Probing the Pharmacophore for Allosteric Ligands of Muscarinic M_2 Receptors: SAR and QSAR Studies in a Series of Bisquaternary Salts of Caracurine V and Related Ring Systems

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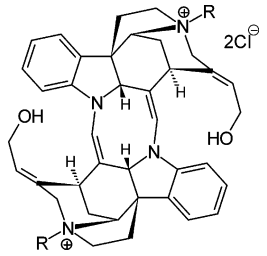
Allosteric effects on muscarinic acetylcholine M_2 receptors were examined in a series of bisquaternary salts of the *Strychnos* alkaloid caracurine V (**6**) and related *iso*-caracurine V, tetrahydrocaracurine V, and bisnortoxiferine ring systems. The compounds inhibited dissociation of the orthosteric antagonist [^{3}H]*N*-methylscopolamine (NMS) from porcine cardiac M_2 receptors with $EC_{0.5,diss}$ values from 4 to 3270 nM. The majority of compounds hardly changed [^{3}H]NMS equilibrium binding, indicating similar binding affinities in free and NMS-occupied M_2 receptors. The most potent agents were found in the caracurine V, *iso*-caracurine V, and tetrahydrocaracurine V series and carried nonpolar alkyl groups with a maximal chain length of three carbon atoms. 3D QSAR (CoMSIA) analysis explained the wide range of binding affinities by steric and electrostatic properties of the side chains. Furthermore, the findings suggest that the spatial orientation of the "caracurine" aromatic rings compared with the bisnortoxiferine ring skeleton is favorable to optimal allostere−receptor interactions.

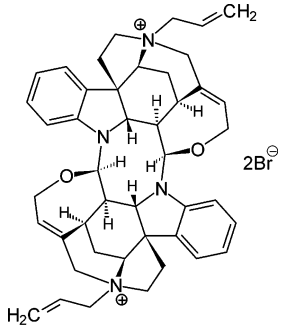
Introduction

Apart from the conventional orthosteric binding site, to which the endogenous ligand acetylcholine and competitive antagonists such as *N*-methylscopolamine and atropine bind, all five muscarinic receptor subtypes (M_1−M_5) contain a second, allosteric binding site.[1−7] Due to greater sequence differences between the amino acids within the allosteric sites on different muscarinic subtypes, ligands for the allosteric site appear to have a higher potential for subtype selectivity than orthosteric ligands. Furthermore, allosteric agents may achieve subtype selectivity not only by different binding affinities to the allosteric sites but also by a subtype selective cooperativity with the orthosteric ligand, i.e., the equilibrium binding of an orthosteric ligand can be either enhanced or reduced or left unaffected by the allosteric modulator in a subtype-dependent fashion. The cardiac M_2 receptor appears to be especially sensitive to allosteric modulation.[8−10] Its allosteric site is well-defined, [9,11,12] and affinity data for a number of structurally different allosteric modulators have been reported for M_2 receptors occupied by [^{3}H]-*N*-methylscopolamine ([^{3}H]NMS).[13] So far, SAR studies have been carried out in several series of compounds derived from hexamethonium,[14] bispyridinium TMB-4,[15] W84,[16−18] WDUO and IWDUO,[19] strychnine and brucine,[20] truxillic acid,[21,22] thiazoloandrostane,[23] pentacyclic carbazolones,[24] and bisquaternary dimers of strychnine and brucine.[25] Molecular modeling studies led to a hypothesis of the pharmacophore consisting of two positively charged nitrogen atoms at a distance of approximately 10 Å surrounded by two aromatic ring systems.[26−28] These pharmacophoric elements are fixed in the very rigid ring skeleton of the neuromuscular blockers toxiferine I **1** and alcuronium **2**.



Toxiferine I **1**, R = methyl
Alcuronium **2**, R = allyl

Alcuronium is a well-known, very potent classical enhancer of NMS binding to muscarinic M_2 receptors.[29] Its cyclization product, diallylcaracurinium salt V **3**, was identified as an allosteric agent with a similar high binding affinity for the M_2 receptor.[30]



Diallylcaracurinium V **3**

Studies at M_2/M_5 chimeric receptors and site-directed mutagenesis in wild-type and chimeric receptors allowed

* To whom correspondence should be addressed. Tel. +49 931-888-5489; fax +49 931-888-5494; e-mail: zlotos@pzlc.uni-wuerzburg.de.
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identification of the M_2 receptor epitopes responsible for the high M_2/M_5 selectivity of **2** and **3**.[12,31] **3** derives M_2/M_5 selectivity in NMS-occupied receptors from two amino acids: tyrosine-177 at the second outer loop of M_2 and threonine-423 at the top of the seventh transmembrane domain. In case of **2**, only an epitope contained in the second outer loop is responsible for high M_2/M_5 selectivity. Molecular modeling studies revealed the $\pi-\pi$ interaction between one of the aromatic rings of **3** and the benzene ring of tyrosine-177 to be essential in the ligand−receptor complex.[31] The divergent binding properties of **2** and **3** could be due to a different relative spatial arrangement of the pharmacophoric elements in both ring systems. The stereochemistry of caracurine V,[32] toxiferine I, and the related *iso*-caracurine V[33] and tetrahydrocaracurine V ring systems was recently determined by means of NMR spectroscopy and semiempirical calculations.[34] While the relative orientation of the aromatic indole rings is similar in all three "caracurine" ring systems, opening of both tetrahydrooxepine rings of caracurine V to give the bisnortoxiferine ring skeleton leads to the conformational change of the central eight-membered ring from a crown form to a boat form, resulting in a considerably changed geometry of the whole ring skeleton. Thus, **3** and related agents appear to be useful tools to study the molecular mechanisms involved in ligand recognition by the muscarinic allosteric site.

The purpose of this study is to examine how the stereochemical difference in the above-mentioned ring systems affects their affinity for the allosteric site of muscarinic M_2 receptors. Our preliminary report on the bisquaternary caracurine V series[30] as well as studies in the monoquaternary strychnine and brucine series[20] indicated that the affinity for the allosteric site is very sensitive to the properties of the N-substituent. To find the best affinity-increasing groups, caracurine V was first quaternized with a great number of N-substituents of different size, lipophilicity, and electronic properties. The affinities of the resulting compounds for the allosteric site of NMS-occupied M_2-receptors were analyzed by means of 3D-QSAR CoMSIA study. The findings should result in an improved understanding of the allosteric pharmacophore and help develop better tools for probing the M_2 allosteric binding site.

Chemistry

Caracurine V (**6**) was obtained from the dimerization of the Wieland−Gumlich aldehyde (**5**) using pivalic acid according to the modified procedure of Battersby and Hodson.[35] **5** was prepared from strychnine by nitrosation at C-22 using isoamyl nitrite/sodium ethoxide and subsequent second-order Beckmann rearrangement of the resulting *E*-22-oximinostrychnine (*E*-**4**) with thionyl chloride as previously described by Hyman et al.[36] However, a much more convenient method for preparing *E*-**4** involved using *tert*-butyl nitrite/t-BuOK as a nitrosation agent. Apart from the major *E*-stereoisomer of **4** (64%), a small amount of *Z*-**4** (17%) was obtained. The stereochemistry was determined by NMR spectroscopy. The H-17 resonance signal of the major *E*-**4** isomer appeared at δ 5.03 ppm, downfield by 0.32 ppm from the corresponding signal of the minor *Z*-**4** isomer due to the anisotropic deshielding effect of the oxime hydroxyl group.[37] The *E*-configuration of the major isomer was confirmed by the chemical shift of C-17 at δ 74.9 ppm, upfield by 4.3 ppm from the corresponding signal of the *Z*-isomer owing to γ-interaction between the hydroxyl group and C-17 in *E*-**4**.[38] The *E*-configuration of the major oxime is opposite to the stereochemistry proposed previously by Hyman et al.[36] Only *E*-**4** was used as a starting material for the synthesis of the key intermediate Wieland−Gumlich aldehyde (**5**).[36] **5** exists in a DMSO solution as an approximately 1:1 mixture of two C-17 epimers as indicated by two sets of signals in both ^{1}H and ^{13}C NMR spectra (400 MHz, DMSO-d_6).

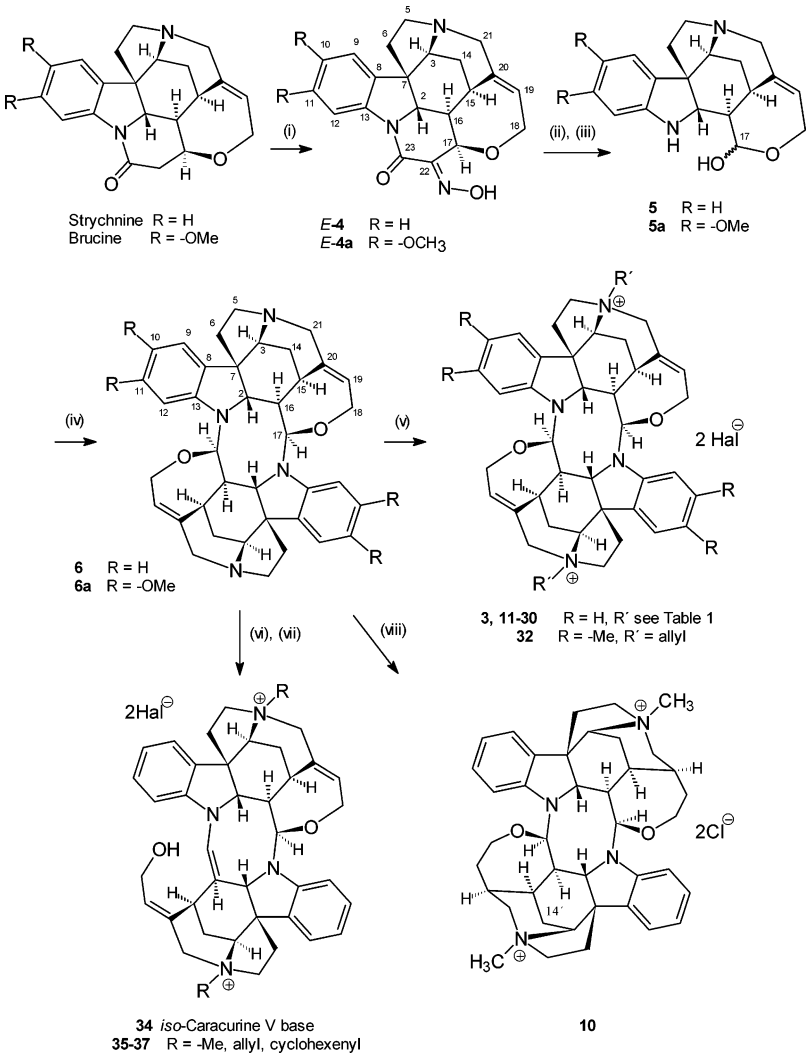
Tetramethoxycaracurine V (**6a**) was prepared similarly to **6** (Scheme 1). Nitrosation of brucine, recently decribed by Zlotos et al.,[25] gave two isomeric oximes, *E*-**4a** (89%) and Z-**4a** (2%). Interestingly, treatment of *E*-**4a** with thionyl chloride and subsequent heating in aqueous HCl provided the expected dimethoxy-Wieland−Gumlich-aldehyde (**5a**) in only 17%. The very low yield of the desired **5a** can be explained by a different course of the second-order Beckmann rearrangement of *E*-**4a** compared to *E*-**4**. While in *E*-**4** the C17−C22 bond was preferentially cleaved, cleavage of the C22−C23 bond, probably induced by the electron-donating methoxy group in position 10 of the aromatic ring, was the major reaction of *E*-**4a**. The resulting cyano group in position 17 was subsequently hydrolyzed by aqueous HCl, giving a water-soluble amino acid which could not be isolated from the reaction mixture. Treatment of the reaction mixture upon evaporation of thionyl chloride with steam at pH 3 instead of aqueous HCl yielded the unchanged nitrile **7** (Scheme 2).

