Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1481–1485

6*H*,13*H*-Pyrazino[1,2-*a*;4,5-*a*′]diindole analogs: Probing the pharmacophore for allosteric ligands of muscarinic M_2 receptors

Darius P. Zlotos,[a,*] Christian Tränkle,[b] Aliaa Abdelrahman,[c] Daniela Gündisch,[c] Krzysztof Radacki,[d] Holger Braunschweig[d] and Klaus Mohr[b]

[a]*Pharmaceutical Institute, University of Würzburg, Am Hubland, 97074 Würzburg, Germany*
[b]*Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Bonn, Gerhard-Domagk-Str. 3, 53121 Bonn, Germany*
[c]*Pharmaceutical Institute, University of Bonn, Kreuzbergweg 26, 53115 Bonn, Germany*
[d]*Institute of Inorganic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany*

Received 21 November 2005; revised 12 December 2005; accepted 12 December 2005
Available online 4 January 2006

Abstract—A series of 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole analogs was synthesized in order to probe the pharmacophore hypothesis for allosteric ligands of muscarinic M_2 receptors. The 3D structure of the novel ring system was determined by means of NMR spectroscopy and X-ray diffraction revealing a totally flat geometry. Low binding affinities for the [^{3}H]*N*-methylscopolamine-occupied M_2 receptors (reflected by $EC_{50,diss}$) indicated that the spatial arrangement of the pharmacophore elements (two aromatic rings flanked by two cationic centers) incorporated in the bisquaternary analogs **5** and **6** is unfavorable for strong ligand–receptor interactions. Due to the structural similarity of the novel compounds to neuromuscular-blocking agents, their affinities (reflected by K_i) to the muscle type of nicotinic acetylcholine receptors were also determined. The dimethyl and diallyl analogs **5** and **6** exhibited rather high affinities to the muscle type of nicotinic acetylcholine receptors, suggesting a pronounced neuromuscular-blocking activity. Compound **5** showed a 34-fold higher affinity for the muscle type nAChR than for the allosteric site of M_2 receptors.

Apart from the conventional acetylcholine binding site, all five muscarinic receptor subtypes (M_1–M_5) contain a second, allosteric binding domain. Allosteric ligands could offer several advantages over classical ones, including greater subtype selectivity and saturability of their effect.[1,2] According to a pharmacophore hypothesis, potent allosteric ligands of muscarinic M_2 receptors should incorporate two positively charged nitrogen atoms at a distance of approximately 10 Å flanked by two aromatic ring systems. The relative spatial arrangement of the pharmacophoric elements was recently examined using the rigid caracurine *V* and related ring systems.[3,4] SAR and QSAR studies suggested that the relative orientation of the aromatic rings as given in the caracurine *V* scaffold as well as N-quaternization with non-polar alkyl groups of a maximal chain length of three carbon atoms are required for an optimal ligand–receptor interaction.

To explore the pharmacophore model, we synthesized several analogs of the 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole ring system and determined their binding affinities to cardiac muscarinic M_2 receptors. The novel ring system incorporates the pharmacophore elements in a considerably different 3D arrangement when compared to the caracurine *V* scaffold. Due to the structural similarity of the novel compounds to neuromuscular-blocking agents, we also determined their binding constants to the muscle-type of the nicotinic acetylcholine receptors. The findings reported here are important for the development of potent allosteric modulators of muscarinic M_2 receptors with negligible neuromuscular blocking activity (Fig. 1).

Condensation of the commercially available (2*S*)-(−)-indoline-2-carboxylic acid using DCC in THF afforded the 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole-6,13-dione **1**,[5] which was converted to 6a,7,13a,14-tetrahydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole **2** by reduction of both carbonyl groups with borane in THF.[6] Dehydrogenation of the indoline moieties of compound **2** yielding 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole **3** was

Keywords: Muscarinic M_2 receptors; Allosteric ligands.
* Corresponding author. Tel.: +0931 8885489; fax: +0931 8885494; e-mail: zlotos@pzlc.uni-wuerzburg.de

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.030

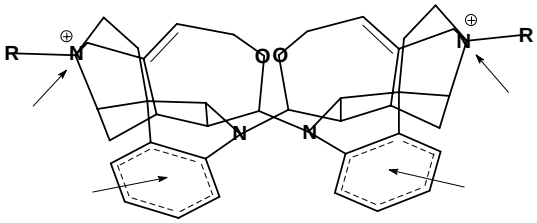


Figure 1. 3D-structure of caracurinium *V* salts[3] with pharmacophore elements marked by arrows.

