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Bisquaternary Caracurine V Derivatives as Allosteric Modulators of Ligand Binding to M_2 Acetylcholine Receptors

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Abstract—The allosteric effect on muscarinic acetylcholine M_2 receptors of 11 bisquaternary salts of the *Strychnos* alkaloid caracurine V was determined. The effect was indicated by the concentration which retarded the rate of dissociation of the antagonist [^{3}H]-*N*-methylscopolamine from porcine cardiac cholinoceptors by a factor of 2 (EC_{50}). The most potent compounds carry allyl and propargyl substituents, respectively. Introduction of more bulky substituents (e.g., benzyl groups) resulted in a considerably reduced allosteric potency. The wide range of EC_{50} values (3 nM for R = allyl, 1750 nM for R = 2-naphthyl) suggests a sterically restricted binding pocket. Molecular modeling studies indicated that the caracurine V ring system satisfies the pharmacophore model for the allosteric interaction.

Structurally different compounds from various pharmacological groups are known to retard the dissociation of the antagonist [^{3}H]-*N*-methylscopolamine, ([^{3}H]NMS) from cardiac muscarinic receptors.[1] This effect is based on an allosteric modulation of the radioligand binding caused by a modulator occupying a site apart from the common antagonist binding area.[2–4] So far, SAR studies in several series of quaternary molecules derived from hexamethonium,[5,6] bispyridinium compound TMB-4,[7,8] W84, WDUO, IWDUO,[9] strychnine and brucine,[10] truxillic acid[11,12] and thiazoloandrostane[13] have been carried out. Molecular modeling studies revealed two positively charged nitrogens at a distance of ca. 10 Å surrounded by two aromatic ring systems to be essential elements of a high allosteric potency.[14]

One of the most potent allosteric modulators is the muscle relaxant alcuronium. However, its neuromuscular blocking activity is an obstacle for studies in intact organisms and makes the therapeutic use as an allostere impossible (Scheme 1).

Diallylcaracurine V, the cyclization product of alcuronium, has recently been reported to exert virtually the same allosteric effect as alcuronium at cardiac muscarinic receptors.[15a–b] Both compounds retard the dissociation of [^{3}H]NMS from M_2 receptors half-maximally at EC_{50} of about 3.5 nM (buffer conditions as indicated below). On the other hand, the neuromuscular blocking activity of caracurine V dimethochloride was reported to be about 50-fold lower than that of toxiferine (Scheme 1).[16] Until now, no SAR studies of bisnortoxiferine and caracurine V ring systems have been carried out. Thus, the purpose of this work was to synthesize and pharmacologically evaluate a number of bisquaternary caracurine V derivatives. The systematic variation of *N*-substituents at the caracurine V ring system is an approach to separate the allosteric effect on M_2 receptors from the neuromuscular blocking activity.

Caracurine V (**1**) was prepared according to the procedure of Battersby and Hodson[17] as previously described by Zlotos.[18] The quaternization of caracurine V base with a 2.5-fold excess of methyliodide, allylbromide, propargylbromide, a number of different substituted benzylbromides, dimethoxybenzylchloride and 2-naphthylbromide readily proceeded in a chloroform solution at room temperature within 30 min. Introduction of propyl[19] and phthalimidopropyl[20] substituents required the corresponding alkyliodides and higher temperatures. The separated caracurine V ammonium salts were filtered and washed with chloroform until no alkyl halide could be detected by TLC. No further purification was necessary as indicated by high resolution ^{1}H NMR and FABMS spectra.[21]

