (−90 °C) electric organs of *Torpedo ocellata* (obtained fresh from the Mediterranean near Alexandria, Egypt) were homogenized in an equal volume of 50 mM Tris-HCl buffer (pH 7.4), which contained 0.1 mM phenylmethylsulfonyl fluoride, 0.02% NaN_3, and 1 mM EDTA, to reduce proteolytic breakdown. The homogenate was centrifuged at 3000 rpm for 10 min in a Sorvall SS-34 rotor, and the pellet was homogenized again in two volumes of the same buffer and centrifuged again under the same conditions. The two supernatants were pooled and centrifuged at 17 000 rpm for 60 min, and the pellets were collected and resuspended in 50 mM Tris-HCl buffer containing 0.02% NaN_3 so that the final protein concentration was ~1 mg/mL as determined by the method of Lowry et al.[8] Diisopropylfluorophosphonate (DFP) was added during membrane preparation at 0.1-mM range in order to inhibit, irreversibly, all cholinesterases that might be present in the preparation without affecting acetylcholine (ACh) receptor binding. This preparation was usually stable for binding measurements for up to one week of storage at 0 °C.

[^{3}H]Acetylcholine Binding—Equilibrium dialysis was used to study the binding of [^{3}H]ACh (specific activity 49.5 mCi/mmol; New England Nuclear) to its receptor sites in *Torpedo ocellata* electric organ membranes. Dialysis was carried out in 10 mL of modified Krebs-Ringer phosphate solution containing 0.1 μM [^{3}H]ACh and 0.1 mM DFP, as previously reported by Albuquerque et al.[4]

[^{3}H]Phencyclidine Binding—Binding of [^{3}H]PCP (specific activity 48 Ci/mmol; New England Nuclear) to the allosteric channel site of the nicotinic ACh receptor of *Torpedo ocellata* electric organ membranes was measured by incubating 2 mM [^{3}H]PCP with the membrane in 1 mL of 50 mM Tris-HCl buffer at 22 °C and then filtering them over GF/B filters that were previously dipped in 1% Prosil-28 (organosilicone solution) to reduce binding to filters. The filtered membrane preparations were rinsed with 10 mL of 50 mM Tris-HCl buffer (4 °C). The filter was placed in 5 mL of toluene-based liquid scintillation cocktail and its radioactivity was counted after 6 h in a Packard Tri-Carb liquid scintillation spectrometer (model 3255).

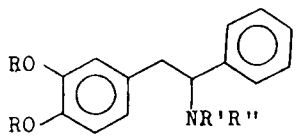
Results and Discussion

The binding of [^{3}H]PCP (2 nM) to *Torpedo ocellata* electric organ membranes was time dependent. In presence of carbamylcholine (i.e., stimulated binding), equilibrium was reached faster and was at higher level than that of the unstimulated binding (Figure 1A). The dissociation of [^{3}H]PCP (Figure 1B) was fit by a single rate constant (4.1×10^{-3} s^{-1}).

The effects of a concentration of 10^{-3} M for each of the newly synthesized *N*-methyl, *N*-alkyl-α-phenyl-β-(3,4-dimethoxy)- and *N*-methyl, *N*-alkyl-α-phenyl-β-(3,4-dihydroxy)-phenethylamines (**Va–d**, and **VIIa–d**) on the binding interactions of 0.1 μM [^{3}H]ACh and 2 nm [^{3}H]PCP to membrane preparations from the electric organ of *Torpedo Ocellata* fish were monitored. The particular compound was included in the incubation medium and the results are an average of five experiments and were calculated as the percent change of control ± standard deviation (SD). In all cases, $p < 0.05$.

The data for the binding of [^{3}H]ACh to the isolated nicotinic ACh receptors (Figure 2) indicated that the 3,4-

Table I—(±)α-Phenyl-β-(3,4-dimethoxy)- and (±)α-Phenyl-β-(3,4-dihydroxy)phenethylamines