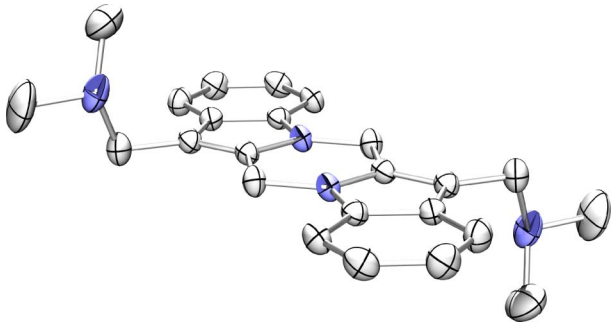


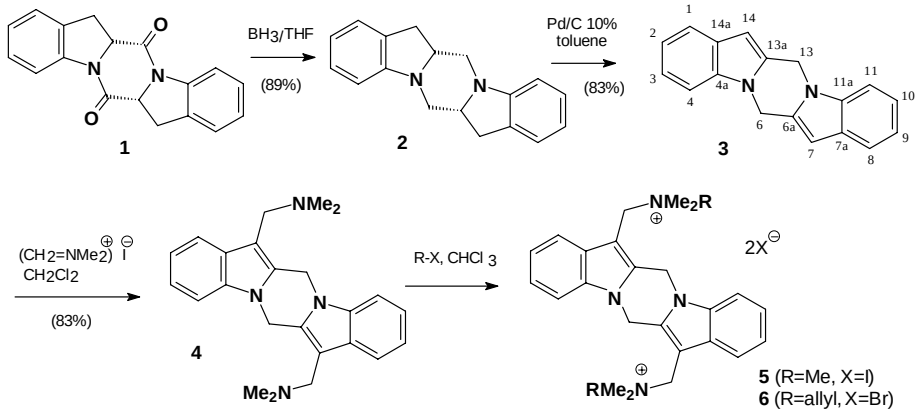
Figure 2. X-ray structure (Ortep-Plot 50%) of compound **4**.[11]

accomplished by heating compound **2** at reflux in toluene with Pd/C 10%.[7] The methylamine side chains were introduced by means of Mannich reaction using dimethylmethylenammonium iodide (Eschenmoser's salt) in CH_2Cl_2.[8] The resulting Mannich base **4** was double quaternized using methyliodide and allylbrimide in $CHCl_3$ yielding the methyl and the allyl ammonium salts **5** and **6**, respectively.[9] (see Scheme 1).

To determine the relative spatial arrangement of the pharmacophore elements, the 3D-structure of compound **4** was elucidated by means of NMR spectroscopy, semiempirical calculations, and single crystal X-ray diffraction. Both ^1H and ^{13}C NMR spectra of **4** displayed only a single set of signals, indicating a symmetrical 3D-structure. Moreover, the methylene protons within the central six-membered ring (6-CH_2 and 13-CH_2) appeared as a singlet (4H, $\delta = 5.4$ ppm). The magnetic equivalence of the protons at C6 (and C13) is only possible, when the central piperazine ring adopts a fully flat conformation which implies a totally flat geometry of the whole 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole ring system. The plane 3D structure was also obtained as a result of semiempirical AM1 calculations (PC SPARTAN[10]) irrespective of the starting conformations of the central piperazine ring. The proposed flat 3D structure was confirmed by X-ray crystallography of compound **4**.[11] In the crystal structure, the methylamine side chains are arranged symmetrically at the opposite sides of the pentacyclic ring system. Consequently, the whole molecule possesses an inversion center located in the middle of the central piperazine ring (see Fig. 2).