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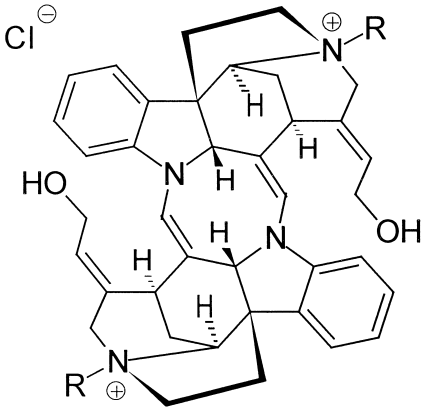
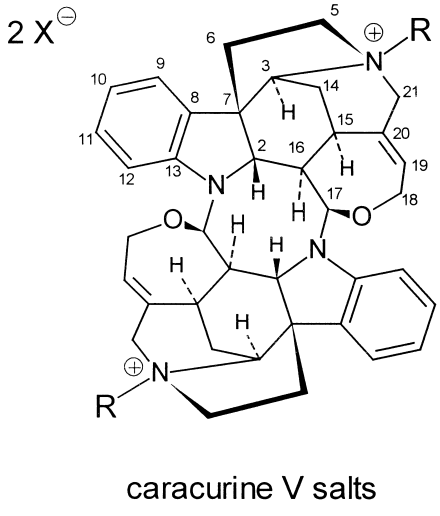
In order to investigate the binding mode of caracurine derivatives with both nicotinic and muscarinic acetylcholine receptors, knowledge of the exact stereochemistry of caracurine V skeleton is necessary. The 3D structure of caracurine V has recently been elucidated using semiempirical calculations and NMR spectroscopy.[18] Since the caracurine V skeleton is a highly fused and rigid ring system, the bisquaternary derivatives are likely to have the same stereochemistry as the free base. Analogous to caracurine V, ^{1}H and ^{13}C NMR spectra of all bisquaternary derivatives show only single sets of signals indicating that a 2-fold symmetry axis remained after the quaternization. The proton coupling constants also correspond to the respective coupling constant values found for caracurine V.

Due to identical allosteric potencies of alcuronium and diallylcaracurine, both compounds are supposed to satisfy the pharmacophore model of two positively charged nitrogens and two aromatic systems at distinct distances to each other. With the aim of comparing the geometries of both ring systems, the 3D structures of alcuronium and diallylcaracurine V were generated using PC SPARTAN 1.1, Wavefunction, Inc.

The starting geometries were based on the X-ray data of strychnine, which is the starting compound for the synthesis of caracurine V. Semiempirical calculations (AM1) revealed a considerable different stereochemistry of the central eight-membered rings. The indole nitrogen in alcuronium adopts, due to a conjugation with the double bond, a nearly planar geometry, whereas its arrangement in caracurine is pyramidal. Consequently, the relative spatial arrangement of the aromatic indole rings changes considerably. Nevertheless, the distance between the N^{+}-centers is virtually unchanged (alcuronium 9.75 Å, diallylcaracurine 9.65 Å). The distances between the positively charged nitrogens and the centers of the adjacent aromatic rings are also the same in both molecules (alcuronium 5.0 Å, diallylcaracurine 5.1 Å). The superposition of alcuronium and diallylcaracurine displayed in Figure 1 shows that the caracurine V ring system satisfies the pharmacophore model for a high allosteric potency.

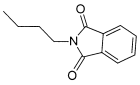
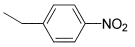
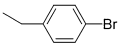
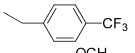
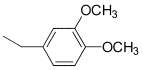
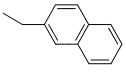




Scheme 1.

In order to determine the allosteric potency of the caracurine V derivatives, their ability to allosterically retard the dissociation of [^{3}H]NMS from porcine cardiac M_2 receptors was measured. Dissociation assays were conducted in a buffer composed of 4 mM Na_2HPO_4 and 1 mM KH_2PO_4 (pH 7.4) at 23 °C. Cardiac membranes were preincubated with [^{3}H]NMS (0.2 nM) for 30 min; radioligand dissociation was then revealed by the addition of 1 μM atropine, in the presence or absence of the allosteric modulator. The time course of dissociation was observed by withdrawing aliquots at various times over a period of 120 min. Membranes were separated by

Table 1.