Compound	R	R′	R″	mp, °[a]	Yield, %	Molecular Formula	Analysis, %	
							Calc.	Found
IVa	CH_3	H	CH_3	164–165[b]	70	$C_{17}H_{22}ClNO_2$[c]	—	—
IVb	CH_3	H	C_2H_5	191–192	79	$C_{18}H_{24}ClNO_2$[c]	C 67.2	67.3
							H 7.5	7.5
							Cl 11.0	11.0
							N 4.4	4.7
IVc	CH_3	H	n-C_3H_7	185–187	70	$C_{19}H_{26}ClNO_2$[c]	C 67.9	68.0
							H 7.8	8.0
							Cl 10.6	10.9
							N 4.2	3.7
IVd	CH_3	H	n-C_4H_9	169–170	84	$C_{20}H_{28}ClNO_2$[c]	C 68.7	68.6
							H 8.1	8.2
							Cl 10.1	10.5
							N 4.0	3.6
Va	CH_3	CH_3	CH_3	218–220[d]	78	$C_{18}H_{24}ClNO_2$[c]	—	—
Vb	CH_3	CH_3	C_2H_5	194–196	87	$C_{19}H_{26}ClNO_2$[c]	C 67.9	67.4
							H 7.8	7.4
							Cl 10.6	11.0
							N 4.2	4.1
Vc	CH_3	CH_3	n-C_3H_7	198–200	98	$C_{20}H_{28}ClNO_2$[c]	C 68.6	68.6
							H 8.1	8.1
							N 4.0	4.2
Vd	CH_3	CH_3	n-C_4H_9	195–196	80	$C_{21}H_{30}ClNO_2$[c]	Cl 9.8	9.6
							N 3.9	4.2
VIa	H	H	CH_3	163–165	71	$C_{15}H_{18}ClNO_2$[c]	Cl 12.7	13.0
							N 5.0	4.8
VIb	H	H	C_2H_5	174–175	78	$C_{16}H_{20}ClNO_2$[c]	Cl 12.1	12.2
							N 4.8	4.4
VIc	H	H	n-C_3H_7	182–183	73	$C_{17}H_{22}ClNO_2$[c]	Cl 11.6	11.9
							N 4.6	4.2
VId	H	H	n-C_4H_9	195–196	76	$C_{18}H_{24}ClNO_2$[c]	Cl 11.0	10.9
							N 4.4	4.8
VIIa	H	CH_3	CH_3	243–244	91	$C_{16}H_{20}ClNO_2$[c]	Cl 12.1	12.0
							N 4.8	4.4
VIIb	H	CH_3	C_2H_5	180–182	87	$C_{17}H_{22}ClNO_2$[c]	Cl 11.6	12.0
							N 4.6	4.2
VIIc	H	CH_3	n-C_3H_7	197–198	92	$C_{18}H_{24}ClNO_2$[c]	Cl 11.0	11.2
							N 4.4	4.8
VIId	H	CH_3	n-C_4H_9	213–214	82	$C_{19}H_{26}ClNO_2$[c]	Cl 10.6	10.5
							N 4.2	4.0
VIIIa	CH_3	CH_3	$(CH_3)_2$[e]	228–230[f]	63	$C_{19}H_{26}INO_2$[g]	—	—
VIIIb	CH_3	CH_3	$(CH_3)C_2H_5$[e]	204–205	72	$C_{20}H_{28}INO_2$[g]	N 3.2	3.4
VIIIc	CH_3	CH_3	(CH_3)n-C_3H_7[e]	208–210[h]	64	$C_{21}H_{30}INO_2$[g]	N 3.1	2.8
VIIId	CH_3	CH_3	(CH_3)n-C_4H_9[e]	207–209	48	$C_{22}H_{32}INO_2$[g]	N 3.0	3.4

[a] Unless otherwise specified, recrystallization was done from methanol:ether. [b] Lit.[1] mp 164–165 °C. [c] Hydrochloride salt. [d] Lit.[1] mp 218–220 °C. [e] Quaternary ammonium compound. [f] Lit.[1] mp 228–230 °C. [g] Iodide salt. [h] Recrystallized from benzene:petroleum ether (bp 60–80 °C).

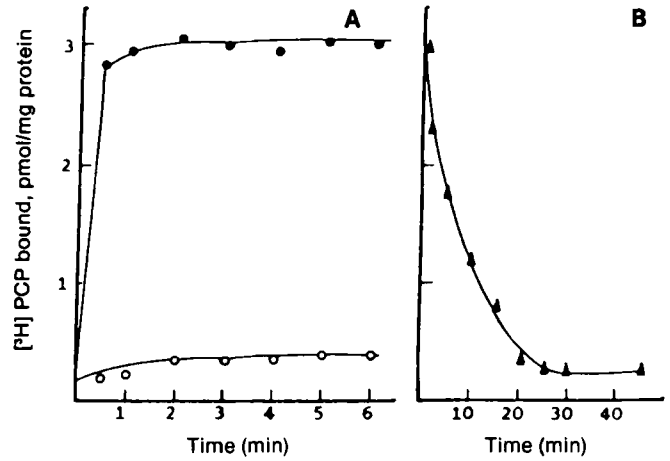


Figure 1—*Binding of 2 nM [³H]PCP to ACh receptors from Torpedo electric organ; in presence (●) and in absence (○) of 100 μM carbamylcholine (A) as a function of time, and its dissociation rate (▲) upon addition of 100 μM cold PCP (B).*

