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Bisquaternary Dimers of Strychnine and Brucine. A New Class of Potent Enhancers of Antagonist Binding to Muscarinic M_2 Receptors

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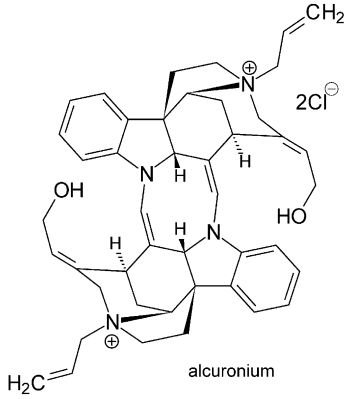
Abstract—Bisquaternary dimers of strychnine and brucine were synthesized and their allosteric effect on muscarinic acetylcholine M_2 receptors was examined. The compounds retarded the dissociation of the antagonist [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) from porcine cardiac cholinoceptors. This action indicated ternary complex formation. All compounds exhibited higher affinity to the allosteric site of [^{3}H]NMS-occupied M_2 receptors than the monomeric strychnine and brucine, while the positive cooperativity with NMS was fully maintained. SAR studies revealed the unchanged strychnine ring as an important structural feature for high allosteric potency.

Introduction

All five muscarinic receptor subtypes have allosteric binding domains in addition to the orthosteric conventional ligand binding site.[1–5] The cardiac M_2 receptor appears to be especially sensitive to allosteric modulation;[2,6,7] its allosteric site is well-defined[8–10] and affinity data for a number of structurally different compounds have been reported for M_2 receptors occupied by [^{3}H]*N*-methylscopolamine ([^{3}H]NMS).[11] Orthosteric and allosteric ligands and the receptor interact with each other according to the ternary complex model of allosteric interactions.[1,12] The allosteric effect on ligand equilibrium binding reflects the cooperativity of interaction. Negative cooperativity is indicated by a reduction of equilibrium binding; neutral cooperativity means no change of equilibrium binding upon formation of the ternary complex; positive cooperativity is indicated by an increase of equilibrium binding. In the case of muscarinic allosteric compounds, the formation of a ternary complex generally results in an inhibition of dissociation of the orthosteric ligand, such as [^{3}H]NMS. Thus, an inhibition of [^{3}H]NMS dissociation is indicative of an allosteric interaction.

Since the neuromuscular blocker alcuronium has been shown to increase the binding of NMS to the M_2 subtype of muscarinic receptors,[13] a number of other positively cooperative compounds have been identified. The alkaloids strychnine,[14,15] brucine,[16] eburnamonine[17] and a series of quaternary strychnine and brucine analogues[16] were reported to be positively cooperative with NMS at M_2 receptors. However, their affinities for liganded M_2 receptors were considerably lower than that of alcuronium, which limits their usefulness as research tools. Recently, some very potent pentacyclic carbazolones also showed positive cooperativity with NMS at M_2 receptors.[18] The poor allosteric potency of strychnine



alcuronium

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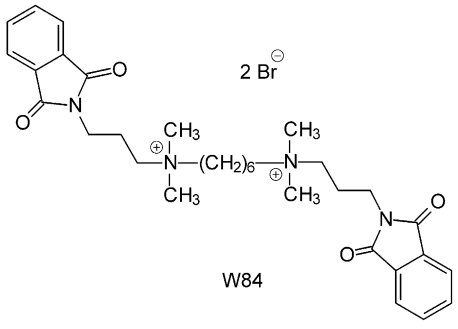
and its quaternary analogues compared to alcuronium is in accordance with the pharmacophore model for highly potent ligands at NMS-occupied M_2 receptors.[19,20] The pharmacophoric elements are two positively charged nitrogens at a distance of about 10 Å surrounded by two aromatic ring systems. A monoquaternary strychnine analogue, which in simplest terms can be regarded as a half of alcuronium, has only one positively charged nitrogen and one aromatic indole ring and should, thus, interact with fewer receptor binding domains than alcuronium.

Bis(ammonio)alkane-type allosteric modulators derived from the lead compound W84[21–23] were for a long time regarded as negatively cooperative with NMS at M_2 receptors. Even the highly potent analogue dimethyl-W84 revealed a weak inhibitive effect on NMS binding.[9] Recently, we reported that an introduction of methyl groups into the propyl side chain of W84 and its derivatives[24,25] as well as the exchange of one positively charged nitrogen by a silicon atom[26] switched the allosteric action from negative to positive cooperativity.



W84

In this paper, we examined the effect of quaternization of two strychnine, dihydrostrychnine, brucine and 22-oximinobrucine molecules with various dibromohalogenides on the affinity and cooperativity with the antagonist NMS at muscarinic M_2 receptors. The resulting bisquaternary strychnine and brucine dimers include all pharmacophoric elements for highly potent allosteric modulators and are therefore expected to exhibit a higher affinity to the allosteric binding site than strychnine, brucine and their monoquaternary analogues. With respect to cooperativity, the new compounds may take advantage of two structural features found in other positively cooperative agents. They comprise strychnine or brucine units and can be regarded as W84 analogues with completely rigid side chains.