Compd	R	Anion	EC_{50} [nM]
1	—	—	1187
2	—CH_3	Cl^-	8
3		Cl^-	30
4		Br^-	3
5		Br^-	4
6		Cl^-	376
7		Br^-	69
8		Br^-	315
9		Br^-	507
10		Br^-	1748
11		Cl^-	342
12		Br^-	1558

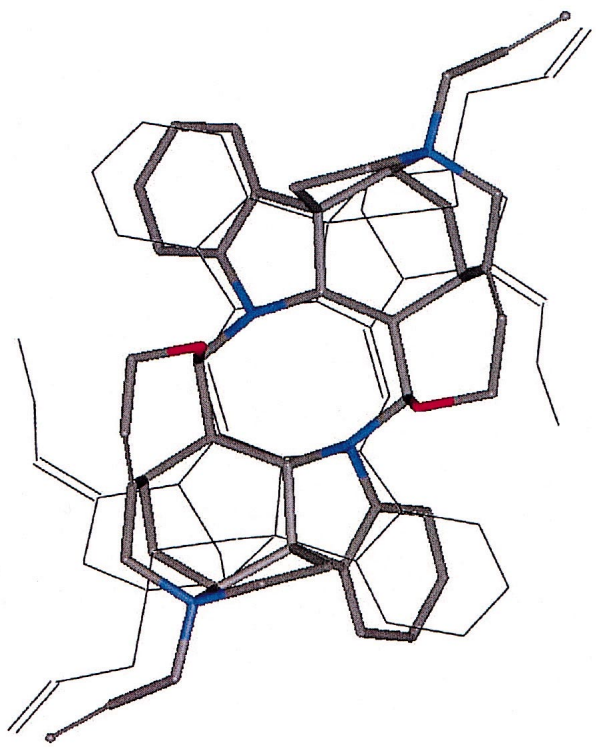


Figure 1. Superposition of diallylcaracurine V (stick model) onto alcuronium (RMS fit of HyperChem 5.1 ChemPlus Extension, Hypercube, Inc.), fitting atoms: $2\times N^+$, two carbon atoms of each indole ring — zC9, C12, C9′,C12′.

vacuum filtration and membrane bound radioactivity was determined by liquid scintillation counting. Experimental results were analyzed by nonlinear regression analysis (Prism 2.01, Graph Pad®). Dissociation data were fitted using a monoexponential decay function that yielded the apparent rate constant of dissociation k_{-1}. To obtain concentration–effect curves for the retardation of radioligand dissociation, curve fitting was based on a four parameter logistic function. The concentration which retarded [^{3}H]NMS dissociation by a factor of 2 (EC_{50}) served as a measure of allosteric potency.

All investigated caracurine V derivatives were able to retard the dissociation of [^{3}H]NMS from porcine cardiac membranes. Nevertheless, we observed a wide range of allosteric potency with EC_{50} values from 3 nM to 1750 nM, indicating that the allosteric effect on [^{3}H]NMS binding to M_2 receptors is very sensitive to the properties of the *N*-substituent at the caracurine V ring system (Table 1). The most potent compounds carry allyl and propargyl substituents respectively. Substitution of the allyl group with the propyl group resulted in a 10-fold lower potency. Thus, the double or triple bond is essential for high activity. Introduction of more bulky substituents (e.g., benzyl groups) resulted in a considerably reduced allosteric potency. This suggests a sterically restricted receptor binding pocket. However, volume of the substituent can not be the only factor determining the allosteric effect as shown by comparison of bis(trifluoromethyl)benzyl caracurine V **10** with the more bulky dimethoxybenzyl derivative **11**; EC_{50} values 1748 nM and 342 nM, respectively. The very poor allosteric potency of **10** could be due to the high lipophilicity of the trifluoromethyl group. Compound **12**, carrying the very lipophilic and voluminous naphthyl group is also a poor allosteric modulator, EC_{50} 1558 nM. Introduction of the phthalimidopropyl group which is also a part of potent allosteric modulators W84 and C7/3′-phth, EC_{50} values 24 nM and 11 nM, respectively,[1] led to the less potent compound **6**, EC_{50} 376 nM. This indicates that the allosteric interaction with the receptor protein is mediated through caracurine indole rings as postulated in the pharmacophore model.