The absolute configuration of C-17 was unchanged in the course of the rearrangement as indicated by the coupling constant between H-17 and H-16 (J = 2.3 Hz) which reflects the cis-orientation of the hydrogen atoms. 19,20,19′,20′-Tetrahydrocaracurine V (**8**) was obtained by catalytic hydrogenation of **6** in ethanol using PtO_2 as catalyst as described in our preliminary report.[34] Since quaternization attempts of **8** with methyl iodide and allyl bromide led to decomposition of the tetrahydrocaracurine V ring system, dimethyltetrahydrocaracurinium V dichloride (**10**) was prepared by the catalytic hydrogenation of dimethylcaracurinium V dichloride (**11**) with PtO_2 in water. The double quaternization of caracurine V (**6**) using 2.5-fold excess of methyl iodide and a number of alkyl bromides readily proceeded in a chloroform solution at room temperature. Introduction of propyl and phthalimidopropyl substituents required the corresponding alkyl iodides and higher temperatures. The resulting bisquaternary caracurinium V salts **3** and **11**−**30** are displayed in Table 1.

N-Allylcaracurinium V bromide (**31**), the only monoquaternary compound in this series, was obtained by treatment of allyl bromide with a large excess of **6**. Quaternization of tetramethoxycaracurine V (**6a**) with allyl bromide in chloroform gave the only tetramethoxycaracurinium V salt (**32**).

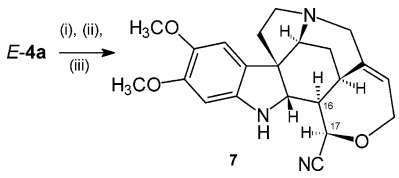
Caracurine V dioxide (**33**) was prepared by N-oxidation of **6** using hydrogen peroxide as previously described by Verpoorte.[39] *Iso*-caracurine V (**34**) was obtained from decomposition of **6** with TFA according to Zlotos.[33] It could be readily double-alkylated with methyl iodide, allyl bromide, and 3-bromocyclohexene in

Scheme 1[a]



[a] Reagents and conditions: (i) *tert*-butyl nitrite/t-BuOK/toluene, 50 °C, 2 h; (ii) $SOCl_2$, rt, 2.5 h, (iii) 2 M HCl, 100 °C, 3 h; (iv) pivalic acid, 120 °C, 14 h; (v) 2 R'-Hal, $CHCl_3$, rt, 30 min.; (vi) TFA, 15 h; (vii) 2 R-Br, $CHCl_3$, rt, 30 min; (viii) 1. MeI, $CHCl_3$, rt, 30 min; 2. IRA-400 resin, chloride phase; 3. H_2 (50 bar)/PtO_2, H_2O, 16 h.

Scheme 2[a]



[a] Reagents and conditions: (i) $SOCl_2$, rt, 2.5 h; (ii) H_2O; 1 M Na_2CO_3 to pH 3; (iii) steam, 90 °C, 1.5 h.

chloroform to give the bisquaternary analogues **35**−**37** (Scheme 1).

The Calabash-curare alkaloid toxiferine I (**1**) was prepared from the dimerization of Wieland−Gumlich aldehyde methochloride using pivalic acid as described by Battersby and Hodson.[35]

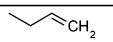
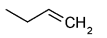
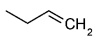
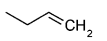
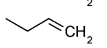
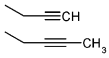
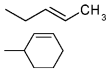
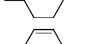
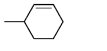
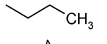
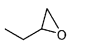
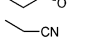
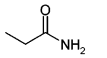
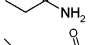
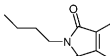
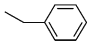
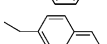
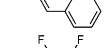
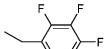
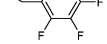
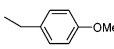
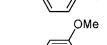
Pharmacological Studies

The allosteric interaction of the test compounds with muscarinic M_2 receptors was studied in homogenates of porcine heart ventricles with [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) as the ligand for the orthosteric receptor site.

Kinetic dissociation binding experiments were conducted, and the allosteric effect of the test compounds on the dissociation of [^{3}H]NMS was determined (two to four experiments for each compound). The concentration of the test compounds that inhibits [^{3}H]NMS dissociation half-maximally, $EC_{0.5,diss}$, can be taken as a measure of the equilibrium dissociation constant of allosteric agent binding to [^{3}H]NMS-occupied receptors.[40,9] The $EC_{0.5,diss}$ values are compiled in Table 1.

Equilibrium binding experiments were performed to get insight in affinities of the test compounds to NMS-free receptors and to obtain information on cooperative effects between the allosteric and the orthosteric ligand. The effect of the test compounds on [^{3}H]NMS equilibrium binding was investigated at a selected concentration of allosteric modulator ($EC_{0.25,diss}$) that reduced the rate of [^{3}H]NMS dissociation by about 75% down to a level of 25% of the control. The equilibrium binding data (from two to five independent experiments with quadruplicated values) were expressed relative to the control binding in the absence of modulator, which was set as 100%. These data are included in Table 1. [^{3}H]-NMS equilibrium binding of approximately 100% indicates neutral cooperativity, which means that [^{3}H]NMS

Table 1. Pharmacological Data for All Investigated Compounds (for details, see text)

cpd	R′	$pEC_{0.5,diss}$	n_H	[^{3}H]NMS equilibrium (%) at $EC_{0.25,diss}$
alcuronium 2		8.65 ± 0.02	-0.94 ± 0.05	115.9 ± 3.3
36[a]		8.57 ± 0.05	-0.99 ± 0.10	118.8 ± 2.0
3		7.95 ± 0.08	-1.30 ± 0.25	108.8 ± 4.5
31[b]		7.74 ± 0.03	-1.35 ± 0.13[e]	81.4 ± 1.4
32[c]		6.69 ± 0.05	-0.78 ± 0.07[e]	70.8 ± 3.8
toxiferine I 1	-CH_3	7.02 ± 0.05	-0.66 ± 0.05[e]	104.2 ± 3.3
35[a]	-CH_3	7.87 ± 0.06	-0.90 ± 0.11	119.0 ± 6.6
11	-CH_3	8.09 ± 0.02	-0.94 ± 0.05	115.1 ± 1.9
10[d]	-CH_3	8.44 ± 0.04	-0.75 ± 0.05[e]	104.4 ± 3.5
6	-	6.36 ± 0.04	-1.03 ± 0.10	95.8 ± 1.5
12		8.36 ± 0.02	-1.02 ± 0.04	104.8 ± 2.4
13		7.36 ± 0.11	-0.92 ± 0.20	103.1 ± 2.8
14		7.14 ± 0.08	-1.09 ± 0.19	100.3 ± 6.7
15		7.70 ± 0.04	-1.41 ± 0.13[e]	108.8 ± 4.6
37[a]		8.07 ± 0.05	-0.82 ± 0.07[e]	117.8 ± 6.1
16		7.55 ± 0.02	-1.02 ± 0.05	99.1 ± 2.7
17		7.86 ± 0.03	-0.96 ± 0.07	109.7± 2.7
18		7.19 ± 0.06	-1.01 ± 0.13	110.3± 0.9
19		6.69 ± 0.04	-0.91 ± 0.09	85.3 ± 1.0
20		6.65 ± 0.06	-0.78 ± 0.10	87.8 ± 3.1
21		6.43 ± 0.06	-1.17 ± 0.06[e]	88.4 ± 5.5
22		7.12 ± 0.04	-1.07 ± 0.10	86.4 ± 2.1
23		5.82 ± 0.03	-1.81 ± 0.18[e]	89.0 ± 6.3
24		7.27 ± 0.08	-1.40± 0.121	110.6 ± 0.7
25		5.95 ± 0.08	-1.13 ± 0.28	70.4 ± 3.5
26		6.47 ± 0.06	-1.20 ± 0.06[e]	100.3 ± 2.4
27		6.47 ± 0.04	-1.03 ± 0.09	99.8 ± 3.5
28		6.29 ± 0.07	-1.16 ± 0.07[e]	113.3 ± 5.0
29		5.76 ± 0.01	-1.77 ± 0.09[e]	87.0 ± 5.4
30		5.49 ± 0.07	-1.34 ± 0.19	6.1 ± 1.0
33	-O^-	non-active		-

[a] *iso*-Caracurine V ring system. [b] *N*-Allylcaracurinium V bromide. [c] Tetramethoxycaracurine V ring system. [d] Tetrahydrocaracurine V ring system. [e] n_H significantly different from unity (*F*-test, $p < 0.05$).

is unchanged despite allosteric agent binding to the receptor. In this case, the affinity of the test compound to the free receptor equals its affinity to the [^{3}H]NMS-occupied receptor. Values greater or lower than 100% indicate positive or negative cooperativity, respectively, between the allosteric and the orthosteric ligand. As can be seen from Table 1, the test compounds except diphenacylcaracurinium V salt (**30**) hardly affected [^{3}H]-NMS equilibrium binding. Thus, the test compounds appear to have a very similar affinity for free receptors as compared to NMS-occupied receptors. **30**, however, that is clearly negatively cooperative with NMS, seems to have a higher affinity for free than for NMS-liganded receptors. For the majority of compounds it can be concluded that structure−activity-relationships derived for NMS-occupied receptors also refer to the interaction with NMS-free receptors.