Results and Discussion

Chemistry

19,20-Dihydrostrychnine (**1**) was obtained by catalytic hydrogenation of strychnine in 10% aqueous acetic acid using Pd/C 10% in 95% yield according to the procedure of Oxford et al.[27] Due to single sets of signals in both ^1H and ^{13}C NMR spectra of **1**, addition of hydrogen to the C19–C20 double bond proceeded diastereo-

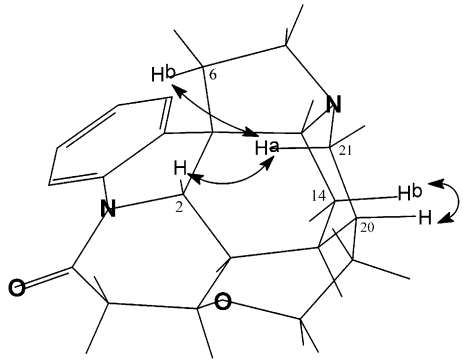
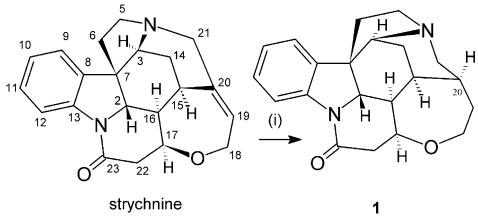


Figure 1. NOE interactions ($CDCl_3$, 600 MHz) indicating the chair conformation of the piperidine ring in dihydrostrychnine (**1**).

selectively from the less hindered side of the strychnine ring system (Scheme 1). The absolute configuration of the new chiral atom C-20 was determined by a 600 MHz NOESY experiment. A strong NOE effect between H-14b (δ 2.14), which has an axial orientation to the piperidine ring, and H-20 (δ 2.35) revealed the axial position of H-20 in the piperidine ring. The resulting (*R*)-configuration for C-20 is in agreement with the stereochemistry of the C19-C20 bond determined in 19,20-dihydrostrychnine crystals by X-ray analysis.[28] Moreover, like in the crystal structure, the piperidine ring conformation has changed upon hydrogenation from a boat form in strychnine to a chair form in **1** as indicated by NOE interactions displayed in Figure 1.

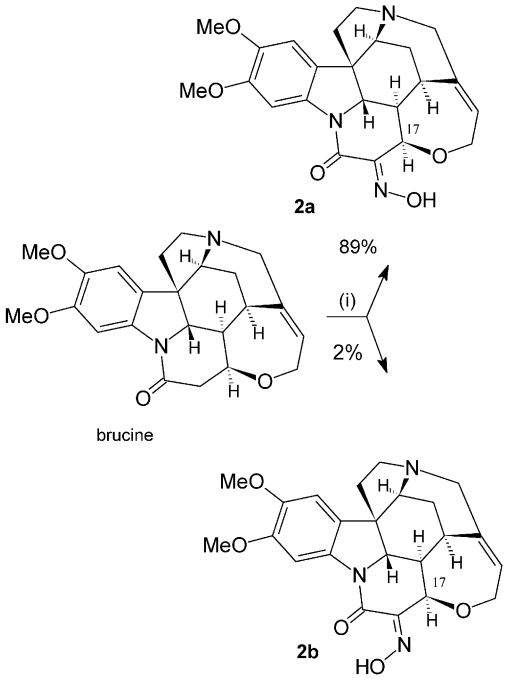
22-Oximinobrucine (**2**) was synthesized by isonitrosation of brucine with *tert*-butyl nitrite/*t*-BuOK in toluene following the procedure reported for oxohomoeburnane (Scheme 2).[29] The major *E*-oxime (**2a**) (89%) could be separated from the *Z*-oxime (**2b**) (2%) by column chromatography on silica gel. The stereochemistry was determined by ^1H and ^{13}C NMR spectroscopy. The H-17 resonance signal of the major isomer appeared at δ 5.00 ppm, downfield by 0.31 ppm from the corresponding signal of the minor isomer due to the anisotropic deshielding effect of the oxime hydroxy group.[30] The *E*-configuration of the major isomer was confirmed by the chemical shift of C-17 at δ 75.2 ppm, upfield by 4.7 ppm from the corresponding signal of the *Z*-isomer owing to γ-interaction between the hydroxy group and C-17 in the *E*-oxim (**2a**).[31]

The bisquaternary title compounds **3–10** were synthesized by refluxing an excess of strychnine, brucine and dihydrostrychnine (**1**) with the respective dibromoalkane in dry acetonitrile for 14 h in 58–90% yield. Due to a low solubility of oxime (**2a**) in acetonitrile,



strychnine 1

Scheme 1. (i) H_2/Pd/C 10%, 10% AcOH, 48 h.



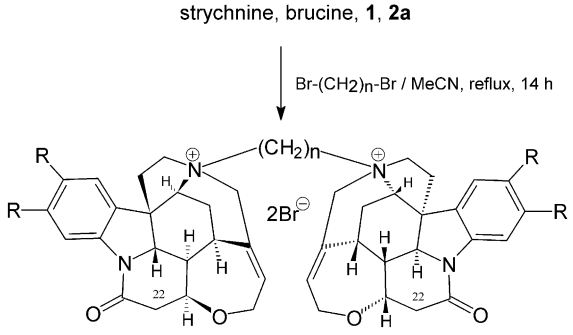
Scheme 2. (i) *tert*-Butylnitrite/*t*-BuOK/toluene, 2 h.

its quaternization was carried out in a 1:2 mixture of chloroform and acetonitrile giving compound **10** in 27% yield. The identities of the NMR-spectroscopically pure compounds **1–10** were established by elemental analysis (C, H, N) and ^{1}H and ^{13}C NMR studies. The NMR assignments were based on HH-COSY, HMQC and HMBC experiments.

Pharmacological studies

The allosteric action of the test compounds on the dissociation of the orthosteric radioligand [^{3}H]*N*-methylscolamine ([^{3}H]NMS) and their effect on [^{3}H]NMS equilibrium binding were studied in homogenates of porcine heart ventricles. Dissociation assays were used to determine the affinity of the allosteric agents at receptors in which access to the orthosteric binding site was blocked by [^{3}H]NMS. It is thus ensured that only allosteric effects are being observed. [^{3}H]NMS dissociation was monophasic both in the absence and in the presence of the test compounds. All investigated compounds 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.

