Systematic Development of High Affinity Bis(ammonio)alkane-type Allosteric Enhancers of Muscarinic Ligand Binding

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Bis(ammonio)alkane compounds carrying lateral phthalimidopropyl substituents on the nitrogen atoms belong to the archetypal muscarinic allosteric agents. Herein, a series of symmetrical and nonsymmetrical compounds was synthesized in which the phthalimide residues were replaced by differently substituted imide moieties. The allosteric action was measured in porcine heart muscarinic M_2 receptors using [^{3}H]*N*-methylscopolamine (NMS) as a ligand for the orthosteric receptor site in equilibrium binding and dissociation experiments. 1,8-Naphthalimido residues conferred an up to 100-fold gain in affinity leading into the low nanomolar range, while the inhibition of NMS binding was maintained. Additional propyl chain methylation was accompanied by an allosteric elevation of orthosteric ligand binding. In general, the gain in allosteric activity achieved by ring variation plus propyl chain methylation on one side of the molecule could not be augmented by symmetrical variations. The elevation of the ligand binding can be explained by different quantitative structure−activity relationships for the affinities to the free and the orthoster-liganded receptor.

Introduction

Allosteric modulators of receptors are capable of enhancing or decreasing ligand binding to receptors which offers novel therapeutic perspectives.[1,2] Intensive efforts were and still are directed at the allosteric modulation of muscarinic receptors opening new concepts for the therapy of organophosphate poisoning, pain, or Alzheimer's disease.[3−8]

The effect on ligand binding depends on the type of allosteric modulator, the type of ligand, and the subtype of muscarinic receptor. To guide a rational design of appropriate allosteric agents, structure−activity relationships have been elucidated. Often, the allosteric inhibition of the dissociation of the orthosteric ligand serves to indicate occupancy of the allosteric site.[9−11] In addition it is of high interest to know whether the allosteric agents elevate or reduce orthosteric ligand equilibrium binding or leave it unchanged. According to the ternary complex model of allosteric interactions[12] as formulated by Ehlert,[13] the cooperativity factor α is the alloster binding constant for the interaction with the orthoster (agonist or antagonist) occupied receptor divided by the alloster binding constant for the free receptor. Thus, $\alpha < 1$, $\alpha = 1$, and $\alpha > 1$ indicate a positive, neutral, or negative cooperativity, respectively, i.e., an enhanced, unchanged, or diminished equilibrium binding of the orthosteric ligand in the presence of the allosteric agent.

Whereas the alloster binding constant for the free muscarinic receptor is independent of the orthosteric radioligand used as a probe, the alloster binding constant for the orthoster-occupied receptor depends on the type of orthosteric ligand. Often, [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) has been used by others and by us as the orthosteric radioligand, but structure−activity-relationships for cooperative interactions with this orthosteric probe have not yet been unravelled.

Many allosteric modulators of the muscarinic receptor protein influencing the binding of the antagonist [^{3}H]-NMS are structurally symmetrical, for example alcuronium,[14] caracurine V derivatives,[15] tubocurarine,[16,17] methoctramine,[18] obidoxime,[11] and a wide range of the bis(ammonio)alkane derivatives, such as W84,[19] and its congeners dimethyl-W84,[20] CHIN 3/6,[21] and IWDUO[22] (see Scheme 1).

Within the series of symmetrical compounds having an bis(ammonio)alkane middle chain, the lateral moieties, mostly aromatic imides, were found to govern the allosteric potency, i.e., the retardation of [^{3}H]NMS-dissociation from M_2 muscarinic receptors. In the following, the term allosteric potency refers to the experimentally observed effects of an allosteric agent on orthosteric ligand binding; the term allosteric affinity is used when effects are interpreted in terms of binding affinities.

Variation of the lateral moieties resulted in the following structure−activity relationships: saturation of the aromatic imide and shortening of the imide at both ends of the molecule resulted in a substantial decrease in potency.[23] Replacement of one carbonyl of the imide with alkoxy, benzyl, or benzylidene groups, again at both ends revealed the allosteric potency to be dependent on a high polarizibility and the presence of a sp^2 hybridized carbon atom, and along with this, rigidity in this position.[24] The variation of the aromatic

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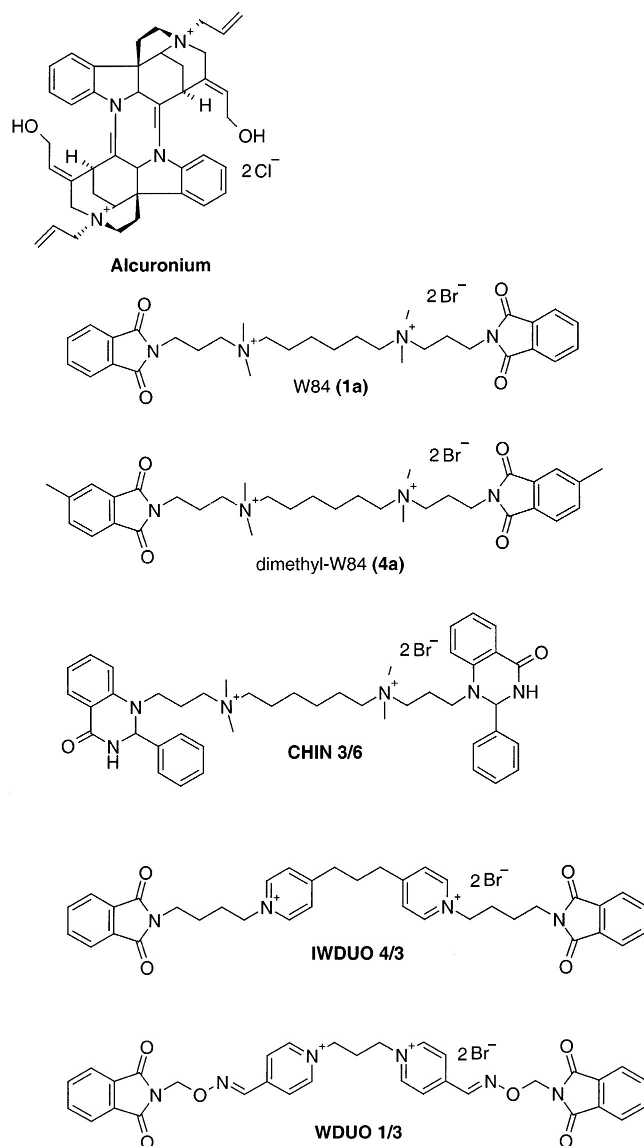
Scheme 1. Structural Formulas of Various Allosteric Modulators