Results and Discussion

SAR in the Caracurine V Series. Except caracurine V dioxide (**33**), all investigated compounds in the caracurine V series were able to retard the dissociation of [^{3}H]NMS from porcine cardiac membranes. Since **33** is the only compound with positive charges on nitrogen atoms fully compensated by the negative charged oxygen atoms, ammonium cations seem to be important for the receptor−ligand interaction. In the series of 22 bisquaternary caracurinium V salts a wide range of allosteric potency with $EC_{0.5,diss}$ values from 4 nM to 3270 nM was observed. This indicates that the allosteric effect on [^{3}H]NMS binding to M_2 receptors is very sensitive to the properties of N-substituents at the caracurine V ring system. The most potent caracurine V analogues carry propargyl (**12**, 4 nM) and allyl (**3**, 10 nM) substituents. Saturation of the propargyl triple bonds of **12** to give the dipropyl analogue **16** resulted in a 8-fold lower binding affinity. Thus, a triple or double bond is essential for high binding. Increase in bulk, generated by cyclopropylmethyl (**17**, 14 nM) and cyclohexenyl (**15**, 20 nM) substituents, did not affect the high binding affinity remarkably. Substitution of the cyclopropane ring of **17** by an oxirane ring (**18**, 65 nM) decreased affinity (4.5-fold). Exchange of ethinyl groups of **12** with isosteric cyano groups (**19**) and with acetamide moieties (**20**) caused a dramatic decrease of allosteric potency (50-fold for **19** and 70-fold for **20**). While extension of propargyl and allyl substituents by one methyl group to give **13** and **14**, respectively, led to considerably lower binding affinity (11-fold for **13** and 7-fold for **14**), reduction of the chain length to only one methyl group resulted in only marginal change in allosteric potency (**11**, 8 nM). The absence of both N-substituents as given for the caracurine V base **6** (442 nM) produced a dramatic decrease in affinity relative to the most potent bisquaternary analogues **12** (110-fold), **3** (44-fold), and **11** (55-fold)**.** In contrast**,** removal of only one *N*-allyl group of **3** to give the monoquaternary compound **31** (18 nM) hardly affected the high allosteric potency. Introduction of longer and more bulky substituents, such as different substituted benzyl groups, resulted in a strong reduction of binding affinity. For example, dibenzylcaracurinium V dibromide (**22**) (69 nM) is about 7 times less potent than the diallyl analogue **3**. A further increase in bulk generated by different benzyl substituents such as methoxy (**25**, **26**), nitro (**27**), bromo (**28**), and trifluoromethyl (**29**) groups, as well as by an expansion to the naphthyl group (**23**), resulted in an 5−25-fold decrease of allosteric potency. The only exception is the pentafluorobenzyl derivative **24** (54 nM) with similar binding affinity as the benzyl analogue **22** (69 nM), probably due to a small volume of the fluorine atoms. The electrical effect of the benzyl substituent does not seem to be crucial for the receptor−ligand interaction as indicated by similar binding affinities of the nitro (**27**), bromo (**28**), and dimethoxy (**26**) analogues. The compound with the lowest binding

affinity in this series is the very long and bulky phenacyl analogue (**30**).

Introduction of two methoxy goups at both aromatic rings of diallylcaracurinium V dibromide (**3**) resulted in about 20-fold decrease of allosteric potency (**32**, 204 nM). This is in agreement with our findings observed in a series of bisquaternary dimers of strychnine and brucine[25] and suggests that the allosteric binding site does not tolerate additional methoxy groups at the lateral heterocyclic sides of the caracurine V ring system.

In summary, alkylation of caracurine V with simple alkyl groups resulted in a dramatic increase in affinity at the NMS-liganded M_2 receptors. The allosteric binding site on muscarinic M_2 receptors seems to best accommodate aliphatic N-substituents with a maximal length corresponding to three carbon atoms. Double or triple bonds are favorable for best allosteric potency. Increase in bulk not exceeding the substituent's length of three carbon atoms does not affect binding affinity. Introduction of longer and more bulky substituents leads to reduced allosteric potency.

Comparison with the SAR in a Quaternary Strychnine Series. The affinity and allosteric properties at the M_1-M_4 subtypes of muscarinic receptors of 15 quaternary strychnine derivatives with a number of substituents also used in this study was recently published.[20] However, the SAR of binding affinities at NMS-liganded M_2 receptors found by Gharagozloo et al. is different from those observed in our bisquaternary caracurine V series. Strychnine and caracurine V (**6**), which in simplest terms can be regarded as a dimeric strychnine, show similar binding affinities (strychnine: 525 nM,[25] caracurine V: 442 nM). Whereas N-alkylation of strychnine with simple alkyl groups such as methyl, ethyl, propyl, allyl, and propargyl was reported to result in marginal changes in affinity except for the allyl substitution (4-fold increase),[20] we observed a dramatic (15−110-fold) increase of binding affinity after double quaternization of caracurine V with identical substituents. Moreover, while CH_2CN substitution of strychnine slightly reduced binding, we observed an about 2-fold increase of allosteric potency after the same substitution of caracurine V. Finally, N-substitution of strychnine with a very bulky naphthyl group caused a 6-fold increase in binding affinity. In contrast, the corresponding dinaphthyl caracurinium V analogue (**23**) is 3.5 times less potent than caracurine V itself.

The considerably higher affinity of the most potent bisquaternary caracurine V analogues relative to the corresponding strychnine derivatives can be explained based on the pharmacophore hypothesis consisting of two positively charged groups surrounded by two aromatic ring systems.[27] The presence of only two of these pharmacophoric elements, i.e., one positive charge and one indole ring, gives rise to limited interactions of the monoquaternary strychnines with the receptor protein and, in consequence, to reduced binding affinity. On the other hand, expansion of the caracurine V ring system by double alkylation with very bulky substituents provides large-sized compounds which probably no longer fit into the receptor binding pocket.

Comparison of Caracurine V, *iso*-Caracurine V, Bisnortoxiferine, and Tetrahydrocaracurine V Ring Systems. The stereochemistry of caracurine V, toxiferine I, *iso*-caracurine V, and tetrahydrocaracurine V was recently determined by means of NMR spectroscopy and semiempirical calculations.[34] In all four ring systems, the pharmacophore is fixed in a highly fused and rigid heterocyclic skeleton. In caracurine V and *iso*-caracurine V, the pharmacophoric elements (two positively charged nitrogens surrounded by two aromatic systems) adopt a nearly identical spatial arrangment in terms of the internitrogen distance (dimethylcaracurinium V dichloride **11**: 9.67 Å; dimethyl-*iso*-caracurinium V diiodide **35**: 9.54 Å) and of the relative position of the indole rings. In the bisnortoxiferine ring system, the N^+-N^+-distance remained the same (toxiferine **1**: 9.68 Å), while the relative spatial position of the aromatic rings dramatically changed. In dimethyltetrahydrocaracurinium V dichloride (**10**), the N^+-N^+-distance is reduced to 8.80 Å, while the orientation of the aromatic rings is similar to that observed for **11** and **35**. The reduced internitrogen distance and the absence of both double bonds in **10** has no significant influence on the allosteric potency as indicated by an $EC_{0.5,diss}$ value of **10** (4 nM) which is in the same range as the binding affinities of **11** (8 nM) and **35** (14 nM). Interestingly, the allosteric potency of toxiferine I (**1**) (96 nM) is much lower than those observed for other *N*-methyl-substituted compounds suggesting that the relative orientation of the aromatic rings as given in the caracurine V and *iso*-caracurine V ring systems is required for an optimal ligand−receptor interaction. This assumption could not be confirmed in the allyl series. Alcuronium (diallylbisnortoxiferine) **2** (5 nM) is nearly as potent as the other allyl-substituted caracurine V **3** (10 nM) and *iso*-caracurine V **36** (3 nM) compounds. However, because of differences in receptor epitopes involved in binding of alcuronium (**2**) and diallylcaracurinium V (**3**),[12] the bisnortoxiferine and caracurin V ring systems should not be considered in the same (Q)SAR analysis.

In summary, the three highly potent *iso*-caracurine V analogues **35**−**37**, as well as the dimethyltetrahydrocaracurinium V salt (**10**), derive affinity from nearly the same relative spatial arrangement of indole rings as given for the caracurine V ring system. Reduction of the internitrogen distance from 9.7 Å (caracurine V salts) to 8.8 Å (**10**) did not change the high allosteric potency.

CoMSIA Studies. To supplement and validate the SARs found so far, and to obtain a model that can be used for the prediction of unsynthesized compounds, a CoMSIA[41] study was performed (for geometry optimization and alignment, see Experimental Section). Since alcuronium (**2**) was identified to bind in a different mode to the allosteric binding site,[12] the bisnortoxiferine analogues **1** and **2** were excluded from the analysis. In addition to that, **33** was excluded from the data set since no biological activity could be measured. For outlier detection leave-one-out cross-validation (LOO-CV) residuals were visually inspected and caracurine V (**6**) was identified as outlier.