The allosteric action of the test compounds on the dissociation of the conventional, orthosteric radioligand [^{3}H]*N*-methylscolamine ([^{3}H]NMS) was studied in homogenates of porcine heart ventricles in Na,K,Pi-buffer (4 mM Na_2HPO_4, 1 mM KH_2PO_4, pH 7.4, 23 °C).[12] Dissociation assays were used to determine the affinity of the allosteric agents at receptors in which access to the orthosteric site was blocked by [^{3}H]NMS. All compounds investigated were able to retard the dissociation of [^{3}H]NMS concentration dependently. To generate concentration–effect curves, the apparent rate constant of dissociation k_{-1} was expressed as a percentage of the value under control conditions. The concentration of an allosteric agent for a half-maximum effect on orthosteric ligand dissociation ($EC_{50,diss}$) corresponds to a 50% occupancy of the liganded receptors by the respective allosteric test compound.[12] Thus, $EC_{50,diss}$ indicates the equilibrium dissociation constant (K_D) of allosteric ligand binding to the [^{3}H]NMS-occupied receptor and reflects the affinity of the test compound to the allosteric binding site. $pEC_{50,diss}$-values of compounds **4**–**6** as well as of the equally substituted caracurine *V* analogs[4] are compiled in Table 1.

Both tertiary analogs **4** and caracurine *V* exhibited relatively poor allosteric potencies ($pEC_{50,diss}$ = 6.36 nM and 6.35, respectively). Whereas the double N-alkylation of caracurine *V* with methyl and allyl groups produced a pronounced increase of allosteric potency (55-fold for R = Me, 44-fold for R = allyl), the corresponding



Scheme 1.

Table 1. Binding constants for the inhibition of [^{3}H]NMS dissociation from muscarinic M_2 receptors by the indicated compounds in Na,K,Pi-buffer (for details, see text)

Compound	$pEC_{50,diss}$[a]
Caracurine *V*[4]	6.36 ± 0.04
Dimethylcaracurine *V*[4]	8.09 ± 0.02
Diallylcaracurine *V*[4]	7.95 ± 0.08
4	6.35 ± 0.05
5	6.64 ± 0.09
6	6.96 ± 0.04

[a] Given are mean values ± SEM of three experiments performed as duplicate determinations.

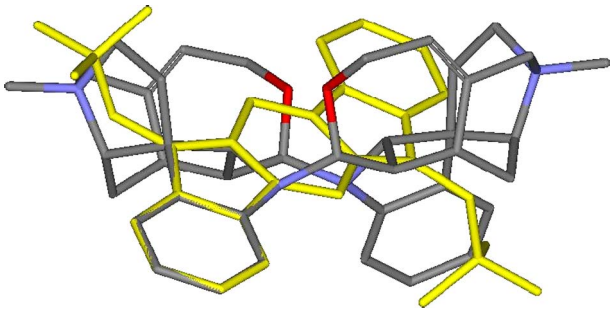


Figure 3. Superposition of compound **5** (yellow) onto dimethylcaracurine *V* (fitting atoms are all six carbon atoms of one aromatic ring).

bisquaternary pyrazinodiindole analogs **5** ($pEC_{50,diss}$ = 6.64 nM) and **6** ($pEC_{50,diss}$ = 6.96) showed only slightly higher binding affinity than the tertiary compound **4**. The findings are likely to be explained by considerably different positions of the pharmacophore elements in both ring systems. As shown in Figure 3, while one aromatic ring and the adjacent quaternary nitrogen adopt similar spatial orientation in both caracurine *V* and pyrazinodiindole analogs, the position of the aromatic ring and the quaternary head on the opposite side are totally different in both ring systems. However, despite different 3D structures, the intercationic distance is the same in both ring scaffolds (9.7 Å).[3,13]

Possessing a bisquaternary structure with the positive charges separated by a distance of ca. 10 Å, the pyrazinodiindole ammonium salts **5** and **6** are likely to exhibit neuromuscular-blocking activity[14] which would limit their usefulness as muscarinic research tools. To assess their neuromuscular-blocking potential, the equilibrium binding constants (K_i) of the compounds at the muscle type of nicotinic ACh receptors (nAChR) from the membrane fractions of *Torpedo californica* electric organ were determined in a binding assay using (±)-[^{3}H]epibatidine as a radioligand.[15] pK_i-values of compounds **4**–**6** as well as of the equally substituted caracurine *V* analogs[15] are compiled in Table 2.