imide moiety within a unilaterally varied series showed the dependence of the potency on the volume of this skeleton.[25] Till now only one comparison of a corresponding contralateral variation of the imides, i.e., a comparison of symmetrical versus nonsymmetrical compounds, has been performed, actually within the WDUO series[26,27] which is derived from the acetylcholinesterase reactivators obidoxime and TMB-4. The agents carry phthalimido substituents at both ends of the bispyridinium middle chain, which were stepwise replaced with dichlorobenzyl residues. The bisphthalimido compound WDUO (see Scheme 1) reduces the dissociation of [^{3}H]-NMS from the receptor protein half-maximally at an EC_{50} value of 0.7 μM. Replacement of one phthalimido residue with a dichlorobenzyl substituent increases the EC_{50} value to 1.3 μM and the replacement of both phthalimido substituents further elevates the EC_{50} value to 4.7 μM. This finding indicates a superiority of the symmetrical imide compounds in this series. Yet, none of the aforementioned bis(ammonio)alkane-type compounds have shown considerable positive cooperativity with [^{3}H]*N*-methylscopolamine. However, in phthalimidopropyl-substituted bis(ammonio)alkane series we recently discovered induction of positive cooperativity by methylation of the lateral propyl chains[28] or by replacement of one quaternary nitrogen by a silicon atom.[29]

To study structure–activity relationships (SAR) within the bis(ammonio)alkane series in detail and in an attempt to tailor a highly potent and positive cooperative muscarinic allosteric modulator, we set out to synthesize and to test symmetrical and nonsymmetrical novel compounds **2d**, **3b**–**d**, **5a**–**d**, **6a**, **6b**, **6d**, **7**, **9**, **14**–**20** carrying on charged nitrogens various substituted and unsubstituted aromatic imidopropyl moieties. In addition, also some already known compounds **1a**, **1b**, **1d**, **2a**–**c**, **3a**, **4a**, **4b**, **4d**, **8**, **10**–**13** were synthesized to be included in the study.

The methylation of the propyl chain was made on the most active compounds and the lead compound W84 with the aim to induce positive cooperativity with [^{3}H]-NMS. SARs will be derived from the observed effects concerning the affinities of the allosteric agents to interact with free and NMS liganded muscarinic M_2 receptors, respectively, because these affinities underly the extent and direction of cooperativity with the orthosteric ligand.