The results of the CoMSIA analysis are given in Table 2. Overall model quality in terms of the cross-validated squared multiple correlation coefficient R^2_{CV-1}, often referred to as q^2, was good ($q^2 = 0.70$). To assess the robustness of the model, a repetitive 5-fold cross-

Table 2. Results of the CoMSIA Study

model	k[a]	R^2_{CV-k}[b]	RMSEP$_{CV-k}$[c]	R^2[d]	RMSEC[e]	n/n_{sel}[f]	LV[g]
CoMSIA-LOO	1	0.70	0.48	0.91	0.28	8159/n.a.	4
CoMSIA-5-fold	20%	0.57[h]	0.67[h]	0.91	0.28	8159/n.a.	3.94[h]

Abbreviations: LOO: leave-one-out cross-validation; 5-fold: repetitive 5-fold cross-validation; LMO: leave-multiple-out cross-validation. [a] k: number of objects left out. [b] R^2_{CV-k}: leave-k-out cross-validated coefficient of determination. [c] RMSEP$_{CV-k}$: leave-k-out cross-validated root-mean-squared error of prediction. [d] R^2: coefficient of determination. [e] RMSEC: root-mean-squared error of calibration. [f] n/n_{sel}: number of variables/number of selected variables. [g] LV: number of latent variables. [h] Mean value of 81 repetitions.

validation[42] was performed (81 repetitions). The resulting mean $R^2_{CV-20\%}$ of 0.57 (minimum: 0.42, maximum: 0.66) indicates that the model is quite robust since 20% of the data can be removed from the training data and can in turn be predicted with sufficient accuracy.

Interpretation of the CoMSIA plots highlighted two structural features as important for biological activity. Combined interpretation of the steric and the electrostatic field allows differentiation of the optimal substituent size at the quaternary nitrogen. Additionally, the hydrogen-bond acceptor field emphasizes the detrimental effect of atoms which are capable of hydrogen-bonding close to the quaternary nitrogen. The described characteristics are best explained with the help of examples.

Figure 1 displays the low active compound **23** (large N-substituent) and the two highly active compounds **3** and **16** (rather small N-substituents). The yellow contours of the CoMSIA plot highlight regions in space which, when occupied by a substituent, reduce biological activity. It can easily be seen that this is the case for compound **23**. When comparing compounds **3** and **16**, it can be concluded that the maximum length of the substituent lies between these two substructures, since the biological activity of **3** which is not extending inside the yellow contour is slightly higher than for **16** which is extending inside the contour. The nitrogen substitution is further described by the electrostatic field. Here, substituents need to be inspected in different regions (see Figure 2).

On one hand, the partial charge of the carbon atom directly connected to the quaternary nitrogen should be negative (red regions). This is the case when no electron-withdrawing groups (e.g. carbonyl, benzyl) are connected to this carbon atom. On the other hand, electron rich parts of the substituent should not extend within the blue colored regions of the CoMSIA plot. If this is the case (e.g. for benzyl substituents), allosteric potency is drastically decreased. One reason for this phenomenon could be that the ionic interaction of the quaternary nitrogen atom with the receptor is reduced owing to a compensation of the respective positive charge with partial negative charges of the substituent. Another explanation for this observation could be drawn from the hydrogen-bond acceptor contour map. This map shows that atoms with hydrogen-bond capabilities (e.g. the carbonyl oxygen of compound **30**, see Figure 2) which are located close to the quaternary nitrogen are detrimental for a high biological activity. Since hydrogen-bond acceptor atoms are usually incorporated in partially negative charged groups, this plot is in accordance with the electrostatic contour map.

Unexpectedly, the hydrogen-bond acceptor map, did not highlight differences in the skeleton of the structures (open ring and closed ring systems), even though

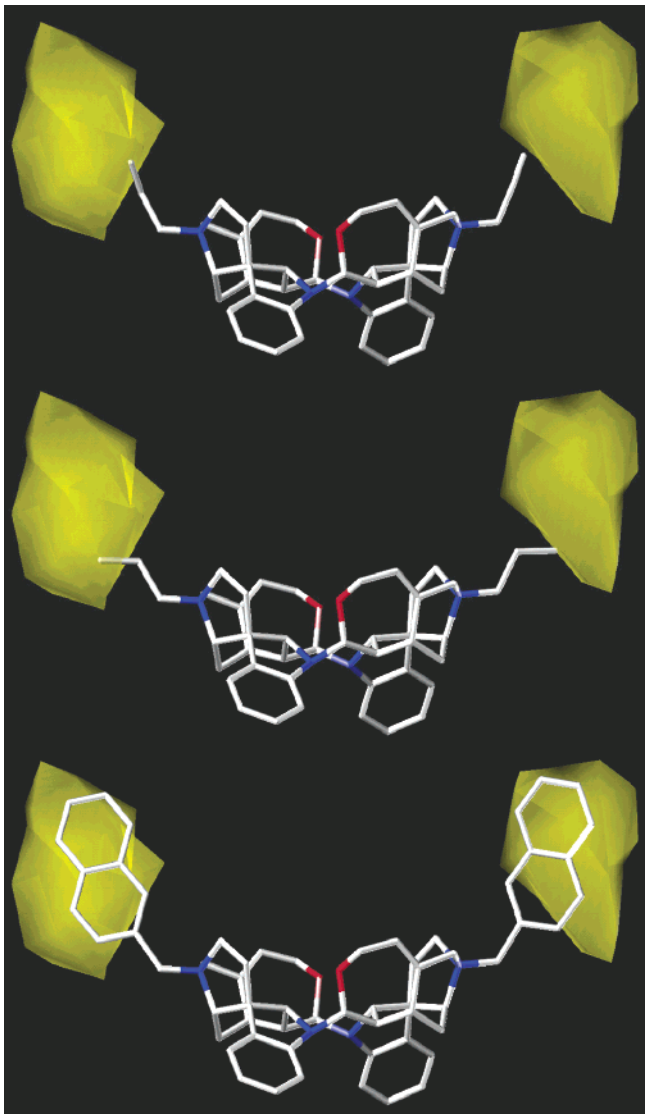


Figure 1. CoMSIA steric contour map with sterically disfavored regions colored yellow. For reasons of clarity, hydrogen atoms are not shown. Substituent size of the quaternary nitrogen is crucial for biological activity. The maximum substituent size is located between an allyl (**3**, $EC_{50} = 10$ nM, top) and a propyl group (**16**, $EC_{50} = 30$ nM, middle). Compounds with larger substiuents (e.g. **23**, $EC_{50} = 1558$ nM, bottom) show a decreased biological activity.

the oxygen atoms were identified as relevant for binding affinity (magenta colored regions in the center of Figure 3). One reason for this might be the cluster in activity-space of compounds with the *iso*-caracurine skeleton, i.e., all such compounds (**35**, **36**, **37**) included in the analysis show high allosteric potency. It should be mentioned that a 2D-QSAR analysis using a topological SESP descriptor[43] obtained a QSAR model of a comparable quality (see Supporting Information for details)

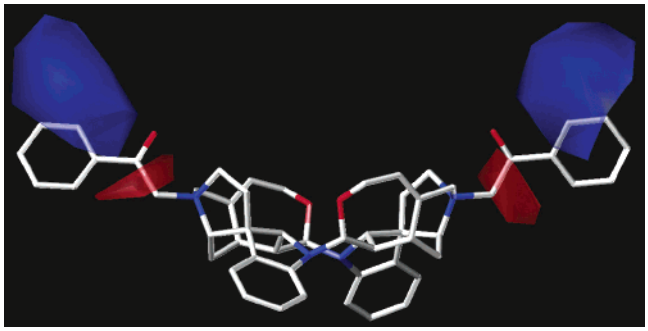


Figure 2. CoMSIA electrostatic contour map (hydrogens suppressed). Regions where partially positive and negative charge increases biological activity are colored blue and red, respectively. Electron-withdrawing substituents such as phenacyl (**30**, $EC_{0.5,diss} = 3270$ nM) reduce biological activity in two ways. On one hand they increase the partial positive charge in the red colored region, and on the other hand they extend into regions which favor a positive charged group.

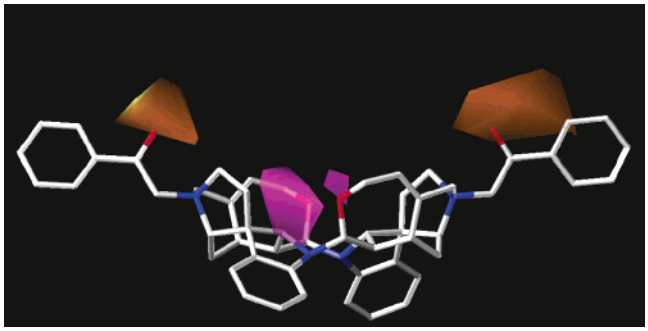


Figure 3. CoMSIA hydrogen-bond acceptor contour map (hydrogens suppressed). Corresponding regions to ligand−acceptor sites that increase or decrease biological activity are colored magenta and orange, respectively. Substituents which possess a hydrogen-bond acceptor close to the orange colored region show a low biological activity. This observation is in accordance with the electrostatic map and is found not only for the displayed compound (**30**, $EC_{0.5,diss} = 3270$ nM) but also for compounds with smaller substituents (e.g. **18**, **19**, **20**).

Summary and Outlook

In this paper we have been able to better define the pharmacophore for allosteric ligands of muscarinic M_2 receptors in terms of the relative spatial arrangement of the aromatic rings and the properties of the N-substituents using bisquaternary analogues of the alkaloids caracurine V, *iso*-caracurine V, tetrahydrocaracurine V, and bisnortoxiferine. Double quaternization of the *Strychnos* alkaloid caracurine V with nonpolar alkyl groups of a maximal chain length of three carbon atoms yielded compounds with up to 100-fold higher binding affinity for the allosteric binding site of NMS-occupied muscarinic M_2 receptors. Notable compounds are dimethyl (**3**), diallyl (**11**), and dipropargyl (**12**) caracurine V analogues. These findings are in contrast to SARs found in the monoquaternary strychnine series and confirm the pharmacophore hypothesis of two positively charged nitrogen atoms in a distance of about 10 Å surrounded by two aromatic ring systems. Two chemical modifications of the caracurine V ring system, i.e., reduction of both double bonds yielding dimethyltetrahydrocaracurinium V salt (**10**) and opening of one tetrahydrooxepine ring to give the *iso*-caracurinium V salts (**35**−**37**), maintained the high allosteric potency. This finding can be explained by the nearly identical spatial arrangement of the aromatic indole rings in all three "caracurine V" ring systems. In contrast, opening of both tetetrahydrooxepine rings of dimethylcaracurinium V dichloride (**11**) to give toxiferine I (**1**) caused a 10-fold decrease of binding affinity due to a considerable conformational change of the whole ring system. The findings suggest that the relative orientation of the aromatic rings as given in the "caracurine V" ring systems is required for an optimal ligand−receptor interaction. The SAR results could be confirmed by 3D-QSAR (CoMSIA) anylysis. The wide range of binding affinities observed for all investigated compounds could be explained by steric and electrostatic properties of the N-substituents.