As expected, the bistertiary pyrazinodiindole analog **4** exhibited a very poor affinity to the muscle type nAChR, suggesting a poor neuromuscular-blocking activity (pK_i = 4.97). In contrast, for the bisquaternary dimethyl analog **5** a pK_i-value of 6.48 was determined, indicating high binding affinity. The diallyl analog **6** (pK_i = 6.26) was only slightly less potent than the dimethyl derivative. Both compounds showed affinities comparable to that of the neuromuscular-blocking drug alcuronium (*N*,*N*′-diallylbisnortoxiferine,[16] pK_i = 6.62).[15] The high binding affinities of the pyrazinodiindole analogs **5** and **6** to the muscle type nAChR suggest their neuromuscular-blocking potential being considerably higher than those of the corresponding caracurine *V* analogs.

To assess the muscarinic/nicotinic selectivity of the new compounds, one has to compare the binding constants for the allosteric binding site of the M_2 receptor ($EC_{50,diss}$) with the binding constants at the muscle type nAChR (K_i). However, as the aforementioned binding assays were conducted under different buffer conditions ($EC_{50,diss}$ in 'Na,K,P_i-buffer': 4 mM Na_2HPO_4, 1 mM KH_2PO_4, pH 7.4; K_i in 'Hepes-buffer':15 mM Hepes, 120 mM NaCl, 5.4 mM KC1, 0.8 mM $MgCl_2$, and 1.8 mM $CaCl_2$, pH 7.4), and especially the $EC_{50,diss}$-values are known to be strongly affected by the buffer conditions,[17] the direct comparison of the values from Tables 1 and 2 could not be appropriate. Therefore, the $EC_{50,diss}$ values of the pyrazinodiindole analogs **4**–**6** were determined additionally under the Hepes-buffer conditions used in the studies with nAChRs (Table 3).

Whereas the rank order of potency (**6** > **5** > **4**) observed in the Na,K,P_i-buffer (Table 1) remained the same in the Hepes-buffer (Table 3), the buffer switch was accompanied by a loss of allosteric potency which is in agreement with previous studies with other allosteric ligands.[17] In this respect, it has to be mentioned that, for example, Mg^{2+}, a component of the Hepes-buffer, is known to be an allosteric antagonist at M_2 receptors[18,19] and thus decreases modulator potency. Anyhow, under identical conditions the dimethyl analog **5** shows a 34-fold higher affinity for the muscle type nAChR than for the allosteric site of M_2 receptors. The diallyl analog **6** displays a

Table 2. Binding constants of the indicated compounds for the muscle type nAChR in Hepes-buffer (for details, see text)

Compound	pK_i[a]
Caracurine *V*[15]	<4.0
Dimethylcaracurine *V*[15]	5.28
Diallylcaracurine *V*[15]	5.82
4	4.97 ± 0.02
5	6.48 ± 0.03
6	6.26 ± 0.02

[a] Given are mean values ± SEM of three experiments performed as duplicate determinations.

Table 3. Comparison of the binding constants for the allosteric site of muscarinic M_2 receptors ($EC_{50,diss}$) and for the muscle type nAChR (K_i) in Hepes buffer (for details, see text)

Compound	$pEC_{50,diss}$[a]	pK_i[a]	EC_{50}/K_i
4	4.78 ± 0.04	4.97 ± 0.02	1.5
5	4.95 ± 0.03	6.48 ± 0.03	34
6	5.64 ± 0.05	6.26 ± 0.02	4.2

[a] Given are mean values of three experiments performed as duplicate determinations.

rather low nicotinic selectivity ($EC_{50}/K_i = 4$). The data have revealed that the bistertiary compound **4** exhibits equal affinities toward the muscarinic and nicotinic receptors tested.

In summary, to probe the pharmacophore hypothesis for allosteric ligands of muscarinic M_2 receptors, analogs of the 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole ring system **4**–**6** were synthesized. The 3D structure of the novel ring system was determined by means of NMR spectroscopy and X-ray diffraction revealing a totally flat geometry. Low binding affinities to the [^{3}H]NMS-occupied M_2 receptors ($EC_{50,diss}$) indicated that the spatial arrangement of the pharmacophore elements (two aromatic rings flanked by two cationic centers) incorporated in the bisquaternary analogs **5** and **6** is unfavorable for strong ligand–receptor interactions when compared to the corresponding caracurine *V* analogs. Interestingly, compounds **5** and **6** exhibited higher affinities (K_i) to the muscle type of nicotinic acetylcholine receptors compared with M_2 receptors whose orthosteric site is blocked by NMS, suggesting a pronounced neuromuscular-blocking activity. The largest difference was seen with the dimethyl analog **5** possessing a 34-fold higher affinity for the muscle type nAChR than for the allosteric site of M_2 receptors.