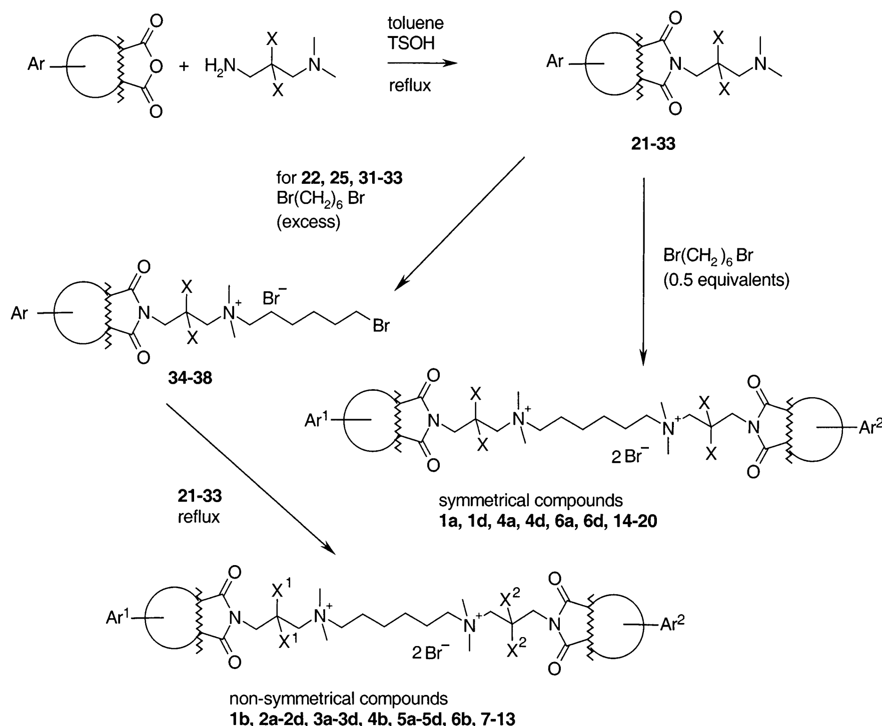
Chemistry

To synthesize the series of symmetrical bis(ammonio)-alkane compounds **14**–**20** and the nonsymmetrical analogues **7**–**13** the commercially nonavailable starting anhydrides were synthesized by conversion of the 1,2-dicarboxylic acids by acetic anhydride according to procedures described in the literature.[30–33] The phthalimido-like propylamine derivatives **21**–**27**, **29**, and **31**–**32**, and 1,2- and 1,8-naphthalimidopropylamines **28**, **30**, and **33** were obtained by refluxing, in toluene and catalytic amount of *p*-toluenesulfonic acid, equimolar amounts of the phthalic-like anhydrides and the naphthalic anhydrides, respectively, with the corresponding N^1,N^1-dimethylpropane-1,3-diamines, using a water separator (Scheme 2). To obtain the nonsymmetric W84-derivatives, the phthalimidopropylamines **22**, **25**, and **31**–**33**, respectively, were stirred with 1,6-dibromohexane for several days at room temperature and 50 °C, respectively, to give the alkylated compounds **34**–**38** (Scheme 2). By using a huge excess of the alkylating agent a double amination of dibromohexane could be avoided.[25] Next, equimolar amounts of **21**–**33** and **34**–**38** were reacted by refluxing in acetonitrile for several days to give the nonsymmetrical compounds **1b**, **2a**–**d**, **3a**–**d**, **4b**, **5a**–**d**, **6b**, **7**–**13** in varying yields (Scheme 2). The symmetrical bis(ammonio)alkane-type compounds **1a**, **1d**, **4a**, **4d**, **6a**, **6d**, and **14**–**20** were obtained by refluxing 2 mol of **21**–**33** and 1 mol of 1,6-dibromohexane in acetonitrile for several days (Scheme 2). The identities were established by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopic experiments (see Tables 6–8 in Supporting Information).

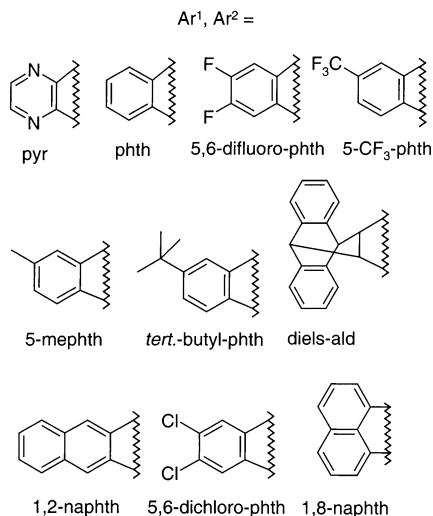
Pharmacology

Binding experiments were carried out in homogenates of porcine heart ventricles containing muscarinic acetylcholine receptors of the M_2 subtype. The applied

Scheme 2. Synthesis Pathway[a]



compd	Ar	X
21	pyr	H
22, 34	phth	H
23	5,6-difluoro-phth	H
24	5-CF_3-phth	H
25, 35	5-mephth	H
26	*tert.*-butyl-phth	H
27	diels-ald	H
28	1,2-naphth	H
29	5,6-dichloro-phth	H
30	1,8-naphth	H
31, 36	phth	CH_3
32, 37	5-mephth	CH_3
33, 38	1,8-naphth	CH_3



[a] The detailed substitution pattern of the compounds **1**−**20** is given in Table 1 and Table 2, respectively.

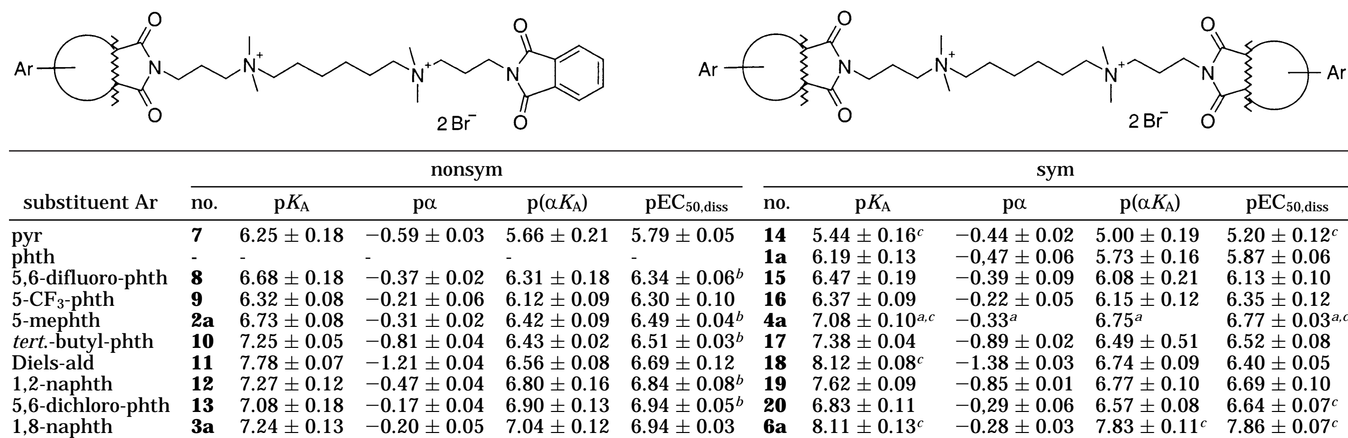
buffer (2.7 mM $MgHPO_4$, 45 mM TrisHCl, 37 °C, pH 7.3) yields allosteric activities that are similar to those found under organ bath conditions.[15,34] We used the orthosteric antagonist [^{3}H]*N*-methylscopolamine ([^{3}H]-NMS) as a probe to measure the interaction of the test compounds with the allosteric site of the M_2 receptors. [^{3}H]NMS-dissociation experiments revealed the allosteric stabilization of the [^{3}H]NMS-receptor complexes and allowed computation of the incubation time that is required for the equilibrium binding experiments with [^{3}H]NMS in the presence of the allosteric agents.

Results and Discussion

All compounds synthesized were subjected to the pharmacological evaluation. The $pEC_{50,diss}$ values, p(αK_A) values, pK_A values, and the cooperativity factors pα in connection with the substitution pattern are summarized in Tables 1 and 2.