Table 1. Parameters characterizing the allosteric interaction of the indicated test compounds with [^{3}H]-*N*-methylscopolamine at porcine heart M_2 receptors (for details see text)



	n	R	$pEC_{0.5,diss}$	[^{3}H]NMS equilibrium binding (%) at $EC_{0.25,diss}$
Alcuronium	—	—	8.61 ± 0.05[a]	—
W84	—	—	7.67 ± 0.04[b]	—
Strychnine	—	—	6.28 ± 0.06[c]	124.1 ± 3.7
3	6	H	7.99 ± 0.07[d,e]	121.5 ± 5.4
4	7	H	8.30 ± 0.05[d,f]	122.8 ± 6.5
5	8	H	8.38 ± 0.06[e]	125.2 ± 4.3
9[k]	7	H	7.87 ± 0.03[f]	111.0 ± 3.4
Brucine	—	—	5.49 ± 0.04[g]	114.8 ± 2.9
6	6	OMe	7.54 ± 0.03[h]	107.8 ± 4.0
7	7	OMe	7.53 ± 0.04[h,i]	114.3 ± 4.6
8	8	OMe	7.57 ± 0.04[h]	112.0 ± 3.1
10[l]	7	OMe	7.24 ± 0.05[i,j]	114.0 ± 1.8

[a] Ref 34.
[b] Ref 35.
[c] $n_H = -0.79 \pm 0.08$ [significantly different from unity (*F*-test, $p < 0.05$)].
[d] $pEC_{0.5,diss}$ of Compounds **3** and **4** significantly different (*t*-test, $p < 0.05$).
[e] $pEC_{0.5,diss}$ of Compounds **3** and **5** significantly different (*t*-test, $p < 0.05$).
[f] $pEC_{0.5,diss}$ of Compounds **4** and **9** significantly different (*t*-test, $p < 0.05$).
[g] $n_H = -0.73 \pm 0.05$ [significantly different from unity (*F*-test, $p < 0.05$)].
[h] $pEC_{0.5,diss}$ of Compounds **6**, **7** and **8** *not* significantly different from each other (*t*-test, $p > 0.05$).
[i] $pEC_{0.5,diss}$ of Compounds **7** and **10** significantly different (*t*-test, $p < 0.05$).
[j] Min = 8.69 ± 0.05% [significantly different from min = 0% (*F*-test, $p < 0.05$)].
[k] Double bonds of both strychnine units hydrogenated.
[l] *E*-oxime groups at C-22 of both brucine units.

The concentration of an allosteric agent for a half-maximum effect on orthosteric ligand dissociation ($EC_{0.5,diss}$) corresponds to a 50% occupancy of the liganded receptors by the respective allosteric test compound.[8] Thus, $EC_{0.5,diss}$ indicates the equilibrium dissociation constant (K_D) of alloster binding to the ligand-occupied receptor and reflects the affinity of the test compound to the allosteric binding site. $pEC_{0.5,diss}$-values (means ± SE, $n = 3$ experiments in triplicate determinations) are compiled in Table 1.

The effect of the allosteric test compounds on [^{3}H]NMS equilibrium binding was investigated at equieffective concentrations $EC_{0.25,diss}$. $EC_{0.25,diss}$ is the concentration of test compound at which the rate of [^{3}H]NMS dissociation is reduced to 25% of the control value. Equilibrium binding data in the presence of allosteric modulator were expressed as a percentage of the value under control conditions, which was set as 100%. [^{3}H]NMS equilibrium binding greater than 100% indicates positive cooperativity between the allosteric and orthosteric ligand. The latter was found with all test compounds in this series (Table 1; means ± SE, $n = 3$–5 experiments in quadruplicate determinations). In the case of W84, [^{3}H]NMS binding is lowered indicating negative cooperativity with [^{3}H]NMS.[35]

SAR discussion

The $pEC_{0.5,diss}$ values and the effect on [^{3}H]NMS equilibrium binding at equieffective concentrations $EC_{0.25,diss}$ for all investigated compounds are given in Table 1.

The allosteric modulators in this series with highest potency to affect [^{3}H]NMS dissociation are the bisquaternary double strychnines **3**–**5**. Compounds **4** and **5** exhibited nearly the same affinity to NMS-occupied M_2 receptors as alcuronium ($EC_{0.5,diss} = 4$ nM). While quaternization of strychnine with simple alkyl groups (i.e., methyl, ethyl, propyl) was reported to result in virtually unchanged affinity to NMS-liganded M_2 receptors,[16] the bridging of two strychnine moieties via C_6–C_8 alkyl chains carried out here caused a dramatic (50-fold for **3**, 105-fold for **4** and 131-fold for **5**) increase of affinity. The considerably higher affinity of the bisquaternary double strychnines relative to the monomeric analogues can be explained based on the pharmacophore for potent allosteric modulators consisting of two positively charged groups at a distance of about 10 Å surrounded by two aromatic ring systems.[19,20] The presence of only two of these pharmacophoric elements, that is, one positive charge and one indole ring, gives rise to limited interactions of the monoquaternary strychnines with the receptor protein and to reduced binding affinity.

The findings observed in the strychnine series also apply for the brucine analogues. Thus, *N*-substitution of two brucine units with C_6–C_8 alkyl chains led to a nearly 115-fold increase in affinity. However, brucine and its bisquaternary dimer analogues **6**–**8** are about 5 times less potent than strychnine and its corresponding bisquaternary derivatives.