In conclusion, we demonstrated that the caracurine V skeleton is an excellent pharmacological tool for exploring the allosteric mechanism of the ligand–receptor interaction. The rigid ring system satisfies the pharmacophore model for allosteric interactions and the effect on [^{3}H]NMS/M_2 receptor-complexes is very sensitive to the side chain properties. The wide range of EC_{50} values is a very good basis for further QSAR studies. However, for reliable QSAR results, some more differently substituted caracurine V derivatives have to be synthesized.

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19. Compound **3** was obtained by refluxing caracurine V with propyliodide in acetone for 30 min. The separated dipropylcaracurine V diiodide was transferred to the corresponding chloride using Amberlite IRA-400 resin.
20. Compound **6** was obtained by refluxing caracurine V with phthalimidopropyliodide in chloroform for 1 h. The separated iodide was transferred to the corresponding chloride using Amberlite IRA-400 resin.
21. All compounds in this communication gave spectroscopic data in full accord with their assigned structures. Representative example: Compound **4**: white solid. ^{1}H NMR (500 MHz, D_2O/CD_3OD) δ: 7.32 (2H, m, H-9 and H-10), 7.03 (1H, ddd, $J=8.3$, 7.1, 1.2 Hz, H-11), 6.77 (1H, dd, $J=8.3$, 1.0 Hz, H-12), 6.48 (1H, br m, H-19), 6.28 (1H, m, -CH_2-C$\underline{H}$=CH_2), 5.90 (1H, d, $J=15.0$ Hz, -CH_2-CH=C$\underline{H}$H), 5.87 (1H, d, $J=9.2$ Hz, -CH_2-CH=CH$\underline{H}$), 4.99 (1H, d, $J=2.0$ Hz, H-17), 4.56 (1H, s br, H-3), 4.40 (1H, dd, $J=14.4$, 7.1 Hz, H-18^{b}), 4.25 (3H, m, H-21^{b} + -C$\underline{H_2}$-CH=CH_2), 4.21 (1H, d, $J=11.2$ Hz, H-2), 4.12 (1H, dd, $J=14.4$, 4.7 Hz, H-18^{a}), 3.89 (1H, dd, $J=13.0$, 7.5 Hz, H-5^{b}), 3.81 (1H, d, $J=13.8$ Hz, H-21^{a}), 3.72 (1H, ddd, 13.7, 13.7, 5.7 Hz, H-5^{a}), 3.25 (1H, s br, H-15), 2.54 (1H, dt, $J=15.5$, 3.8, 3.8 Hz, H-14^{b}), 2.45 (1H, dm, $J=13.7$ Hz, H-6^{b}), 2.12 (1H, dt, $J=11.0$, 2.2, 2.2 Hz, H-16), 1.98 (1H, ddd, $J=13.7$, 13.7, 5.7 Hz, H-6^{a}), 1.90 (1H, d, $J=15.5$ Hz, H-14^{a}). ^{13}C NMR (125 MHz, D_2O/CD_3OD) δ: 152.19 (C-13), 136.29 (C-19), 134.96 (C-8), 131.12 (C-9), 131.02 (C-20), 130.55 (-CH_2-CH=$\underline{C}H_2$), 126.02 (C-10), 122.93 (-CH_2-$\underline{C}$H=CH_2), 122.21 (C-11), 113.04 (C-12), 98.54 (C-17), 73.14 ($\underline{C}$-3), 69.03 (-$\underline{C}H_2$-CH=CH_2), 66.50 (C-18), 62.85 (C-21), 60.51 (C-5), 57.22 (C-7), 57.08 (C-2), 51.57 (C-16), 38.74 (C-6), 32.21 (C-15), 24.94 (C-14). FABMS (MNOBA matrix) m/z 747, 745 $[M-Br]^{+}$.