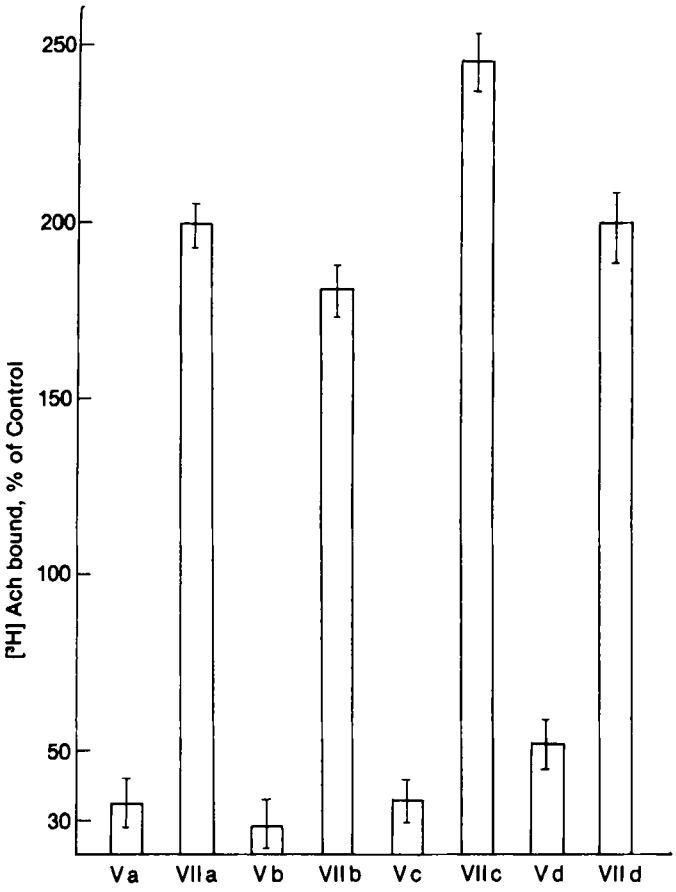


Figure 2—*Effect of 10^{-3} M* **Va–d** *and* **VIIa–d** *on the binding of 10^{-6} M [³H]ACh to acetylcholine receptors from Torpedo electric organ.*

dimethoxy derivatives (**Va–d**) acted as inhibitors, while the 3,4-dihydroxy analogues (**VIIa–d**) potentiated the binding interaction. The sequence of the percent bound ± SD was of the order **VIIc** (246.7% ± 3.24) > **VIId** (200.9% ± 3.92) > **VIIa** (199.5% ± 2.16) > **VIIb** (181.0% ± 3.02) > **Vd** (52.0% ± 2.82) > **Vc** (36.9% ± 2.66) > **Va** (34.4% ± 2.84) > **Vb** (28.7% ± 3.42).

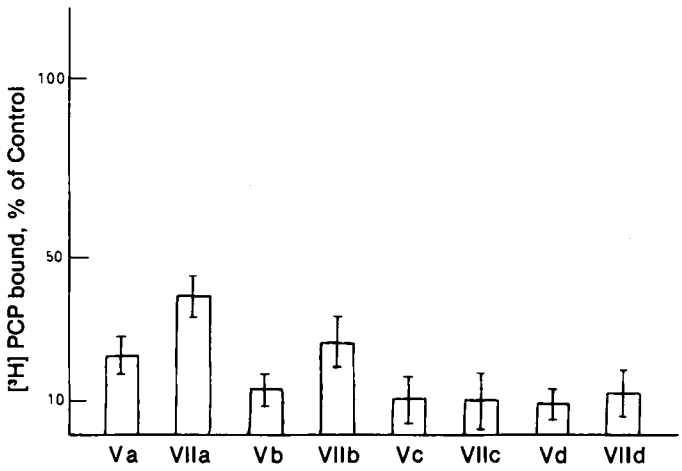


Figure 3—*Effect of 10^{-3} M* **Va–d** *and* **VIIa–d** *on the binding of 2 nM [³H]PCP to carbamyocholine-stimulated ACh receptors from Torpedo electric organ.*

The data for [³H]PCP binding to the ion channel molecule (Figure 3) indicated that all tested compounds (**Va–d** and **VIIa–d**) gave blockade to the binding mode. The sequence of the percent bound ± SD was of the order **VIIa** (39.1% ± 2.22) > **VIIb** (26.8% ± 2.68) > **Va** (22.1% ± 2.04) > **Vb** (13.0% ± 1.82) > **VIId** (12.2% ± 2.46) > **Vc** (10.1% ± 2.62) > **VIIc** (10.0% ± 3.02) > **Vd** (9.8% ± 1.84).

These results revealed that the newly described phenethylamines (**Va–d** and **VIIa-d**) could be considered as potential probes for the nicotinic ACh receptor-ion channel molecule.

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Acknowledgments

The authors are grateful to Prof. Dr. Nabil A. Monsour and Dr. Mahmoud Morshedy, Faculty of Agriculture, University of Alexandria, for providing laboratory facilities and for their kind help.