The influence of the length of the interquaternary alkyl chain on the allosteric potency was different in both series. While affinities of the brucine analogues **6**–**8** remained almost unchanged with increasing lengths of the middle chain, the allosteric potency of the strychnine derivatives with C_7 and C_8 middle chains **4** and **5**, respectively, were about 2-fold higher than for the C_6 analogue **3**. The data in the strychnine series are in agreement with our previous studies in the W84, WDUO and IWDUO series in which the minimal essential distance between the positive charges was found to be 10 Å, equivalent to seven methylene groups.[21]

In order to examine which structural elements of the strychnine ring system are responsible for high allosteric potency, two chemical modifications of the strychnine ring skeleton have been carried out: reduction of the C19–C20 double bond (compound **9**) and introduction of the oxime function at C-22 (compound **10**). Both structural changes resulted in a significant reduction of binding affinity. Hydrogenation of both double bonds in **4** led to about 3-fold decrease in affinity. A similar decrease of binding affinity (2-fold) resulted from the introduction of *E*-oxime groups at C-22 in both brucine moieties of **7**. The findings indicate that the unchanged strychnine ring is an optimal structural feature responsible for high allosteric potency. Since expansion of the strychnine ring system by two methoxy groups and an oxime function led to significant decrease of affinity, the volume of the strychnine ring skeleton seems to be optimal to fit into the binding pocket of the receptor protein.

Strychnine, brucine and their quaternary analogues are known to be positively cooperative with NMS at M_2 receptors.[16] Screening of the [^{3}H]NMS equilibrium binding at equieffective concentrations showed that the positive cooperativity with NMS remained unchanged after bridging of two strychnine or brucine units with C_6–C_8 alkyl chains. This finding suggests that all compounds in this series have higher affinity for the ligand-occupied receptor compared with the free receptor and that the ratio of these affinities is hardly affected by the structural modifications.

Conclusion

In this study we have been able to demonstrate that bridging of two strychnine and brucine moieties with C_6–C_8 alkyl chains leads to a pronounced increase in affinity for the allosteric site of the muscarinic M_2 receptors, while the positive cooperativity with the antagonist NMS is fully maintained. SAR studies have revealed the unchanged strychnine ring as an important structural feature for high allosteric potency. Compounds **4** and **5** ($EC_{0.5,diss}$ 5 or 4 nM, respectively) are among the most potent enhancers of NMS binding to M_2 receptors and may serve as useful pharmacological tools in exploring allosteric interactions at muscarinic receptors. Apparently, the allosteric binding site of M_2 receptors nicely accommodates bulky substituents such as strychnine rings as lateral heterocyclic moieties of bis(ammonio)alkane-type allosteric agents.

Experimental

General procedures

Melting points were determined with a Gallenkamp melting point apparatus (Sanyo) and were not corrected. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 400 and AV 600 instruments. TLC was carried out on silica gel 60 F_{254} aluminium sheets; Merck. Proton chemical shifts are referred to $CHCl_3$ (7.24 ppm) and DMSO-d_6 (2.55 ppm); carbon chemical shifts are referred to $^{13}CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.50 ppm). IR spectra were obtained using a Biorad PharmalyzIR FT-IR spectrometer. 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.

(20*R*)-19,20-Dihydrostrychnine (1). One gramme Pd/C 10% was added to a solution of strychnine (10 g, 0.03 mol) in 10% acetic acid (200 mL). The reaction mixture was shaken in a Paar-like hydrogenation apparatus under the hydrogen pressure of 5 bar for 48 h. The catalyst was filtered off and washed with 10% acetic acid (2 × 20 mL). The free base was precipitated from the combined aqueous solutions by the dropwise addition of 20% NaOH under ice-cooling and the reaction mixture was extracted with chloroform (3 × 100 mL). The combined chloroform layers were washed with water and dried over $MgSO_4$. Evaporation of the solvent afforded TLC and NMR pure, colorless crystals (9.5 g, 94%), which were used for the next step without further purification; mp 223–225 °C (lit.[27] 220–222 °C); IR (ATR) 2924, 2872, 1663, 1597, 1475, 1390, 1099, 778 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.45 (dddd, J = 15.1, 12.5, 5.3, 2.6 Hz, 1H, H-19a), 1.55 (ddd, J = 11.0, 3.7, 3.7 Hz, 1H, H-16), 1.65 (ddd, J = 13.6, 3.1, 3.1 Hz, 1H, H-14a), 1.91–1.98 (m, 2H, H-6a and H-19b), 2.14 (ddd, J = 13.6, 3.3, 3.3 Hz, 1H, H-14b), 2.24–2.29 (m, 2H, H-15 and H-21a), 2.35 (m, 1H, H-20), 2.45 (ddd, J = 13.9, 8.7, 8.7 Hz, 1H, H-6b), 2.61 (dd, J = 15.9, 5.2 Hz, 1H, H-22a), 2.83–2.91 (m, 2H, H-5a and H-21b), 3.00 (dd, J = 15.9, 8.6 Hz, 1H, H-22b), 3.12 (m, 1H, H-5b), 3.45 (t, J = 3.2 Hz, 1H, H-3), 3.57 (ddd, J = 12.8, 12.8, 3.3 Hz, 1H, H-18a), 3.95 (m, 1H, H-17), 3.99 (d, J = 11.0 Hz, 1H, H-2), 3.99 (m, 1H, H-18b), 7.07 (ddd, J = 7.7, 7.4, 1.0 Hz, 1H, H-10), 7.16 (d, J = 7.7 Hz, 1H, H-9), 7.21 (ddd, J = 7.7, 7.4, 1.0 Hz, 1H, H-11), 8.00 (d, J = 7.7 Hz, 1H, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.2 (C-15), 31.4 (C-14), 32.7 (C-19), 35.0 (C-20), 41.2 (C-22), 46.6 (C-6), 51.6 (C-7), 53.4 (C-16), 53.9 (C-5), 58.0 (C-21), 62.7 (C-3), 67.8 (C-2), 68.6 (C-18), 76.6 (C-17), 115.8 (C-12), 122.0 (C-9), 124.4 (C-10), 128.2 (C-11), 135.2 (C-8), 140.9 (C-13), 169.6 (C-23).