All of the agents except for the negatively cooperative diphenacylcaracurinium V analogue **30** are nearly neutraly cooperative with the antagonist [^{3}H]NMS, i.e., they do not change the [^{3}H]NMS equilibrium binding at the M_2 orthosteric binding site. Thus, any effects of structural modifications on the allosteric potency are only due to changes in receptor binding affinity and not in cooperativity with [^{3}H]NMS. However, one has to keep in mind that the cooperativity between the allosteric modulators included in our study and other orthosteric ligands, especially the endogenous agonist acetylcholine, can be quite different.

Owing to the close structural relationship of caracurine V and *iso*-caracurine V analogues to the strong muscle relaxants toxiferine I and alcuronium, all investigated compounds are likely to exhibit neuromuscular-blocking activity, which would limit their usefulness as research tools and make their therapeutical use impossible. However, recent radioligand binding studies at the muscle type of nicotinic acetylcholine receptors indicated that from all ring systems under investigation, the caracurine V ring skeleton is likely to have the lowest neuromuscular blocking activity.[44] Consequently, reduction of the caracurine V ring skeleton to structural features responsible for good allosteric potency could possibly lead to potent allosteric muscarinic compounds with negligible neuromuscular blocking activity. Therefore, synthetic work on simpler heterocyclic ring systems derived from caracurine V is continued in our laboratory.[45,46]

Experimental Section

General Methods. Starting materials were prepared according to literature procedures as indicated, and if no reference is quoted, they are available commercially. Alcuronium (**2**) was a generous gift from Roche, Basel. Melting points were determined with a Gallenkamp melting point apparatus (Sanyo) and were not corrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian XL 300 and Bruker AV 400 instruments. IR spectra were obtained using a Biorad PharmalyzIR FT-IR spectrometer. IR and NMR data of all investigated compounds are given in the Supporting Information.

TLC was carried out on Merck silica gel 60 F_{254} aluminum sheets. Mass spectra of the tertiary amines were recorded on a Finnigan MAT 8200 spectrometer (70 eV). Mass spectra of the quaternary compounds were run by fast-atom-bombardment on Kratos Concept 1H and Finnigan MAT 90 mass spectrometers in 3-nitrobenzyl alcohol as matrix. Elemental analyses were performed by the microanalytical section of the Institute of Inorganic Chemistry, University of Würzburg. All reactions were carried out under an argon atmosphere.

***E*-22-Oximinostrychnine (*E*-4) and *Z*-22-Oximinostrychnine (*Z*-4).** *tert*-Butyl nitrite (80 mL) and tert-BuOK (8 g) were

added to the solution of strychnine (10.0 g, 0.03 mol) in dry toluene (500 mL). The reaction mixture was stirred at 50 °C for 2 h. After the mixture was cooled to room temp, 10% aqueous NH_4Cl solution (100 mL) was added and vigorous stirring was continued for 15 min. Water and toluene were removed in vacuo, the yellow residue was suspended in $CHCl_3$/MeOH 4:1 (300 mL), and the mixture was stirred for 30 min. The residue obtained upon filtration and evaporation of $CHCl_3$ and MeOH was purified on a silica gel column, eluting with $CHCl_3$/MeOH/25%NH_3 130:10:1 to afford two oximes.

(***Z*-4**) (1.9 g, 17%), yellow crystals; mp > 320 °C (dec); R_f = 0.44 ($CHCl_3$/MeOH/25% NH_3 130:10:1); $[\alpha]^{22}_D$ −471° (*c* 0.57, DMSO); MS *m/e* 363 (M^+, 100), 346 (87), 318 (57). Anal. ($C_{21}H_{21}N_3O_3$) C, H, N.

(***E*-4**) (6.8 g, 62%), yellow crystals; mp > 320 °C (dec); R_f = 0.22 ($CHCl_3$/MeOH/25% NH_3 130:10:1); $[\alpha]^{22}_D$ −265° (*c* 0.58, DMSO); MS *m/e* 363 (M^+, 100), 346 (90), 318 (38).

General Procedure for the Synthesis of Wieland−Gumlich Aldehyde (5) and 10,11-Dimethoxy-Wieland−Gumlich Aldehyde (5a). The appropriate oxime was added to thionyl chloride (100 mL) under ice-cooling within 20 min, and the mixture was stirred at room temperature for 2.5 h. Thionyl chloride was removed under reduced pressure at 40 °C, and the residue was treated with 2 M HCl (200 mL) **(caution!, HCN evolution**). The resulting mixture was stirred for 3 h at 100 °C. The cold solution was made alkaline with 25% ammonia and extracted with $CHCl_3$/*n*-butanol 10:1 (5 × 100 mL). The combined organic layers were washed with water (50 mL), dried over $MgSO_4$, and evaporated to give a brown foam which was chromatographed on neutral alumina containing 10% H_2O, eluting with $CHCl_3$/toluene 5:1.

Wieland−Gumlich Aldehyde (5). 5 was prepared from the reaction of *E*-**4** (6 g, 16.5 mmol) according to the general procedure. Yield 4.1 g (80.0%); mp 215 °C (Lit.[36] 208−210 °C). **5** exists in DMSO solution as a mixture of 17-(*S*) and 17-(*R*) epimers.

10,11-Dimethoxy-Wieland−Gumlich Aldehyde (5a). 5a was prepared from the reaction of *E*-**4a** [25](6 g, 14 mmol) according to the general procedure. Yield 0.9 g (17.0%); mp 210 °C (dec); MS *m/e* 370, (M^+,100), 204 (54), 180 (53). Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

10,11,10′,11′-Tetramethoxycaracurine V (6a). 5a (600 mg, 1.6 mmol) was heated for 14 h with 5 g of pivalic acid at 105 °C in an evacuated sealed tube. Water (50 mL) was added, and the cool reaction mixture was made basic with 25% ammonia. Extraction with $CHCl_3$, drying over $MgSO_4$, and evaporation of the extract gave a brown foam which was chromatographed on neutral alumina containing 10% H_2O, eluting with $CHCl_3$/toluene 3:1 to give **6a** as colorless crystalline solid (105 mg, 18%). mp > 320 °C (dec); MS *m/e* 704 (M^+, 45), 703 (M^+-1, 100), 685 (27). Anal. ($C_{42}H_{48}N_4O_6$) C, H, N.

17-(*R*)-Cyano-19,20-didehydro-11,12-dimethoxy-17,18-epoxycurane (7). *E*-**4a** (8.5 g, 20 mmol) was added to thionyl chloride (100 mL) under ice-cooling within 20 min, and the mixture was stirred at room temperature for 2.5 h. Thionyl chloride was removed under reduced pressure at 40 °C, and water (100 mL) was added, followed by 1 M Na_2CO_3 until pH 3. Steam was passed through the reaction mixture for 1.5 h. **(caution!, HCN evolution**). The cold solution was made alkaline with 25% ammonia and extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with water (50 mL), dried over $MgSO_4$, and evaporated to give a brown foam which was chromatographed on neutral alumina containing 10% H_2O, eluting with $CHCl_3$/toluene 1:1 to give **7** as colorless crystalline solid (1.7 g, 23%). mp 188 °C; $[\alpha]^{22}_D$ −197° (*c* 0.79, $CHCl_3$); MS *m/e* 379 (M^+, 100) (11), 364 (42), 204 (38), 189 (82), HRMS ($C_{22}H_{25}N_3O_3$) found *m/e* 379.1900, calcd 379.1896. Anal. ($C_{22}H_{25}N_3O_3$) C, H, N.

4,4′-Dimethyl-(20*S*,20′*S*)-19,20,19′,20′-tetrahydrocaracurinium V Dichloride (10). PtO_2 (15 mg) was added to an aqueous solution (20 mL) of **11** (75 mg, 0.1 mmol). The reaction mixture was hydrogenated under the hydrogen pressure of 50 bar for 24 h. The catalyst was removed by filtration through Celite, and the filter pad was washed with water (2 × 10 mL). Evaporation of the solvent afforded NMR pure colorless crystals of **10** in quantitative yield, mp > 300 °C (dec); $[\alpha]^{22}_D$ −117° (*c* 0.46, DMSO); MS−FAB *m/e* 655 and 653 ($M^{2+}Cl^-$), 617 (M^{2+} − H^+). Anal. ($C_{40}H_{50}N_4O_2Cl_2$ H_2O) C, H, N.

General Double Quaternization Procedure of Caracurine V (6), *iso*-Caracurine V (34), and Tetramethoxycaracurine V (6a). The solution of the respective halide (0.85 mmol) in chloroform (5 mL) was added to a solution of the alkaloid (100 mg, 0.17 mmol) in chloroform (5 mL). After being stirred at room temperature for 30 min, the crystallized ammonium salt was isolated by filtration. If no crystallization occurred, the product was precipitated by adding diethyl ether. The collected ammonium salt was washed with chloroform or with a chloroform/diethyl ether mixture 1:1 and dried in a vacuum at 80 °C. No further purification was necessary as indicated by TLC (silica gel, mobile phase MeOH/2 M NH_3/2 M aqueous NH_4NO_3 84:24:12) and ^1H NMR spectra.

Melting points of all caracurinium V and *iso*-caracurinium V salts are higher than 300 °C (dec).

4,4′-Diallylcaracurinium V Dibromide (3). Obtained from **6** and allyl bromide (102 mg) as a white solid; yield 130 mg (92%); $[\alpha]^{22}_D$ +95° (*c* 0.54, DMSO); FAB-MS *m/e* 747.2 and 745.2 ($M^{2+}Br^-$), 665.3 (M^{2+} − H^+), 625.1. Anal. ($C_{44}H_{50}N_4O_2Br_2$) C, H, N.

4,4′-Dimethylcaracurinium V Dichloride (11).[34] A solution of the precipitated iodide in water was passed through "Amberlite" IRA-400 resin, chloride phase and water was evaporated under reduced pressure; yield 80 mg (68%); FAB-MS *m/e* 651.3 and 649.3 ($M^{2+}Cl^-$), 613.2 (M^{2+} − H^+), 556.1. $C_{40}H_{46}N_4O_2Cl_2$.