Acknowledgment

The authors are very grateful to Mechthild Kepe (University of Bonn, Institute of Pharmacy) for skillfull technical assistance.

References and notes

1. Birdsall, N. J. M.; Lazareno, S. *Mini-Rev. Med. Chem.* **2005**, *5*, 523.
2. Mohr, K.; Holzgrabe, U. *Recept. Channels* **2003**, *9*, 229.
3. Zlotos, D. P. *Eur. J. Org. Chem.* **2004**, *11*, 2375.
4. Zlotos, D. P.; Buller, S.; Stiefl, N.; Baumann, K.; Mohr, K. *J. Med. Chem.* **2004**, *47*, 3561.
5. Somei, M.; Kawasaki, T. *Heterocycles* **1996**, *42*(1), 281.
6. Synthesis of (6a*S*,13a*S*)-6a,7,13a,14-tetrahydro-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole **2**. 1 M Borane-THF solution (30 mL) was added to an ice-cooled and stirred solution of **1** (1.87 g; 6.44 mmol)[5] in dry THF (150 mL). The reaction mixture was heated for 17 h at reflux, allowed to cool, and 30 mL of 2 M HCl was added under ice-cooling. After heating for 1/2 h at reflux, the reaction mixture was made basic with 25% ammonia under ice-cooling and extracted with CH_2Cl_2. The combined organic layers were washed with water and dried over $MgSO_4$. The solvent was removed in vacuo to give compound **2** (1.50 g, 89%) as a colorless crystalline solid, which was used for the following step without further purification. FT-IR (ATR) ν (cm^{-1}) 2922, 2822, 1601, 1481, 1458; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (m, 4H, H-1, H-3, H-8, H-10), 6.66 (t, 2H, J = 7.3 Hz, H-2, H-9), 6.38 (d, 2H, J = 7.8 Hz, H-4, H-11), 4.45 (dddd, 2H, J = 12.2, 9.6, 3.3, 3.1 Hz, H-6a, H-13a), 3.46 (dd, 2H, J = 10.2, 3.3 Hz, H^b-6, H^b-13), 3.28 (dd, 2H, J = 16.2, 9.6 Hz, H^b-7, H^b-14), 2.97 (2H, dd, J = 12.2, 10.2 Hz, H^a-6, H^a-13), 2.73 (dd, 2H, J = 16.2, 3.0 Hz, H^a-7, H^a-14); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6 (C-4a, C-11a), 128.0 (C-7a, C-14a), 127.6 (C-3, C-10), 124.4 (C-1, C-8), 118.3 (C-2, C-9), 107.8 (C-4, C-11), 56.3 (C-6a, C-13a), 51.3 (C-6, C-13), 32.9 (C-7, C-14).
7. Synthesis of 6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole **3**. 0.85 g Pd/C 10% was added to a solution of compound **2** (1.5 g, 5.8 mmol) in toluene (100 mL). The reaction mixture was heated for 4 h under reflux. Pd/C was filtered off through a pad of Celite®545 and washed with toluene. The solvent was removed in vacuo and the residue was purified by silica gel chromatography (CH_2Cl_2–hexane, 1:2) to give compound **3** (1.23 g, 83%) as a colorless crystalline solid. Mp: 230 °C; FT-IR (ATR) ν (cm^{-1}) 1547, 1470, 1451, 1433; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 2H, J = 7.8 Hz, H-1, H-8), 7.42 (d, 2H, J = 8.1 Hz, H-4, H-11), 7.25 (m, 2H, H-3, H-10), 7.16 (m, 2H, H-2, H-9), 6.52 (s, 2H, H-7, H-14), 5.49 (s, 4H, CH_2-6, CH_2-13); ^{13}C NMR (100 MHz, $CDC1_3$) δ 135.6 (C-4a, C-11a), 130.2 (C-6a, C-13a), 128.4 (C-7a, C-14a), 121.4 (C-3, C-10), 120.6 (C-1, C-8), 120.4 (C-2, C-9), 108.8 (C-4, C-11), 97.4 (C-7, C-14), 40.5 (C-6, C-13); MS (EI, 70 eV) *m*/*z* (rel int) 258.0 [M^+] (84), 257.1 (100), 256.1 (38), 128.1 (46); Anal. Calcd for $C_{18}H_{14}N_2$: C, 83.69; H, 5.46; N, 10.84. Found: C, 83.47; H, 5.65; N, 10.64.