***E*-22-Oximinobrucine (2a) and *Z*-22-oximinobrucine (2b).** *tert*-Butyl nitrite (80 mL) and *tert*-BuOK (8 g) were added to the solution of brucine (11.8 g, 0.03 mol) in dry toluene (500 mL). The reaction mixture was stirred at 50 °C for 2 h. After cooling to room temperature 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.

2b. (0.30 g, 2.4%), yellow crystals; mp > 320 °C (dec.); R_f = 0.24 ($CHCl_3$/MeOH/25% NH_3 130:10:1); $[\alpha]_D^{20}$ −239.6° (*c* 0.25, $CHCl_3$/MeOH 1:3); IR (ATR) 2937, 2863, 1630, 1560, 1479, 1446, 1404, 1285, 1214, 1112, 1001, 848, 750 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (d, J = 14.2 Hz, 1H, H-14a), 1.71 (ddd, J = 10.6, 2.8, 2.8 Hz, 1H, H-16), 1.83 (m, 2H, C$\underline{H}_2$-6), 2.34 (ddd, J = 14.2, 4.3, 4.3 Hz, 1H, H-14b), 2.61 (d, J = 14.4 Hz, 1H, H-21a), 2.73 (m, 1H, H-5a), 3.02 (m, 1H, H-5b), 3.15 (s, br, 1H, H-15), 3.63 (d, J = 14.4 Hz, H-21b), 3.79 (s, 3H, –CH_3), 3.81 (s, 3H, –CH_3), 3.90 (d, J = 10.6 Hz, 1H, H-2), 3.92 (s, br., 1H, H-3), 4.21 (d, J = 6.3 Hz, 2H, C$\underline{H}_2$-18), 4.69 (d, J = 2.8 Hz, 1H, H-17), 5.85 (m, 1H, H-19), 7.08 (s, 1H, H-12), 7.74 (s, 1H, H-9), 13.56 (br, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.1 (C-14), 30.8 (C-15), 42.1 (C-6), 46.0 (C-16), 49.1 (C-5), 51.3 (C-7), 52.3 (C-21), 55.4, 55.7 (2 × CH_3), 58.8 (C-2), 59.1 (C-3), 64.2 (C-18), 79.9 (C-17), 106.6 (C-9), 100.5 (C-12), 125.3 (C-8), 126.2 (C-19), 138.3 (C-20), 141.2 (C-13), 145.3 (C-22), 146.2 (C-10), 148.3 (C-11), 157.4 (C-23). Anal. calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92; found : C, 64.91; H, 6.05; N, 9.75.

2a. (11.3 g, 89%); mp > 320 °C (MeOH/CH_2Cl_2) (dec.); R_f = 0.13 ($CHCl_3$/MeOH/25% NH_3 130:10:1); $[\alpha]_D^{20}$ −186.8° (*c* 0.25, $CHCl_3$/MeOH 1:3); IR (ATR) 2951, 2900, 2870, 2847, 1655, 1492, 1298, 1274, 1117, 1035, 869, 744 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (d, br., J = 14.2 Hz, 1H, H-14a), 1.58 (ddd, J = 10.6, 2.5, 2.5 Hz, 1H, H-16), 1.81 (m, 2H, C$\underline{H}_2$-6), 2.33 (ddd, J = 14.2, 4.3, 4.3 Hz, 1H, H-14b), 2.62 (d, J = 14.2 Hz, 1H, H-21a), 2.74 (m, 1H, H-5a), 3.02 (dd, J = 9.6, 7.3 Hz, 1H, H-5b), 3.09 (s, br., 1H, H-15), 3.63 (d, J = 14.2 Hz, 1H, H-21b), 3.79 (s, 3H, –CH_3), 3.81 (s, 3H, –CH_3), 3.92 (s, br, 1H, H-3), 4.04 (d, J = 10.6 Hz, 1H, H-2), 4.13 (dd, J = 13.9, 6.1 Hz, 1H, H-18a), 4.18 (dd, J = 13.9, 7.1 Hz, 1H, H-18b), 5.00 (d, J = 2.3 Hz, 1H, H-17), 5.87 (m, 1H, H-19), 7.05 (s, 1H, H-12), 7.78 (s, 1H, H-9), 12.79 (br, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.4 (C-14), 31.4 (C-15), 42.4 (C-6), 44.7 (C-16), 49.3 (C-5), 51.2 (C-7), 52.5 (C-21), 55.7, 55.9 (2 × CH_3), 59.0 (C-2), 59.1 (C-3), 65.3 (C-18), 75.2 (C-17), 106.9 (C-9), 100.3 (C-12), 124.5 (C-8), 126.6 (C-19), 135.6 (C-20), 140.0 (C-13), 149.2 (C-22), 146.3 (C-10), 148.5 (C-11), 158.9 (C-23). Anal. calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92; found: C, 64.86; H, 6.02; N, 9.80.

General procedure for the synthesis of compounds 3–10

The solution of the alkaloid (1.25 mmol) and the respective dibromoalkane (0.5 mmol) in dry acetonitrile (50 mL) was heated to reflux for 14 h. The precipitated colorless crystalline product was collected by filtration, washed with small amounts of acetonitrile and dried in 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.