Figure 1 illustrates essential aspects of the present study. The effect of three test compounds on [^{3}H]NMS equilibrium binding is shown. The parent compound **1a** reduced [^{3}H]NMS binding as was expected from previous studies (e.g., ref 28). Analysis of the curve according to the ternary complex model of allosteric interactions yields the affinity value pK_A for the binding of **1a** to free M_2 receptors, in which the orthosteric site is not occupied by [^{3}H]NMS (Table 2). Furthermore, the factor α of cooperativity between **1a** and [^{3}H]NMS is derived from the curve. In Table 2 we indicate the minus log value of α, because α is log-normally distributed[35] and because the minus sign indicates the direction of cooperativity. The value p(αK_A) reflects the binding affinity of **1a** for [^{3}H]NMS-occupied M_2 receptors. This affinity

Table 1. Substitution Pattern and Parameters Characterizing the Allosteric Interactions of the Compounds **1a**, **2a**, **3a**, **4a**, **6a**, and **7**−**20** with the Binding of [^{3}H]*N*-Methylscopolamine at Porcine Heart M_2 Receptors (mean values ± SEM from 3−12 independent experiments; for details, see text)



substituent Ar	nonsym no.	pK_A	pα	p(αK_A)	pEC$_{50,diss}$	sym no.	pK_A	pα	p(αK_A)	pEC$_{50,diss}$
pyr	**7**	6.25 ± 0.18	−0.59 ± 0.03	5.66 ± 0.21	5.79 ± 0.05	**14**	5.44 ± 0.16[c]	−0.44 ± 0.02	5.00 ± 0.19	5.20 ± 0.12[c]
phth	-	-	-	-	-	**1a**	6.19 ± 0.13	−0,47 ± 0.06	5.73 ± 0.16	5.87 ± 0.06
5,6-difluoro-phth	**8**	6.68 ± 0.18	−0.37 ± 0.02	6.31 ± 0.18	6.34 ± 0.06[b]	**15**	6.47 ± 0.19	−0.39 ± 0.09	6.08 ± 0.21	6.13 ± 0.10
5-CF$_3$-phth	**9**	6.32 ± 0.08	−0.21 ± 0.06	6.12 ± 0.09	6.30 ± 0.10	**16**	6.37 ± 0.09	−0.22 ± 0.05	6.15 ± 0.12	6.35 ± 0.12
5-mephth	**2a**	6.73 ± 0.08	−0.31 ± 0.02	6.42 ± 0.09	6.49 ± 0.04[b]	**4a**	7.08 ± 0.10[a,c]	−0.33[a]	6.75[a]	6.77 ± 0.03[a,c]
tert.-butyl-phth	**10**	7.25 ± 0.05	−0.81 ± 0.04	6.43 ± 0.02	6.51 ± 0.03[b]	**17**	7.38 ± 0.04	−0.89 ± 0.02	6.49 ± 0.51	6.52 ± 0.08
Diels-ald	**11**	7.78 ± 0.07	−1.21 ± 0.04	6.56 ± 0.08	6.69 ± 0.12	**18**	8.12 ± 0.08[c]	−1.38 ± 0.03	6.74 ± 0.09	6.40 ± 0.05
1,2-naphth	**12**	7.27 ± 0.12	−0.47 ± 0.04	6.80 ± 0.16	6.84 ± 0.08[b]	**19**	7.62 ± 0.09	−0.85 ± 0.01	6.77 ± 0.10	6.69 ± 0.10
5,6-dichloro-phth	**13**	7.08 ± 0.18	−0.17 ± 0.04	6.90 ± 0.13	6.94 ± 0.05[b]	**20**	6.83 ± 0.11	−0,29 ± 0.06	6.57 ± 0.08	6.64 ± 0.07[c]
1,8-naphth	**3a**	7.24 ± 0.13	−0.20 ± 0.05	7.04 ± 0.12	6.94 ± 0.03	**6a**	8.11 ± 0.13[c]	−0.28 ± 0.03	7.83 ± 0.11[c]	7.86 ± 0.07[c]

[a] Data taken from ref 28. [b] Data taken from ref 25. [c] Significantly different from the parameter value of the nonsymmetrical compound, $p < 0.05$.

Table 2. Substitution Pattern and Parameters Characterizing the Allosteric Interactions of the Compounds **1**−**6** with the Binding of [^{3}H]*N*-Methylscopolamine at Porcine Heart M_2 Receptors (mean values ± SEM from 3−12 independent experiments; for details, see text)