8. Synthesis of 6,14-bis[dimethyl(aminomethyl)]-6*H*,13*H*-pyrazino[1,2-*a*;4,5-*a*′]diindole **4**. Dimethylmethylenammonium iodide (350 mg, 1.9 mmol) was added to a solution of compound **3** (200 mg, 0.77 mmol) in dry CH_2Cl_2 (150 mL). After heating for 1 h at reflux, the reaction mixture was made basic with 25% ammonia. The organic layer was separated, washed with water, and dried over $MgSO_4$. The solvent was removed in vacuo and the residue was purified by silica gel chromatography ($CHCl_3$–methanol–25% ammonia, 100:10:1) to give compound **4** (240 g, 83%) as a colorless crystalline solid. Mp: 212 °C; FT-IR (ATR) ν (cm^{-1}) 2938, 2760, 1614, 1570, 1461, 1444; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, 2H, J = 7.8 Hz, H-1, H-8), 7.47 (d, 2H, J = 8.1 Hz, H-4, H-11), 7.26 (m, 2H, H-3, H-10), 7.18 (m, 2H, H-2, H-9), 5.51 (s, 4H, CH_2-6, CH_2-13), 3.69 (s, 4H, 2× CH_2–NMe_2), 2.31 (s, 12H, 2× NMe_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.4 (C-4a, C-11a), 128.7 (C-6a, C-13a), 128.5 (C-7a, C-14a), 121.4 (C-3, C-10), 120.2 (C-2, C-9), 119.0 (C-1, C-8), 108.8 (C-4, C-11), 107.0 (C-7, C-14), 53.5 (2× *C*H_2–NMe_2), 45.4 (2× NMe_2), 39.4 (C-6, C-13); MS (CI, 70 eV) *m*/*z* (rel int) 327.1 [M^+−$HNMe_2$] (8), 282.1 (100); HRMS (ESI, pos.) $C_{24}H_{29}N_4{\cdot}H^+$: *m*/*z* calcd 373,2392, *m*/*z* found 373,2395.
9. Synthesis of compounds **5** and **6**. 0.5 mL MeI and allylbromide, respectively, was added to a solution of compound **4** (75 mg, 0.20 mmol) in $CHCl_3$ (10 mL). After stirring at rt for 30 min, the precipitated colorless ammonium salts **5** (100 mg, 76%), and **6** (110 mg, 89%), respectively, were isolated by filtration, washed with cold $CHCl_3$, and dried in vacuo at 100 °C. Compound **5**: FT-IR (ATR) ν (cm^{-1}) 1615, 1562, 1473, 1448; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.95 (d, 2H, J = 7.8 Hz, H-1, H-8), 7.89 (d, 2H, J = 8.1 Hz, H-4, H-11), 7.43 (m, 2H, H-3, H-10), 7.33 (m, 2H, H-2, H-9), 5.86 (s, 4H, CH_2-6, CH_2-13), 4.91 (s, 4H, 2× CH_2–NMe_3^+), 3.21 (s, 18H, 2× NMe_3^+); ^{13}C NMR (100 MHz, DMSO-d_6): δ 135.2 (C-4a, C-11a), 135.1 (C-6a, C-13a) 128.1 (C-7a, C-14a), 122.1 (C-3, C-10), 121.2 (C-2, C-9), 118.9 (C-1, C-8), 110.2 (C-4, C-11), 97.8 (C-7, C-14), 59.2 (2× *C*H_2–NMe_3^+), 51.4 (2× NMe_3^+), 39.3 (C-6, C-13); Anal. Calcd for $C_{26}H_{34}N_4I_2H_2O$: C, 46.31; H, 5.38; N, 8.31. Found: C, 46.85; H, 5.24; N, 8.32. Compound **6**: FT-IR (ATR) ν (cm^{-1}) 1615, 1562, 1473, 1445; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, 2H, J = 8.1 Hz, H-4, H-11), 7.96 (d, 2H, J = 7.8 Hz, H-1, H-8), 7.40 (m, 2H, H-3, H-10), 7.32 (m, 2H, H-2, H-9), 6.26 (m, 2H, 2× C*H*=CH_2) 5.94 (s, 4H, CH_2-6, CH_2-13), 5.73 (m, 4H, J = 15.2 Hz, –CH=CH_2), 5.03 (s,