(1,6-Hexylene)-4,4′-distrychninium dibromide (3). 0.37 g (82%) was obtained from strychnine and 1,6-dibromohexane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +6.8° (*c* 0.5, DMSO); IR (ATR) 2955, 2847, 1681, 1595, 1480, 1458, 1388, 1327, 1112, 1051, 766 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.52–1.58 (m, 6H, 2 × $N^+CH_2CH_2C\underline{H}_2$ and 2 × H-16), 1.70 (d, *J* = 15.4 Hz, 2H, 2 × H-14a), 1.90–2.10 (m, 4H, 2 × $N^+CH_2C\underline{H}_2$), 2.26 (m, 4H, 2 × $C\underline{H}_2$-6), 2.71 (m, 2H, 2 × H-22a), 2.73 (m, 2H, 2 × H-14b), 3.02 (dd, *J* = 17.3, 7.8 Hz, 2H, 2 × H-22b), 3.55 (ddd, *J* = 12.9, 12.9, 6.1 Hz, 2H, 2 × H-5a), 3.38 (s, br, 2H, 2 × H-15), 3.75 (m, 4H, 2 × $N^+C\underline{H}_2$), 3.95 (m, 2H, 2 × H-5b), 3.97 (d, 2H, *J* = 13.1 Hz, 2 × H-21a), 4.21 (m, 2H, 2 × H-18a), 4.22 (d, *J* = 10.9 Hz, 2H, 2 × H-2), 4.29 (dd, *J* = 14.3, 6.3 Hz, 2H, 2 × H-18b), 4.37 (d, *J* = 13.1 Hz, 2H, 2 × H-21b), 4.43 (m, 2H, 2 × H-17), 4.53 (s, br, 2H, 2 × H-3), 6.43 (m, 2H, 2 × H-19), 7.22 (t, *J* = 7.6 Hz, 2H, 2 × H-10), 7.38 (dd, *J* = 8.1, 7.6 Hz, 2H, 2 × H-11), 7.79 (d, *J* = 7.6 Hz, 2H, 2 × H-9), 7.99 (d, *J* = 8.1 Hz, 2H, 2 × H-12); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.4 ($N^+CH_2\underline{C}H_2$), 24.4 (C-14), 25.3 ($N^+CH_2CH_2\underline{C}H_2$), 29.0 (C-15), 38.9 (C-6), 46.0 (C-16), 40.9 (C-22), 51.9 (C-7), 58.1 (C-5), 58.2 (C-2), 60.7 (C-21), 63.2 (C-18), 65.3 ($N^+\underline{C}H_2$), 73.0 (C-3), 75.6 (C-17), 115.2 (C-12), 123.6 (C-9), 123.9 (C-10), 129.4 (C-11), 129.5 (C-8), 132.9 (C-20), 135.4 (C-19), 141.2 (C-13), 168.9 (C-23). Anal. calcd for $C_{48}H_{56}N_4O_4Br_2$: C, 63.16; H, 6.18; N, 6.14; found: C, 62.89; H, 6.42; N, 5.71.

(1,7-Heptylene)-4,4′-distrychninium dibromide (4). 0.35 g (76%) was obtained from strychnine and 1,6-dibromoheptane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +11.4° (*c* 0.5, DMSO); IR (ATR) 2943, 2838, 1653, 1499, 1445, 1398, 1284, 1215, 1198, 1111, 847, 757 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.52 (m, 2H, $N^+CH_2CH_2CH_2C\underline{H}_2$), 2.20 (m, 2H, 2 × H-6a), 2.28 (m, 2H, 2 × H-6b), chemical shifts and coupling constants for all other hydrogen atoms coincide with the δ values for the corresponding atoms of **3** within ±0.02 ppm and ±0.1 Hz, respectively; ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.0 ($N^+CH_2CH_2CH_2\underline{C}H_2$), chemical shifts for all other carbon atoms coincide with the δ values for the corresponding atoms of **3** within ±0.1 ppm. Anal. calcd for $C_{49}H_{58}N_4O_4Br_2$: C, 63.50; H, 6.31; N, 6.04; found: C, 63.05; H, 6.09; N, 5.63.

(1,8-Octylene)-4,4′-distrychninium dibromide (5). 0.37 g (78%) was obtained from strychnine and 1,6-dibromooctane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +6.8° (*c* 0.5, DMSO); IR (ATR) 2912, 2869, 1684, 1596, 1481, 1464, 1387, 1109, 1051, 762 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.50 (m, 4H, 2 × $N^+CH_2CH_2CH_2C\underline{H}_2$), 2.18 (m, 2H, 2 × H-6a), 2.24 (m, 2H, 2 × H-6b), chemical shifts and coupling constants for all other hydrogen atoms coincide with the δ-values for the corresponding atoms of **3** within ±0.02 ppm and ±0.1 Hz, respectively; ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.5 ($N^+CH_2CH_2CH_2\underline{C}H_2$), chemical shifts for all other carbon atoms coincide with the δ values for the corresponding atoms of **3** within ±0.1 ppm. Anal. calcd for $C_{50}H_{60}N_4O_4Br_2$: C, 63.83; H, 6.43; N, 5.95; found : C, 63.40; H, 5.91; N, 5.82.