4,4′-Dipropargylcaracurinium V Dibromide (12). Obtained from **6** and propargyl bromide (101 mg) as a white solid; yield 135 mg (96%); $[\alpha]^{22}_D$ +47° (*c* 0.44, DMSO); FAB-MS *m/e* 743.2 and 741.2 ($M^{2+}Br^-$), 661.2 (M^{2+} − H^+), 623.3. Anal. ($C_{44}H_{46}N_4O_2Br_2$) C, H, N.

4,4′-Bis(2-butyn-1-yl)caracurinium V Dibromide (13). Obtained from **6** and 1-bromo-2-butyne (113 mg) as a white solid; yield 115 mg (79%); $[\alpha]^{22}_D$ +73° (*c* 0.55, DMSO); FAB-MS *m/z* 771.2 and 769.2 ($M^{2+}Br^-$), 689.3 (M^{2+} − H^+), 637.3. Anal. ($C_{46}H_{50}N_4O_2Br_2$), C, H, N.

4,4′-Bis(*trans*-2-buten-1-yl)caracurinium V Dibromide (14). Obtained from **6** and *trans*-1-bromo-2-butene (115 mg) as a white solid; yield 110 mg (75%); $[\alpha]^{22}_D$ +91° (*c* 0.30, DMSO); FAB-MS *m/e* 775.4 and 773.4 ($M^{2+}Br^-$), 693.5 (M^{2+} − H^+), 639.3. Anal. ($C_{46}H_{54}N_4O_2Br_2$), C, H, N.

4,4′-Bis(cyclohexene-3-yl)caracurinium V Dibromide (15). The mixture of three diastereomers was obtained from **6** and 3-bromo-1-cyclohexene (137 mg) as a white solid; yield 140 mg (90%); $[\alpha]^{22}_D$ +37° (*c* 0.58, DMSO); FAB-MS *m/e* 827.4 and 825.4 ($M^{2+}Br^-$), 745.4 (M^{2+} − H^+), 665.4. Anal. ($C_{50}H_{58}N_4O_2Br_2$) C, H, N.

4,4′-Bis(cyclopropylmethyl)caracurinium V Dibromide (17). Obtained from **6** and (bromomethyl)cyclopropane (115 mg) as a white solid; yield 80 mg (55%); $[\alpha]^{22}_D$ +53° (*c* 0.62, DMSO); FAB-MS *m/e* 775.3 and 773.3 ($M^{2+}Br^-$), 693.4 (M^{2+} − H^+), 655.3. Anal. ($C_{46}H_{54}N_4O_2Br_2$) C, H, N.

4,4′-Bis(1-oxiranylmethyl)caracurinium V Dibromide (18). The mixture of three diastereomers was obtained from **6** and epibromohydrine (117 mg) as a white solid; yield 70 mg (48%); $[\alpha]^{22}_D$ +63° (*c* 0.45, DMSO); FAB-MS *m/e* 779.4 and 777.4 ($M^{2+}Br^-$), 697.4 (M^{2+} − H^+), 641.3. Anal. ($C_{44}H_{50}N_4O_4Br_2$) C, H, N.

4,4′-Bis(cyanomethyl)caracurinium V Dibromide (19). Obtained from **6** and bromoacetonitrile (117 mg) as a white solid; yield 120 mg (85%); $[\alpha]^{22}_D$ +69° (*c* 0.56, DMSO); FAB-MS *m/e* 745.3 and 743.3 4 ($M^{2+}Br^-$), 663.3 (M^{2+} − H^+). Anal. ($C_{42}H_{44}N_6O_2Br_2$) C, H, N.

4,4′-Bis(carbamoylmethyl)caracurinium V Dibromide (20). Obtained from **6** and 2-bromoacetamide (117 mg) as a white solid; yield 80 mg (54%); $[\alpha]^{22}_D$ +70° (*c* 0.45, DMSO); FAB-MS *m/e* 781.3 and 779.3 (M^{2+} − Br^-), 699.3 (M^{2+} − H^+). Anal. ($C_{42}H_{48}N_6O_4Br_2$) H, N, C: calcd, 58.61; found, 58.10.

4,4′-Dibenzylcaracurinium V Dibromide (22). Obtained from **6** and benzyl bromide (145 mg) as a white solid; yield

140 mg (88%); $[\alpha]^{22}_D$ +82° (*c* 0.51, DMSO); FAB-MS *m/e* 847.3 and 845.3 ($M^{2+}Br^-$), 765.4 ($M^{2+} - H^+$), 675.4. Anal. ($C_{52}H_{54}N_4O_2Br_2$) C, H, N.

4,4′-Bis(2-naphthyl)caracurinium V Dibromide (23). Obtained from **6** and 2-naphthyl bromide (188 mg) as a white solid; yield 140 mg (80%); $[\alpha]^{22}_D$ +52° (*c* 0.50, DMSO); FAB-MS *m/e* 947.4 and 945.4 ($M^{2+}Br^-$), 865.4 ($M^{2+} - H^+$). Anal. ($C_{60}H_{58}N_4O_2Br_2$) C, H, N.

4,4′-Bis(pentafluorobenzyl)caracurinium V Dibromide (24). Obtained from **6** and pentafluorobenzyl bromide (222 mg) as a white solid; yield 110 mg (58%); FAB-MS *m/e* 1027.9 and 1025.9 ($M^{2+}Br^-$), 945.9 ($M^{2+} - H^+$). Anal. ($C_{52}H_{44}N_4O_2Br_2F_{10}$) C, H, N.

4,4′-Bis(4-methoxybenzyl)caracurinium V Dichloride (25). Obtained from **6** and 4-methoxybenzyl chloride (133 mg) as a white solid; yield 115 mg (75%); $[\alpha]^{22}_D$ +99° (*c* 0.11, DMSO); FAB-MS *m/e* 864.3 and 862.3 ($M^{2+}Cl^-$), 826.4 ($M^{2+} - H^+$), 705.3. Anal. ($C_{54}H_{58}N_4O_4Cl_2$) C, H, N.

4,4′-Bis(3,4-dimethoxybenzyl)caracurinium V Dichloride (26). Obtained from **6** and 3,4-dimethoxybenzyl chloride[47] (160 mg) as a white solid; yield 130 mg (79%); $[\alpha]^{22}_D$ +89° (*c* 0.56, DMSO); FAB-MS *m/e* 924.2 and 922.2 ($M^{2+}Cl^-$), 886.2 ($M^{2+} - H^+$). Anal. ($C_{56}H_{62}N_4O_6Cl_2$) C, H, N.

4,4′-Bis(4-nitrobenzyl)caracurinium V Dibromide (27). Obtained from **6** and 4-nitrobenzyl bromide (184 mg) as a yellow solid; yield 170 mg (96%); $[\alpha]^{22}_D$ +54° (*c* 0.58, DMSO); FAB-MS *m/e*, 937.1 and 935.1 ($M^{2+}Br^-$), 855.4 ($M^{2+} - H^+$). Anal. ($C_{54}H_{52}N_6O_6Br_2$) C, H, N.

4,4′-Bis(4-bromobenzyl)caracurinium V Dibromide (28). Obtained from **6** and 4-bromobenzyl bromide (213 mg) as a white solid; yield 145 mg (78%); $[\alpha]^{22}_D$ +63° (*c* 0.55, DMSO); FAB-MS *m/e* 1007.1, 1005.1, 1003.1 and 1001.1 ($M^{2+}Br^-$), 921.2 and 923.2 ($M^{2+} - H^+$). Anal. ($C_{52}H_{52}N_4O_2Br_4$) C, H, N.

4,4′-Bis-[4-(trifluoromethyl)-benzyl]-caracurinium V Dibromide (29). Obtained from **6** and 4-(trifluoromethyl)-benzyl bromide (203 mg) as a white solid; yield 155 mg (85%); $[\alpha]^{22}_D$ +55° (*c* 0.63, DMSO); FAB-MS *m/e* 984.3 and 982.3 ($M^{2+}Br^-$) 902.3 ($M^{2+} - H^+$). Anal. ($C_{54}H_{52}N_4O_2Br_2F_6$) C, H, N.

4,4′-Diphenacylcaracurinium V Dibromide (30). Obtained from **6** and phenacyl bromide (170 mg) as a white solid; yield 140 mg (81%); $[\alpha]^{22}_D$ +88° (*c* 0.18, DMSO); FAB-MS *m/e* 936.3 and 934.3 ($M^{2+}Br^-$) 854.3 ($M^{2+} - H^+$). Anal. ($C_{54}H_{54}N_4O_6Br_2$) H, N, C: calcd, 63.91; found, 63.46.

4,4′-Dimethyl-*iso*-caracurinium V Diiodide (35). Obtained from **34** and methyl iodide (120 mg) as a white solid; yield 130 mg (88%); FAB-MS *m/e* 741.1 ($M^{2+}I^-$), 613.2 ($M^{2+} - H^+$). Anal.($C_{40}H_{46}N_4O_2I_2$) C, H, N.

4,4′-Diallyl-*iso*-caracurinium V Dibromide (36). Obtained from **34** and allylbromide (103 mg) as a white solid; yield 125 mg (88%); $[\alpha]^{22}_D$ +77° (*c* 0.34, DMSO); FAB-MS *m/e* 747.1 and 745.1 ($M^{2+}Br^-$), 665.2 ($M^{2+} - H^+$), 625.2. Anal. ($C_{44}H_{50}N_4O_2Br_2$) C, H, N.

4,4′-Bis(cyclohexene-3-yl)-*iso*-caracurinium V Dibromide (37). The mixture of three diastereomers was obtained from **34** and 3-bromocyclohexene as a white solid; yield 115 mg (74%); $[\alpha]^{22}_D$ +101° (*c* 0.65, DMSO); FAB-MS *m/e* 827.3 and 825.3 ($M^{2+}Br^-$), 745.3 ($M^{2+} - H^+$), 665.3. Anal. ($C_{50}H_{58}N_4O_2Br_2$) C, H, N.