no.	Ar^1	X^1	X^2	Ar^2	pK_A	pα	p(αK_A)	pEC$_{50,diss}$
1a	phth	H	H	phth	6.19 ± 0.13 *n* = 4	−0.47 ± 0.06[c] *n* = 4	5.73 ± 0.16 *n* = 4	5.87 ± 0.06 *n* = 4
1b	phth	CH$_3$	H	phth	6.90 ± 0.03[a] *n* = 3	−0.04 ± 0.03[a] *n* = 3	6.86 ± 0.01[a] *n* = 3	6.87 ± 0.04[a] *n* = 3
1d	phth	CH$_3$	CH$_3$	phth	7.08 ± 0.09[a] *n* = 3	−0.21 ± 0.02[a,c] *n* = 3	6.87 ± 0.08[a] *n* = 3	6.75 ± 0.04[a] *n* = 3
2a	5-mephth	H	H	phth	6.73 ± 0.08 *n* = 3	−0.31 ± 0.02[c] *n* = 3	6.42 ± 0.09 *n* = 3	6.49 ± 0.04 *n* = 3
2b	5-mephth	CH$_3$	H	phth	7.26 ± 0.03[a] *n* = 3	0.19 ± 0.02[a,b] *n* = 3	7.45 ± 0.03[a] *n* = 3	7.26 ± 0.02[a] *n* = 3
2c	5-mephth	H	CH$_3$	phth	7.20 ± 0.22[a] *n* = 3	−0.13 ± 0.02[a,b] *n* = 3	7.07 ± 0.24[a] *n* = 3	7.15 ± 0.11[a] *n* = 3
2d	5-mephth	CH$_3$	CH$_3$	phth	6.73 ± 0.24 *n* = 4	0.17 ± 0.02[c] *n* = 4	6.90 ± 0.23 *n* = 4	6.92 ± 0.08 *n* = 3
4a	5-mephth	H	H	5-mephth	7.08 ± 0.10[a] *n* = 3	−0.33[a] *n* = 3	6.75[a] *n* = 3	6.77 ± 0.03[a] *n* = 2−5
4b	5-mephth	CH$_3$	H	5-mephth	7.41 ± 0.02[a] *n* = 3	0.03 ± 0.02[a] *n* = 3	7.44 ± 0.0001[a] *n* = 3	7.44 ± 0.06[a] *n* = 3
4d	5-mephth	CH$_3$	CH$_3$	5-mephth	7.21 ± 0.11[a] *n* = 3	0.17 ± 0.02[a,b] *n* = 3	7.38 ± 0.09[a] *n* = 3	7.33 ± 0.10[a] *n* = 3
3a	1,8-naphth	H	H	phth	7.24 ± 0.13 *n* = 4	−0.20 ± 0.05[b] *n* = 4	7.04 ± 0.12 *n* = 4	6.94 ± 0.30 *n* = 6
3b	1,8-naphth	CH$_3$	H	phth	8.29 ± 0.18 *n* = 8	0.37 ± 0.08[c] *n* = 8	8.66 ± 0.14 *n* = 8	8.36 ± 0.09 *n* = 11
3c	1,8-naphth	H	CH$_3$	phth	7.28 ± 0.002 *n* = 3	−0.005 ± 0.004 *n* = 3	7.28 ± 0.00 *n* = 3	7.28 ± 0.08 *n* = 4
3d	1,8-naphth	CH$_3$	CH$_3$	phth	7.41 ± 0.09 *n* = 5	0.56 ± 0.08[c] *n* = 5	7.97 ± 0.10 *n* = 5	7.73 ± 0.09 *n* = 5
5a	1,8-naphth	H	H	5-mephth	8.01 ± 0.12 *n* = 3	−0.22 ± 0.03[b] *n* = 3	7.79 ± 0.10 *n* = 3	7.60 ± 0.05 *n* = 3
5b	1,8-naphth	CH$_3$	H	5-mephth	7.90 ± 0.15 *n* = 3	0.32 ± 0.07[b] *n* = 3	8.23 ± 0.13 *n* = 3	8.12 ± 0.10 *n* = 7
5c	1,8-naphth	H	CH$_3$	5-mephth	7.48 ± 0.02 *n* = 3	−0.04 ± 0.02 *n* = 3	7.44 ± 0.002 *n* = 3	7.44 ± 0.07 *n* = 3
5d	1,8-naphth	CH$_3$	CH$_3$	5-mephth	7.90 ± 0.16 *n* = 3	0.29 ± 0.06[b] *n* = 3	8.19 ± 0.11 *n* = 3	7.85 ± 0.09 *n* = 4
6a	1,8-naphth	H	H	1,8-naphth	8.11 ± 0.13 *n* = 3	−0.28 ± 0.03[b] *n* = 3	7.83 ± 0.11 *n* = 3	7.86 ± 0.07 *n* = 12
6b	1,8-naphth	CH$_3$	H	1,8-naphth	8.07 ± 0.06 *n* = 3	0.17 ± 0.02[b] *n* = 3	8.24 ± 0.08 *n* = 3	8.11 ± 0.10 *n* = 5
6d	1,8-naphth	CH$_3$	CH$_3$	1,8-naphth	7.51 ± 0.08 *n* = 3	0.53 ± 0.06[b] *n* = 3	8.04 ± 0.07 *n* = 3	8.07 ± 0.10 *n* = 4

[a] Data taken from ref 28. [b,c] Cooperativity is significantly different from neutral (pα different from zero, [b] $p < 0.05$, [c] $p < 0.01$).

value can also be derived from the separate [^{3}H]NMS-dissociation experiments as pEC$_{50,diss}$; EC$_{50,diss}$ denotes the concentration of the allosteric agent at which [^{3}H]-NMS-dissociation is half-maximally retarded. For all compounds, the respective values for p(αK_A) and pEC$_{50,diss}$ were equal, thus supporting the validity of the ternary

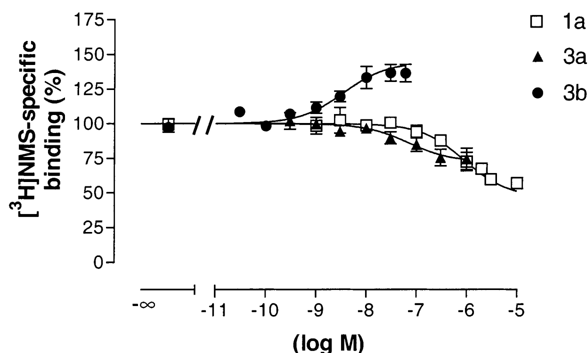


Figure 1. Effects of the indicated test compounds on the equilibrium binding of [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) to porcine heart M_2 receptors. Ordinate: specific [^{3}H]NMS binding in percent of the control value in the absence of test compound as indicated by the starting value of the binding curve. Abscissa: concentration of the test compound. Indicated are mean values ± SEM, $n = 4-8$ independent experiments. Error bars are not visible when smaller than the symbols. Curve fitting according to the ternary complex model of allosteric interactions. For details, see text.

complex model as a quantitative mechanism underlying the experimental findings. The molecular basis for the negative cooperativity between **1a** and [^{3}H]NMS is that **1a** has a higher affinity for free receptors, pK_A = 6.19, than for [^{3}H]NMS-occupied receptors, p(αK_A) = 5.73.