(1,6-Hexylene)-4,4′-dibrucinium dibromide (6). 0.44 g (85%) was obtained from brucine and 1,6-dibromohexane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +30.3° (*c* 0.33, MeOH/H_2O 1:1); IR (ATR) 2927, 2836, 1649, 1649, 1501, 1466, 1440, 1399, 1218, 1196, 1105, 845, 754 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.46 (ddd, *J* = 10.9, 3.1, 3.1 Hz, 2H, 2 × H-16), 1.51 (m, 4H, 2 × $N^+CH_2CH_2C\underline{H}_2$), 1.65 (d, *J* = 14.9 Hz, 2H, 2 × H-14a), 1.86–2.04 (m, 4H, 2 × $N^+CH_2C\underline{H}_2$), 2.13–2.25 (m, 4H, $C\underline{H}_2$-6), 2.62–2.73 (m, 4H, 2 × H-14b, 2 × H-22a), 2.93 (dd, *J* = 17.1, 8.1 Hz, 2H, 2 × H-22b), 3.38 (s, br, 2H, 2 × H-15), 3.44 (m, 2H, 2 × H-5a), 3.73 (m, 4H, 2 × $N^+C\underline{H}_2$), 3.75 (s, 6H, 2 × $C\underline{H}_3$), 3.82 (s, 6H, 2 × $C\underline{H}_3$), 3.90 (m, 2H, 2 × H-5b), 3.91 (d, *J* = 13.4 Hz, 2H, 2 × H-21a), 4.12 (d, *J* = 10.9 Hz, 2H, 2 × H-2), 4.16 (dd, *J* = 14.6, 5.6 Hz, 2H, 2 × H-18a), 4.24 (dd, *J* = 14.6, 6.3 Hz, 2H, 2 × H-18b), 4.30 (d, *J* = 13.4 Hz, 2H, 2 × H-21b), 4.38 (m, 2H, 2 × H-17), 4.46 (s, br, 2H, 2 × H-3), 6.37 (m, 2H, 2 × H-19), 7.39 (s, 2H, 2 × H-9), 7.66 (s, 2H, 2 × H-12); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.4 ($N^+CH_2\underline{C}H_2$), 24.3 (C-14), 25.3 ($N^+CH_2CH_2\underline{C}H_2$), 29.0 (C-15), 38.4 (C-6), 40.3 (C-22), 46.1 (C-16), 51.9 (C-7), 55.7, 56.6 (2 × $\underline{C}H_3$), 57.5 (C-5), 58.4 (C-2), 60.4 (C-21), 63.2 (C-18), 65.4 ($N^+\underline{C}H_2$), 73.2 (C-3), 75.8 (C-17), 100.2 (C-12), 107.9 (C-9), 120.1 (C-8), 132.9 (C-20), 135.4 (C-19), 135.5 (C-13), 145.9 (C-10), 149.6 (C-11), 168.6 (C-23). Anal. calcd for $C_{52}H_{64}N_4O_8Br_2$: C, 60.47; H, 6.25; N, 5.42; found : C, 60.09; H, 6.30; N, 6.34.

(1,7-Heptylene)-4,4′-dibrucinium dibromide (7). 0.48 g (91%) was obtained from brucine and 1,6-dibromoheptane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +27.2° (*c* 0.5, MeOH/H_2O 1:1); IR (ATR) 2937, 2827, 1659, 1591, 1507, 1471, 1452, 1367, 1284, 1201, 1042, 988, 867, 750 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.50 (m, 2H, 2 × $N^+CH_2CH_2CH_2C\underline{H}_2$), chemical shifts and coupling constants for all other hydrogen atoms coincide with the δ values for the corresponding atoms of **6** within ±0.05 ppm and ±0.1 Hz, respectively; ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.0 ($N^+CH_2CH_2CH_2\underline{C}H_2$), chemical shifts for all other carbon atoms coincide with the δ-values for the corresponding atoms of **6** within ±0.1 ppm. Anal. calcd for $C_{53}H_{66}N_4O_8Br_2$: C, 60.80; H, 6.35; N, 5.35; found: C, 60.45; H, 6.45; N, 5.20.

(1,8-Octylene)-4,4′-dibrucinium dibromide (8). 0.35 g (65%) was obtained from brucine and 1,6-dibromooctane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +20.9° (*c* 0.5, MeOH/H_2O 1:1); IR (ATR) 2937, 2827, 1659, 1586, 1469, 1445, 1402, 1283, 1198, 1107, 846, 754 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) chemical shifts and coupling constants for all hydrogen atoms coincide with the δ-values for the corresponding atoms of **7** within ±0.02 ppm and ±0.1 Hz, respectively; ^{13}C NMR (100 MHz, DMSO-d_6) chemical shifts for all carbon atoms coincide with the δ values for the corresponding atoms of **7** within ±0.5 ppm. Anal. calcd for $C_{53}H_{66}N_4O_8Br_2$: C, 60.80; H, 6.35; N, 5.35; found: C, 60.45; H, 6.45; N, 5.20.

(1,7-Heptylene)-4,4′-bis[(20*R*)-19,20-dihydrostrychninium] dibromide (9). 0.42 g (90%) was obtained from (**1**) and 1,6-dibromoheptane; mp >320 °C (dec.); $[\alpha]_D^{20}$ +38.1° (*c* 0.33, DMSO); IR (ATR) 2947, 2908, 1672, 1595, 1479, 1460, 1404, 1347, 1111, 745 cm^{-1}; ^1H NMR

(400 MHz, DMSO-d_6) δ 1.46 (m, 2H, $N^+CH_2CH_2CH_2C\underline{H}_2$), 1.48 (m, 2H, 2 × H-19a), 1.50 (m, 4H, 2 × $N^+CH_2CH_2C\underline{H}_2$), 1.68 (m, 2H, 2 × H-14a), 1.72 (m, 2H, 2 × H-16), 1.80 (m, 4H, 2 × $N^+CH_2C\underline{H}_2$), 2.01 (m, 2H, 2 × H-19b), 2.14 (m, 2H, 2 × H-6a), 2.48 (m, 4H, 2 × H-14b and 2 × H-20), 2.76 (m, 2H, 2 × H-22a), 2.78 (m, 2H, 2 × H-15), 2.86 (m, 2H, 2 × H-22b), 2.89 (m, 2H, 2 × H-6b), 3.19 (m, 2H, 2 × H-5a), 3.56 (m, 2H, 2 × H-5b), 3.61 (m, 2H, 2 × H-21a), 3.66 (m, 2H, 2 × $N^+C\underline{H}H$), 3.72 (m, 2H, 2 × H-18a), 3.98 (m, 2H, 2 × H-21b), 4.00 (m, 4H, 2 × H-18b and 2 × $N^+CH\underline{H}$), 4.13 (m, 2H, 2 × H-17), 4.53 (s, br, 2H, 2 × H-3), 4.66 (d, J = 11.3 Hz, 2H, 2 × H-2), 7.23 (t, J = 7.6 Hz, 2H, 2 × H-10), 7.37 (t, J = 7.6 Hz, 2H, 2 × H-11), 7.82 (d, J = 7.6 Hz, 2H, 2 × H-9), 7.94 (d, J = 7.6 Hz, 2H, 2 × H-12); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.3 ($N^+CH_2\underline{C}H_2$), 25.7 (C-14), 26.01 (N^+CH_2 $CH_2CH_2\underline{C}H_2$), 27.7 (C-20), 27.8 ($N^+CH_2CH_2\underline{C}H_2$), 29.4 (C-15), 31.2 (C-19), 38.9 (C-6), 40.0 (C-22), 50.0 (C-16), 51.1 (C-7), 55.7, 56.6 (2 × CH_3), 57.0 (C-5), 57.9 (C-21), 60.7 (C-18), 64.6 (C-2), 65.2 ($N^+\underline{C}H_2$), 70.6 (C-3), 74.0 (C-17), 114.9 (C-12), 123.6 (C-9), 124.4 (C-10), 129.2 (C-11), 132.1 (C-8), 140.5 (C-13), 169.3 (C-23). Anal. calcd for $C_{49}H_{62}N_4O_4Br_2$: C, 63.23; H, 6.71; N, 6.02; found: C, 63.02; H, 6.70; N, 5.90.