4H, 2× CH_2–NR_3^+), 4.25 (d, 4H, J = 7.1 Hz, 2× –Me_2N^+–CH_2–), 3.12 (s, 12H, 2× NMe_2^+); ^{13}C NMR (100 MHz, DMSO-d_6) δ 135.4 (C-4a, C-11a), 135.2 (C-6a, C-13a) 128.2 (C-7a, C-14a), 127.5 (–CH=CH_2), 126.2 (–CH=CH_2), 122.1 (C-3, C-10), 121.2 (C-2, C-9), 118.9 (C-1, C-8), 110.6 (C-4, C-11), 97.4 (C-7, C-14), 64.7 (Me_2N^+–CH_2–), 58.9 (CH_2–$NRMe_2^+$), 47.9 ($NRMe_2^+$), 39.7 (C-6, C-13); Anal. Calcd for $C_{30}H_{38}N_4Br_2$: C, 58.64; H, 6.23; N, 8.31. Found: C, 59.07; H, 5.88; N, 8.78.

10. PC SPARTAN 1.1, 1996, Wavefunction, Inc., Irvine, CA 92612, USA.
11. The crystal data of **4** were collected at Bruker Apex diffractometer with CCD area detector and graphite monochromated $Mo_{k\alpha}$ radiation. The structure was solved using direct methods, refined with Shelx software package (G. Sheldrick, University of Göttingen 1997), and expanded using Fourier techniques. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned idealized position and were included in structure factor calculations. Crystal data for **4**: $C_{24}H_{28}N_4$, M_r = 372.50, Monoclinic space group $C2/c$, a = 20.372(3) Å, b = 5.2026(7) Å, c = 20.919(3) Å, α = 90.00°, β = 115.541(2)°, γ = 90.00°, V = 2000.6(5) Å^3, Z = 4, ρ_{calcd} = 1.237 g cm^{-3}, μ = 0.074 cm^{-2}, $F(000)$ = 800, T = 193(2) K, R_1 = 0.0684, wR^2 = 0.1560, 1984 independent reflections [$2\theta \leqslant 52.16°$] and 127 parameters. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-284553. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
12. Tränkle, C.; Mohr, K. *Mol. Pharmacol.* **1997**, *51*, 674.
13. The structure of the bisquaternary compound **5** was determined by means of semiempirical calculations (AM1) of PC SPARTAN using the X-ray structure of compound **4** as a starting geometry.
14. Zlotos, D. P. *Mini-Rev. Med. Chem.* **2005**, *5*(6), 595.
15. Zlotos, D. P.; Gündisch, D.; Ferraro, S.; Tilotta, B.; Stiefl, N.; Baumann, K. *Bioorg. Med. Chem.* **2004**, *12*, 6277.
16. Alcuronium can be formally derived from *N*,*N*′-diallylcaracurine V by two intramolecular alcohol eliminations from the central eight-membered ring.[3]
17. Tränkle, C.; Kostenis, E.; Burgmer, U.; Mohr, K. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 926.
18. Burgmer, U.; Schulz, U.; Tränkle, C.; Mohr, K. *Naunyn-Schmiedeberger's Arch. Pharmacol.* **1998**, *357*, 363.
19. Tränkle, C.; Dittmann, A.; Schulz, U.; Weyand, O.; Buller, S.; Jöhren, K.; Heller, E.; Birdsall, N. J.; Holzgrabe, U.; Ellis, J.; Höltje, H. D.; Mohr, K. *Mol. Pharmacol.* **2005**, *68*, 1597.