Compound **3a**, in which one of the phthalimide residues is replaced by naphthalimide, has a 10-fold higher affinity for free M_2 receptors than **1a** (Table 2). The cooperativity with [^{3}H]NMS is negative, i.e., pK_A > p(αK_A), cf. Table 2. Propyl-chain methylation in **3a** next to the naphthalimide residue leads to **3b**; compared with **3a**, the affinity for free receptors is increased by a factor of about 10, and most remarkably, the negative cooperativity with [^{3}H]NMS is switched into a positive cooperative interaction. On a molecular level this means that the affinity of **3b** is higher for [^{3}H]NMS-occupied receptors, p(αK_A) = 8.66, compared with free receptors, pK_A = 8.29 (Table 2). Comparison of **3b** with **1a** and **3a** reveals that the above-mentioned structural modifications increased the affinity for [^{3}H]NMS-occupied receptors, p(αK_A), to a greater extent than the affinity for free receptors, pK_A. In other words, cooperativity is a composite parameter[36] and, for the compounds described here, the underlying molecular events are characterized by divergent structure–activity relationships.

With regard to structure–activity relationships, the symmetrical and nonsymmetrical compounds (**1a**, **2a**, **3a**, **4a**, **6a**, and **7–20**) having *no* methyl groups in the lateral propyl chains will be discussed first. The affinity for NMS-occupied receptors of the nonsymmetrical compounds was found to be in a range of p(αK_A) = 5.7 to 7.0 whereas the affinity range of the symmetrical compounds is slightly larger (5.0 to 7.8). However, comparing pairs of nonsymmetrical and symmetrical compounds reveals, with the exception of the **3a/6a** pair, that there are no significant differences in the affinity to bind to NMS-liganded receptors. Logically, with the exception of the 5-phthalimidomethylated compounds **2a** and **4a**, ranking of both series with respect to the affinity for NMS-occupied receptors gave the same order. In a previous study,[25] that was focused on nonsymmetrical compounds, we found a parabolic correlation between the affinity for NMS-occupied receptors and the volume of the lateral moiety. The data set has now slightly changed: the pyridinimide compound was replaced with pyrazinimide derivatives (**7**, **14**) and compounds substituted with a trifluoromethyl group in position 5 (**9**, **16**) were added. In analogy to the previous study[25] the lipophilicity (log P_1, log P_2), the volume (Vol_1, Vol_2), the surface, the polarizibility (Pol), and the refractivity (Ref) of the corresponding *N*-methylimides were calculated for both lateral imide moieties separately using the Hyperchem/Chemplus software package, and the quantitative structure–activity relationships (QSAR) of the series of symmetrical and nonsymmetrical compounds were analyzed. The best correlations between the physicochemical parameters and the affinities to the free and the NMS-occupied receptors are displayed in Table 3 using the general equation

$$\mathrm{pBE} = \beta_0 + \beta_{\mathrm{Vol}} \cdot x_{\mathrm{Vol1}} + \beta_{\mathrm{Vol}} \cdot x_{\mathrm{Vol2}} + \beta_{\log P1} \cdot x_{\log P1} + \beta_{\mathrm{Pol}} \cdot x_{\mathrm{Pol}} + \beta_{\mathrm{Ref}} \cdot x_{\mathrm{Ref}}$$

pBE is the estimated negative logarithm of the corresponding biological effect. R^2 is the square of the correlation coefficient, s the standard deviation, F the ratio of explained to unexplained variance, Q^2 is the cross validated R^2 by using the leave-one-out procedure (Q^2 can adopt values between 1 and 0), and s_{PRESS} the standard deviation from the predictive residual sum of squares. Only equations with significant correlation coefficients (β) are considered in the following discussion. Interestingly, the correlations found with the affinities for the free and the NMS-occupied receptors are different from each other. The affinity for the free receptor can be described using one parameter, the polarizibility. Adding the lipophilicity reveals a slightly weaker but nonsignificant correlation. In contrast, the correlations found with the affinities to the NMS-occupied receptor are much weaker. Using three parameters, i.e., the volume of one lateral moiety, the lipophilicity, and the polarizibility, a significant correlation for p(αK_A) was found, but it was still weak. Using a parabolic model for the regression analysis[37] did not improve the situation. However, the divergent correlations clearly indicate that the binding sites of the free and NMS-liganded receptor are structurally different and the affinity of this series of compounds for the free M_2 receptor is always higher than for the NMS-liganded receptor, thus leading to a negative cooperativity.

The 21 compounds carrying either phthalimides, methylphthalimides, or naphthalimides at the ends and having a systematic introduction of methyl groups in the propyl chains will be considered in the following. Table 2 clearly shows that the introduction of one methyl group always increases the p(αK_A) value in comparison to the nonalkyl substituted compounds **1a**, **2a**, **3a**, **4a**, **5a**, and **6a**, with the exception of compound **5c**. However, in the series of nonsymmetrical compounds the extent by which the affinity to the NMS-occupied receptor (Table 2) surpasses the affinity to the free receptor depends sensitively on the locus of methylation: if the 2,2-dimethylpropyl chain is neighbored upon the imide which induces a higher affinity to the occupied receptor in comparison to the affinity to the free receptor the cooperativity is positive, and vice versa, if the 2,2-dimethylpropyl chain is neighbored upon the