(1,7-Heptylene)- 4,4′-di-(*E*-22-oximinobrucinium) dibromide (10). 0.15 g (27%) was obtained from **2a** and 1,6-dibromoheptane using chloroform/acetonitrile 2:1 (50 mL) as a reaction solvent; mp > 320 °C (dec.); $[\alpha]_D^{20}$ −54.0° (*c* 0.5, MeOH/H_2O 1:1); IR (ATR) 2940, 2820, 1666, 1500, 1446, 1408, 1285, 1115, 1009 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44–1.60 (m, 6H, 2 × $N^+CH_2CH_2C\underline{H}_2C\underline{H}_2$), 1.74 (d, J = 14.9 Hz, 2H, 2 × H-14a), 1.79 (d, br, J = 11.1 Hz, 2H, 2 × H-16), 1.94 (m, 4H, 2 × $N^+CH_2C\underline{H}_2$), 2.22 (m, 2H, 2 × H-6a), 2.27 (m, 2H, 2 × H-6b), 2.77 (d, J = 14.9 Hz, 2H, 2 × H-14b), 3.38 (s, br, 2H, 2 × H-15), 3.49 (m, 2H, 2 × H-5a), 3.75 (m, 4H, 2 × $N^+C\underline{H}_2$), 3.81 (s, 6H, 2 × CH_3), 3.87 (s, 6H, 2 × CH_3), 3.88 (m, 2H, H-21a), 3.89 (m, 2H, 2 × H-5b), 4.29 (m, 4H, 2 × CH_2-18), 4.38 (d, J = 10.9 Hz, 2H, 2 × H-2), 4.34 (m, 2H, 2 × H-21b), 4.55 (s, br, 2H, 2 × H-3), 5.12 (d, J = 2.0 Hz, 2H, H-17), 6.47 (m, 2H, 2 × H-19), 7.43 (s, 2H, 2 × H-12), 7.79 (s, 2H, 2 × H-9), 12.86 (br, 2H, 2 × OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.7 ($N^+CH_2\underline{C}H_2$), 24.3 (C-14), 25.6 (2 × $N^+CH_2CH_2\underline{C}H_2$), 28.1 ($N^+CH_2CH_2CH_2\underline{C}H_2$), 29.7 (C-15), 38.3 (C-6), 43.3 (C-16), 51.7 (C-7), 55.7, 56.6 (2 × CH_3), 57.2 (C-5), 57.5 (C-2), 60.6 (C-21), 64.5 (C-18), 65.4 ($N^+\underline{C}H_2$), 73.2 (C-3), 74.4 (C-17), 99.9 (C-12), 107.6 (C-9), 120.4 (C-8), 133.6 (C-20), 135.6 (C-13), 146.4 (C-10), 148.6 (C-22), 149.5 (C-22), 158.5 (C-23). Anal. calcd for $C_{53}H_{64}N_6O_{10}Br_2$: C, 57.61; H, 5.84; N, 7.61; found: C, 57.23; H, 5.79; N, 7.27.

Radioligand binding studies

Porcine cardiac homogenates were prepared as described previously.[8] 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, USA) 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 5% of the total binding. Membranes were separated by vacuum filtration through glass fibre filters (Schleicher and Schüll, No. 6; Dassel, Germany) and membrane bound radioactivity was determined by liquid scintillation counting. The pK_D of NMS binding amounted to 9.52 ± 0.04 (mean ± SEM, n = 3). [^{3}H]NMS dissociation proceeded monophasically ($t_{1/2,control}$ = 5.0 ± 0.1 min; mean ± SEM, n = 60). To measure the effect of the test compounds on [^{3}H]NMS dissociation, cardiac membranes were prelabeled with [^{3}H]NMS for 30 min, radioligand dissociation was then revealed by the addition of 1 μM atropine, in the absence or in the presence of allosteric modulator. Two-point kinetic experiments[32] were applied and specific [^{3}H]NMS binding was measured at t = 0 and t = 10 min.

The effect of the allosteric test compounds on [^{3}H]NMS equilibrium binding was investigated at equieffective concentrations $EC_{0.25,diss}$. $EC_{0.25,diss}$ is the concentration of test compound at which the rate of [^{3}H]NMS dissociation is reduced to 25% of the control. The appropriate time of incubation was determined according to Lazareno and Birdsall[33] (eq 31 therein). Five half-lives were taken to ensure equilibrium conditions for binding of the allosteric modulator to the allosteric site. Equilibrium binding data (from 3 to 5 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%. Dissociation data were analysed by nonlinear regression analysis using the Prism program (version 3.02, GraphPAD Software, San Diego, USA).

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