4, 4′-Bisallyl-(10,11,10′,11′-tetramethoxycaracurinium V Dibromide (32). Obtained from **6a** (60 mg, 0.09 mmol) and allylbromide (54 mg, 0.45 mmol) as a white solid; yield 60 mg (85%); $[\alpha]^{22}_D$ +52° (*c* 0.53, DMSO); FAB-MS *m/e* 868.2 and 866.1 ($M^{2+}Br^-$), 786.3 ($M^{2+} - H^+$), 745.3. Anal. ($C_{44}H_{50}N_4O_2Br_2$) C, H, N.

4-Allylcaracurinium V Bromide (31). The solution of diallyl bromide (20 mg, 0.17 mmol) in chloroform (5 mL) was added to a solution of **6** (500 mg, 0.86 mmol) in chloroform (5 mL). After being stirred at room temperature for 30 min, the product was precipitated by adding diethyl ether. The collected ammonium salt was washed with a chloroform/diethyl ether mixture 1:1 and dried in a vacuum at 80 °C. No further purification was necessary as indicated by TLC (silica gel, mobile phase MeOH/2 M NH_3/2 M aqueous NH_4NO_3 84:24:12) and ^{1}H NMR spectrum. **31** was obtainedas a white solid; yield 97 mg (81%); $[\alpha]^{22}_D$ +39° (*c* 0.56, DMSO); FAB-MS *m/e* 625.2 (M^+-Br^-) Anal. ($C_{41}H_{45}N_4O_2Br$) C, H, N.

General Procedure for Synthesis of 4, 4′-Dipropylcaracurinium V Dichloride (16) and 4,4′-Bis[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propyl]caracurinium V Dichloride (21). 6 (100 mg, 0.17 mmol) was added to the solution of propyl iodide (146 mg, 0.86 mmol) and *N*-(3-iodopropyl)-phthalimide (268 mg, 0.86 mmol),[48] respectively, in acetone and $CHCl_3$, respectively. After being heated under reflux for 30 min, the crystallized ammonium salts were isolated by filtration and washed with acetone and $CHCl_3$, respectively. Aqueous solutions of the iodides were passed through "Amberlite" IRA-400 resin, chloride phase, and water was evaporated under reduced pressure. No further purification was necessary as indicated by TLC (silica gel, mobile phase MeOH/2 M NH_3/2 M aqueous NH_4NO_3 84:24:12) and ^{1}H NMR spectra.

16: Obtained as a white solid; yield 84 mg (65%);$[\alpha]^{22}_D$ +129° (*c* 0.26, DMSO); FAB-MS *m/e* 707.4 and 705.4 ($M^{2+}Cl^-$), 669.4 ($M^{2+} - H^+$). Anal. ($C_{44}H_{54}N_4O_2Cl_2$ H_2O) C, H, N.

21: Obtained as a white solid; yield 115 mg (64%); $[\alpha]^{22}_D$ +39° (*c* 0.56, DMSO); FAB-MS *m/e* 997.4 and 995.4 ($M^{2+}Cl^-$) 959.3 ($M^{2+} - H^+$). Anal. ($C_{60}H_{60}N_6O_6Cl_2$ H_2O) C, H, N.

Pharmacology. Porcine cardiac homogenates were prepared as described previously.[11] Binding of [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) (0.2 nM; specific activity 70−83.5 Ci/mmol; Perkin-Elmer Life Sciences, Boston, MA) was measured in a buffer composed of 4 mM Na_2HPO_4 and 1 mM KH_2PO_4 (pH 7.4) at 23 °C. Nonspecific [^{3}H]NMS binding was determined in the presence of 10^{-6} M atropine and was less than 10% of the total binding. Membranes were separated by rapid vacuum filtration through glass fiber filters (Schleicher and Schüll, No. 6; Dassel, Germany). Filters were washed twice with 5 mL of ice-cold distilled water and placed into scintillation vials containing 5 mL of scintillation fluid (Ready protein, Beckman, Palo Alto, CA), and membrane bound radioactivity was determined by liquid scintillation counting in a Beckman LS 6000 counter. The pK_D of NMS binding amounted to 9.52 ± 0.04 (mean ± SEM, $n = 3$).

Two types of experiments were applied to evaluate the effect of the test compounds on the dissociation for [^{3}H]NMS. In the case of "complete dissociation experiments" cardiac membranes were preincubated with 0.2 nM [^{3}H]NMS for 30 min; radioligand dissociation was then revealed by the addition of 1 μM atropine in the presence or absence of allosteric test compound. The time course of dissociation was observed by withdrawing aliquots at appropriate times over a period of 120 min. Data were analyzed by means of nonlinear regression analysis. [^{3}H]-NMS dissociation proceeded monophasically in the absence and in the presence of test compound ($t_{1/2,control} = 4.35 \pm 0.09$ min; mean ± SE, $n = 52$) and was characterized by the apparent rate constant of dissociation k_{-1}. In "two-point kinetic experiments"[49] membranes were incubated with [^{3}H]NMS for 30 min to obtain binding equilibrium. Specific [^{3}H]NMS binding was measured before ($t = 0$ min) and after ($t = 10$ min) the addition of 1 μM atropine alone or in combination with a test compound. Specific [^{3}H]NMS binding at $t = 0$ min and $t = 10$ min served to characterize the monoexponential time course of [^{3}H]NMS dissociation and to obtain the apparent rate constant of dissociation k_{-1}. To generate concentration-effect curves for the allosteric retardation of radioligand dissociation, the apparent rate constant of dissociation k_{-1} was expressed as a percentage of the value under control conditions. Curve fitting was based on a four parameter logistic function (GraphPad Prism Version 3.02; GraphPad, San Diego, CA).

Equilibrium binding expermints were conducted using 0.2 nM [^{3}H]NMS. The effect of the test compounds on [^{3}H]NMS equilibrium binding was investigated at concentrations of allosteric modulator that were shown to effectively reduce the rate of [^{3}H]NMS dissociation to 25% of the control. The appropriate time of incubation was determined according to Lazareno and Birdsall[40] (eq 31 therein). Five half-lives were taken to ensure equilibrium binding conditions. Equilibrium

binding data (from two to five independent experiments with quadruplicated values) in the presence of allosteric modulator were expressed in percent relative to the control binding in the absence of modulator, which was set as 100%.

Molecular Modeling. Geometry Optimization and Alignment. The three different ring skeletons of **6**, **10**, and **34** [34] were selected as template structures for the alignment procedure necessary for CoMSIA studies. As a first step, the structures were aligned using SYBYL's multifit procedure.[50] For this alignment, the torsional angle between atoms C12−C13−N−C17′ found in the AM1 calculations[34] were constrained to their current value. Then the corresponding quaternary nitrogen atoms and the centroids of the aromatic rings C8−C13 and C8′−C13′ were aligned with spring constants of 20 and 10, respectively. The resulting alignment was very good. The remaining compounds of the data set were built based on these template structures. For the latter step, the backbone of the template structures was kept fixed, and only the N-substituents were geometry optimized. For all optimization steps, the Tripos force field with a Powell minimizer was applied for 300 iterations. All other options were set to their respective default values. Compounds with unsubstituted nitrogen were ionized. After the alignment step, AM1 charges were calculated for all structures (MOPAC keywords: 1SCF AM1 MMOK).

3D-QSAR. CoMSIA fields were generated using SYBYL.[50] The CoMSIA region was automatically defined and all available fields (steric, electrostatic, acceptor, donor, and hydrophobic) were calculated. Coefficient contour maps showing the product of standard deviation and coefficient ("StdDev*Coeff") were used for interpretation purposes. To identify appropriate contour levels, field value histograms for each feature were analyzed, and levels that gave meaningful results were applied. It should be noted that contour maps showing steric and electrostatic fields highlight those regions of the ligand which enhance or decrease activity. In contrast to that, CoMSIA plots for hydrogen bonding properties highlight areas where hydrogen-bond donor and hydrogen-bond acceptor features could be located within a hypothetical receptor site.

Only the steric, electrostatic, and hydrogen-bond acceptor CoMSIA field were applied in this study since preliminary knowledge gained from the SAR study motivated their use (see Results and Discussion). The first two fields were thought to be useful in identifying the patterns of the N-substitution, whereas the hydrogen-bond acceptor field was thought to be useful for identifying the different skeletons (open and closed ring systems).