Table 3. Results of the Multidimensional Linear Regression Analysis Representing the Best Correlations between the Physicochemical Parameters and the Affinities for the Free ($y = pK_A$) and the NMS-Occupied Receptor ($y = p[\alpha K_A]$) of the Compounds **1a**, **2a**, **3a**, **4a**, **6a**, and **7**−**20**

$y = p(\alpha K_A)$

Vol_1	Vol_2	$\log P_1$	Pol	β_{Vol1}	β_{Vol2}	$\beta_{\log P1}$	β_{Pol}	β_0	R^2	s	F	Q^2	s_{PRESS}
−	−	+	−	−	−	0.443 ± 0.22	−	5.519 ± 0.51	0.510	0.432	17.696	0.378	0.487
−	−	+	+	−	−	0.327 ± 0.33	0.0349 ± 0.073	5.029 ± 1.15	0.540	0.432	9.372	0.335	0.519
+	−	+	−	0.000742 ± 0.0046	−	0.494 ± 0.39	−	5.850 ± 2.10	0.514	0.444	8.449	0.345	0.515
+	−	+	+	−0.0145 ± 0.0072	−	0.611 ± 0.27	0.251 ± 0.12	8.469 ± 1.89	0.792	0.299	19.131	0.621	0.405
+	+	+	+	−0.0150 ± 0.0074	0.00102 ± 0.0024	0.611 ± 0.27	0.251 ± 0.12	8.250 ± 2.01	0.805	0.301	14.375	0.631	0.414

$y = pK_A$

Vol_1	$\log P_1$	Pol	Ref	β_{Vol1}	$\beta_{\log P1}$	β_{Pol}	β_{Ref}	β_0	R^2	s	F	Q^2	s_{PRESS}
+	−	−	−	0.00693 ± 0.0025	−	−	−	2.875 ± 1.48	0.669	0.414	34.381	0.600	0.455
−	−	+	−	−	−	0.143 ± 0.037	−	3.970 ± 0.80	0.792	0.327	65.013	0.734	0.371
−	+	−	−	−	0.535 ± 0.25	−	−	5.846 ± 0.57	0.548	0.483	20.634	0.404	0.555
−	+	+	−	−	0.135 ± 0.25	0.121 ± 0.055	−	4.150 ± 0.86	0.808	0.324	33.808	0.678	0.421
+	−	+	−	−0.00313 ± 0.0067	−	0.200 ± 0.13	−	4.630 ± 1.62	0.804	0.328	32.962	0.712	0.398
+	+	−	−	0.00536 ± 0.0043	0.166 ± 0.36	−	−	3.456 ± 1.96	0.687	0.414	17.594	0.541	0.502
+	+	+	−	−0.00693 ± 0.0071	0.270 ± 0.27	0.224 ± 0.12	−	5.792 ± 1.86	0.852	0.295	28.594	0.696	0.422
+	+	+	+	−0.00964 ± 0.0070	0.250 ± 0.24	0.240 ± 0.11	0.0208 ± 0.021	5.970 ± 1.70	0.887	0.267	27.409	0.761	0.388

Table 4. Results of the Multidimensional Linear Regression Analysis Representing the Best Correlations between the Physicochemical Parameters and the Affinities to the Free ($y = pK_A$) and the NMS-Occupied Receptor ($y = p[\alpha K_A]$) of the Compounds **1**−**6**

$y = p\alpha$

Vol_1	Vol_2	Met_1	Met_2	β_{Vol1}	β_{Vol2}	β_{Met1}	β_{Met2}	β_0	R^2	s	F	Q^2	s_{PRESS}
−	−	+	−	−	−	0.432 ± 0.180	−	−0.220 ± 0.14	0.573	0.194	25.574	0.485	0.213
−	−	+	+	−	−	0.408 ± 0.170	0.143 ± 0.170	−0.268 ± 0.14	0.635	0.184	15.645	0.511	0.213
+	−	+	−	0.00380 ± 0.0014	−	0.455 ± 0.110	−	−2.402 ± 0.81	0.848	0.119	50.213	0.793	0.139
+	+	+	−	0.00440 ± 0.0015	−0.00130 ± 0.0015	0.466 ± 0.110	−	−2.063 ± 0.86	0.889	0.112	38.765	0.789	0.144
+	−	+	+	0.00367 ± 0.0012	−	0.435 ± 0.099	0.117 ± 0.099	−2.365 ± 0.72	0.889	0.105	45.101	0.815	0.135
+	+	+	+	0.00418 ± 0.0014	−0.00107 ± 0.0014	0.446 ± 0.096	0.105 ± 0.096	−2.089 ± 0.77	0.904	0.100	37.940	0.815	0.139

$y = p(\alpha K_A)$

Vol_1	Vol_2	Met_1	Met_2	β_{Vol1}	β_{Vol2}	β_{Met1}	β_{Met2}	β_0	R^2	s	F	Q^2	s_{PRESS}
+	−	−	−	0.0135 ± 0.0054	−	−	−	−0.294 ± 3.10	0.588	0.463	27.130	0.476	0.523
+	+	−	−	0.0121 ± 0.0061	0.00315 ± 0.0062	−	−	−1.145 ± 3.52	0.613	0.462	14.240	0.449	0.551
+	−	+	−	0.0142 ± 0.0032	−	0.731 ± 0.25	−	−1.111 ± 1.86	0.863	0.274	57.102	0.816	0.319
+	−	+	+	0.0143 ± 0.0033	−	0.753 ± 0.26	−0.120 ± 0.26	−1.149 ± 1.87	0.870	0.274	38.277	0.800	0.341
+	+	+	−	0.0133 ± 0.0037	0.00185 ± 0.0037	0.715 ± 0.26	−	−1.593 ± 2.10	0.872	0.273	38.680	0.798	0.343
+	+	+	+	0.0136 ± 0.0038	0.00162 ± 0.0038	0.734 ± 0.27	−0.103 ± 0.27	−1.568 ± 2.13	0.878	0.276	28.621	0.775	0.374