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Supporting Information Available: IR and ^{1}H and ^{13}C NMR data of all compounds and a 2D-QSAR model. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Stockton, J. M.; Birdsall, N. J. M.; Burgen, A. S. V.; Hulme, E. C. Modification of the binding properties of the muscarinic receptors by gallamine. *Mol. Pharmacol.* **1983**, *23*, 551−557.
(2) Lee, N. H.; El-Fakahany, E. E. Allosteric antagonists of the muscarinic acetylcholine receptor *Biochem. Pharmacol.* **1991**, *42*, 199−205.
(3) Ellis, J. Allosteric binding site on muscarinic receptors. *Drug Dev. Res.* **1997**, *40*, 193−204.
(4) Christopoulos, A.; Lanzafame, A.; Mitchelson, F. Allosteric interactions at muscarinic cholinoceptors. *Clin. Exp. Pharmacol. Phys.* **1998**, *25*, 185−194.
(5) Holzgrabe, U.; Mohr, K. Allosteric modulation of antagonist binding to muscarinic receptors. *Drugs Discovery Today* **1998**, *3*, 214−222.
(6) Christopoulos, A.; Kenakin, T. G-Protein-coupled receptor allosterism and complexing. *Pharmacol. Rev.* **2002**, *54*, 323−374.
(7) Christopoulos, A. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. *Nat. Rev. Drug Discuss.* **2002**, *1*, 198−210.
(8) Ellis, J.; Huyler, J.; Brann, M. R. Allosteric regulations of cloned m1−m5 muscarinic receptor subtypes. *Biochem. Pharmacol.* **1991**, *42*, 1927.
(9) Tränkle, C.; Mies-Klomfass, E.; Botero Cid, M. H.; Holzgrabe, U.; Mohr, K. Identification of a [^{3}H]ligand for the common allosteric site of muscarinic acetylcholine M_2 receptors. *Mol. Pharmacol.* **1998**, *54*, 139−145.
(10) Ellis, J.; Seidenberg, M. Two allosteric modulators interact at a common site on cardiac muscarinic receptors. *Mol. Pharmacol.* **1992**, *42*, 638−641.
(11) Tränkle, C.; Mohr, K. Divergent modes of action among cationic allosteric modulators of muscarinic M_2 receptors. *Mol. Pharmacol.* **1997**, *51*, 674−682.
(12) Buller, S.; Zlotos, D. P.; Mohr, K.; Ellis, J. Allosteric site on muscarinic acetylcholine receptors: a single amino acid in transmembrane region 7 is critical to the subtype selectivities of caracurine V derivatives and alkane-bisammonium ligands. *Mol. Pharmacol.* **2002**, *61*, 160−168.
(13) Tränkle, C.; Kostenis, E.; Burgmer, U.; Mohr, K. Search for lead structures to develop new allosteric modulators of muscarinic receptors. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 926−933.
(14) Bejeuhr, G.; Blaschke, G.; Holzgrabe, U.; Mohr, K.; Sürig, U.; Terfloth, G. Molecular modelling and synthesis of potent stabilizers of antagonist binding to M_2-cholinoceptors. *J. Pharm. Pharmacol.* **1994**, *46*, 108−112.
(15) Botero Cid, M. H.; Holzgrabe, U.; Kostenis, E.; Mohr, K.; Tränkle, C. Search for the pharmacophore of bispyridinium-type allosteric modulators of muscarinic receptors. *J. Med. Chem.* **1994**, *37*, 1439−1445.
(16) Botero Cid, H. M.; Tränkle, C.; Baumann, K.; Pick, R.; Mies-Klomfass, E.; Kostenis E.; Mohr, K.; Holzgrabe, U. Structure−activity relationships in a series of bisquaternary phthalimidine derivatives modulating the muscarinic M_2-receptor allosterically. *J. Med. Chem.* **2000**, *43*, 2155−2164.
(17) Raasch, A.; Scharfenstein, O.; Tränkle, C.; Holzgrabe, U.; Mohr, K. Elevation of ligand binding to muscarinic M_2 acetylcholine receptors by bis(ammonio)alkane-type allosteric modulators. *J. Med. Chem.* **2002**, *45*, 3809−3812.
(18) Muth, M.; Bender, W.; Scharfenstein, O.; Holzgrabe, U.; Balatkova, E.; Tränkle, C.; Mohr, K. Systematic development of high affinity bis(ammonio)alkane-type allosteric enhancers of muscarinic ligand binding. *J. Med. Chem.* **2003**, *46*, 1031−1040.
(19) Nassif-Makki, T.; Tränkle, C.; Zlotos, D.; Bejeuhr, G.; Cambareri, A.; Pfletschinger, C.; Kostenis, E.; Mohr, K.; Holzgrabe, U. Bisquaternary ligands of the common allosteric site of M_2 acetylcholine receptors: optimization of the distances between the pharmacophoric elements. *J. Med. Chem.* **1999**, *42*, 849−858.
(20) Gharagozloo, P.; Lazareno, S.; Popham, A.; Birdsall, N. J. M. Allosteric interactions of quaternary strychnine and brucine derivatives with muscarinic acetylcholine receptors. *J. Med. Chem.* **1999**, *42*, 438−445.
(21) Urbanský, M.; Proška, J.; Ričny, J.; Drašar, P. Truxillic acid derivatives, neuromuscular blocking agents with very high affinity for the allosteric binding site of muscarinic acetylcholine receptors. *Collect. Czech, Chem. Commun.* **1999**, *64*, 1980−1992.
(22) Lysiková, M.; Fuksová, K.; Elbert, T.; Jakubik, J.; Tuček, S. Subtype-selective inhibition of [methyl-3H]-*N*-methylscopolamine binding to muscarinic receptors by alpha-truxillic acid esters. *Br. J. Pharmacol.* **1999**, *127*, 1240−1246.
(23) Urbanský, M.; Proška, J.; Drašar, P. On steroids. CDII. Fused thiazoloandrostanes and their quaternary salts, synthesis and cooperative ligand binding to muscarinic acetylcholine receptor. *Collect. Czech, Chem. Commun.* **1999**, *64*, 1457−1470.
(24) Gharagozloo, P.; Lazareno, S.; Miyauchi, M.; Popham, A.; Birdsall, N. J. M. Substituted pentacyclic carbazoles as novel muscarinic allosteric agents: synthesis and structure-affinity and cooperativity relationships. *J. Med. Chem.* **2002**, *45*, 1259−1274.
(25) Zlotos, D. P.; Buller, S.; Holzgrabe, U.; Mohr, K. Bisquaternary dimers of strychnine and brucine. A new class of potent enhancers of antagonist binding to muscarinic M_2 receptors. *Bioorg. Med. Chem.* **2003**, *11*, 2627−2634.
(26) Holzgrabe, U.; Wagener, M.; Gasteiger, J. Comparison of structurally different allosteric modulators of muscarinic receptors by self-organizing neural networks. *J. Mol. Graphics* **1996**, *14*, 185−193.
(27) Holzgrabe, U.; Hopfinger, A. J. Conformational analysis, molecular shape comparison, and pharmacophore identification of different allosteric modulators of muscarinic receptors. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 1018−1024.

(28) Stiefl, N.; Baumann, K. Mapping property distributions of molecular surfaces: algorithm and evaluation of a novel 3D quantitative structure−activity relationship technique. *J. Med. Chem*. **2003**, *46*, 1390−1407.
(29) Tuček, S.; Musílková, J.; Nedoma, J.; Proška, J.; Shelkovnikov, S.; Vorlíček, J. Positive cooperativity in the binding of alcuronium and *N*-methylscopolamine to muscarinic acetylcholine receptors *J. Mol. Pharmacol.* **1990**, *38*, 674−680.
(30) Zlotos, D. P.; Buller, S.; Tränkle, C., Mohr, K. Bisquaternary caracurine V derivatives as allosteric modulators of ligand binding to M_2 acetylcholine receptors. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2529−2532.
(31) Voigtlander, U.; Jöhren, K.; Mohr, M.; Raasch, A.; Tränkle, C.; Buller, S.; Ellis, J**.;** Höltje, H.-D.; Mohr, K. Allosteric site on muscarinic acetylcholine receptors: identification of two amino acids in the muscarinic M_2 receptor that account entirely for The M_2/M_5 subtype selectivities of some structurally diverse allosteric ligands in *N*-methylscopolamine-occupied receptors *Mol. Pharmacol*. **2003,** *64 (1)* 21−31.
(32) Zlotos, D. P. Stereochemistry of caracurine V. *J. Nat. Prod*. **2000**, *63*, 864−865.
(33) Zlotos, D. P. *iso*-Caracurine V, a novel unexpected decomposition product of caracurine V. *J. Nat. Prod*. **2003**, *66*, 119−120.
(34) Zlotos, D. P. Stereochemistry of caracurine V, *iso*-caracurine V, bisnortoxiferine and tetrahydrocaracurine V ring systems. *Eur. J. Org. Chem*. **2004**, *11*, 2375−2380.
(35) Battersby, A. R.; Hodson, H. F. Alkaloids of Calabash Curare and Strychnos species. Part I. Chemistry and structure of hemitoxiferine-I and toxiferineI, including the preparation of toxiferine-I. *J. Chem. Soc.* **1960**, 736−739.
(36) Hymon, J. R.; Schmid, H.; Karrer; P.; Boller, A.; Els, H.; Fahrni, P.; Fürst, A. Die Chemie des Wieland-Gumlich-Aldehyds und seiner Derivate. *Helv. Chim. Acta* **1969**, 52, 1564−1602.
(37) Jackmann, J. M.; Sternhall, S. In *Application of nuclear NMR spectroscopy in organic chemistry*; Pergamon Press Inc.: New York, 1969; p 226.
(38) Kalinowski, H. O.; Berger, S.; Braun, S. In *^{13}C NMR-Spektroskopie*; Georg Thieme Verlag: Stuttgart, New York, 1984; p 220.
(39) Verpoorte, R.; Baerheim Svendsen, A. Alkaloids of *Strychnos dolichothyrsa* Gilg ex Onochie et Hepper. *J. Pharm. Sci*. **1978**, *67*, 171−174.
(40) Lazareno, S.; Birdsall, N. J. M. Detection, quantitation, and verification of allosteric interactions of agents with labeled and unlabeled ligands at G protein-coupled receptors: interactions of strychnine and acetylcholine at muscarinic receptors *Mol. Pharmacol*. **1995**, *48*, 362−378.
(41) Klebe, G.; Abraham, U,: Mietzner, T. Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. *J. Med. Chem*. **1994**, *37*, 4130−4146.
(42) Baumann, K.; Albert, H.; von Korff, M. A systematic evaluation of the benefits and hazards of variable selection in latent variable regression. Part I: search algorithm, theory and simulations. *J. Chemom*. **2002**, *16*, 339−350.
(43) Baumann, K. An alignment-independent versatile structure descriptor for QSAR and QSPR based on the distribution of molecular features. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 26−35.
(44) Zlotos, D. P.; Gündisch, D.; Ferraro, S.; Tilotta, B. Unpublished results.
(45) Sripha, K., Zlotos D. P. 6,7,14,15-Tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole. Synthesis of a novel pentacyclic ring system. *Tetrahedron* **2003**, *59*, 391−394.
(46) Sripha, K.; Zlotos, D. P.; Buller, S.; Mohr, K. 6,7,14,15-Tetrahydro-*15a*H-azocino[1,2-a:6,5-b′]diindole. Synthesis of a novel pentacyclic ring system. *Tetrahedron Lett*. **2003**, *44*, 7183−7186.
(47) Zlotos D. P., Meise W.: Synthesis and conformational analysis of hexahydroisoquino-[3,2-b][3]benzazepines (*iso*-B-homoberbines). *Heterocycles* **1997**, *45*, 2137−2157.
(48) Ollivier, C.; Renaud, P. A novel approach to formation of carbon−nitrogen bonds: azidation of alkyl radicals with sulfonyl azides. *J. Am. Chem. Soc.* **2001**, *123*, 4717−4727.
(49) Kostenis, E.; Mohr, K. Two-point kinetic experiments to quantify allosteric effects on radioligand dissociation *Trends Pharmacol. Sci*. **1996**, *17*, 280−283.
(50) SYBYL 6.8 Tripos Inc., 1699 South Hanley Rd., St. Louis, MO 63144, 2000.

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