$y = pK_A$

Vol_1	Vol_2	Met_1	Met_2	β_{Vol1}	β_{Vol2}	β_{Met1}	β_{Met2}	β_0	R^2	s	F	Q^2	s_{PRESS}
+	−	−	−	0.01010 ± 0.0041	−	−	−	1.594 ± 2.33	0.590	0.347	27.247	0.479	0.391
+	+	−	−	0.00851 ± 0.0044	0.00359 ± 0.0044	−	−	0.625 ± 2.53	0.646	0.331	16.411	0.483	0.400
+	−	+	−	0.01040 ± 0.0038	−	0.277 ± 0.30	−	1.285 ± 2.21	0.659	0.325	17.421	0.533	0.380
+	−	+	+	0.01070 ± 0.0037	−	0.318 ± 0.29	−0.238 ± 0.29	1.210 ± 2.11	0.709	0.309	13.856	0.537	0.390
+	+	+	−	0.00895 ± 0.0042	0.00314 ± 0.0042	0.249 ± 0.29	−	0.469 ± 2.41	0.702	0.313	13.344	0.510	0.401
+	+	+	+	0.00940 ± 0.0041	0.00268 ± 0.0042	0.290 ± 0.29	−0.210 ± 0.29	0.521 ± 2.33	0.740	0.301	11.393	0.508	0.414

imide which produces the lower affinity to the NMS-liganded receptor the cooperativity remains negative or only changes to neutral. Compare, for example, the nonsubstituted compound **3a** ($p\alpha = -0.2$) with compound **3b** where the affinity-increasing naphthalimide moiety is attached to the methylated propyl chain resulting in a positive cooperativity ($p\alpha = 0.37$) and with compound **3c** in which the alkylated propyl chain is connected to the "less potent" phthalimide resulting in a neutral cooperativity ($p\alpha = -0.005$). The same is true for the series **2a**, **2b**, and **2c** and the series **5a**, **5b**, and **5c**. In addition, the highest affinity for the allosteric site in conjunction with positive cooperativity is found with compounds having at least one naphthyl group attached to a 2,2-dimethylpropyl chain.

To quantify the SARs it was tried to correlate the affinities to the free and NMS-occupied receptors with the aforementioned physicochemical parameters. As an additional parameter we introduced two indicator variables (Met_1, Met_2) which represent the presence or absence of the methyl residues in the propyl chains adopting values of either one or zero. The best correlations are summarized in Table 4 using the general equation

$$pBE = \beta_0 + \beta_{Vol1} \cdot x_{Vol1} + \beta_{Vol2} \cdot x_{Vol2} + \beta_{Met1} \cdot x_{Met1} + \beta_{Met2} \cdot x_{Met2}$$

Again different correlations were found for pK_A and $p(\alpha K_A)$. The affinity to the NMS-liganded receptor, $p(\alpha K_A)$, cannot be characterized with either volume or methylation but it can be described with the volume in connection with the adjacent methyl group with high significance ($R^2 = 0.86$ and $Q^2 = 0.82$). The combination

is in accordance with the qualitative SAR discussed above. Using further parameters leads to weaker correlations. In contrast to this observation and to the findings revealed for the series of symmetrical and nonsymmetrical compounds with a missing methylation (see Table 1) much weaker correlations were found for the affinities to the free receptor. There is no significant difference when using both volumes, the volume and the adjacent methylation, or combinations of three or four of these parameters. These results clearly indicate the importance of the combination of the affinity generating lateral imide moiety with an adjacent methylation with regard to positive cooperativity. A contralateral methylation is not able to induce a positive cooperativity, at the very best a neutral cooperativity is attained.

Previous molecular modeling and QSAR studies revealed the pharmacophore for a high affinity to the NMS-occupied receptor to be composed of two positively charged nitrogens in a distance of about 10 Å and two aromatic imides all together arranged in an S-shaped conformation.[38−40] This pharmacophore hypothesis predicts symmetrical compounds to surpass nonsymmetrical compounds in allosteric potency. However, inspection of the data obtained for the symmetrical/nonsymmetrical collection of compounds (Table 1**)** *generally* revealed similar affinities in both series (except **3a**/**6a**). Furthermore, in the series of compounds displayed in Table 2 (**1**−**6**) the highest allosteric affinities are not found with the symmetrical compounds **1a**, **1d**, **4a**, **4d**, **6a**, and **6d**. These are found with compounds having at least one naphthyl group attached to a 2,2-dimethylpropyl chain, indicating that this moiety may fit perfectly into a binding pocket of the allosteric site. Phthalimide and methylphthalimide skeletons connected to a 2,2-dimethylpropyl chain seem to have less ability to interact with the binding pocket resulting in lower affinities; compare e.g., **1b**, **2b**, **2c**, **4b**, with **3b**. Furthermore, when the phthalimide or the methylphthalimide with a 2,2-dimethylpropyl chain are combined with a naphthyl group attached to a 2,2-dimethylpropyl chain (i.e., **3d** and **5d**), the allosteric potency is found to be less than for **3b** and **5b**, respectively, having only a phthalimidopropyl or a methylphthalimidopropyl chain, but no dimethylation on that side. Thus, the phthalimide and methylphthalimide skeletons by connection with a 2,2-dimethylpropyl chain seem to gain the competence to compete for the binding pocket that is used by the corresponding naphthalimide resulting in a lower affinity to the allosteric site.

Since all affinities to the NMS-occupied receptor were determined in Mg/Tris-buffered medium and magnesium cations were found to be weak allosteric modulators competing with the hexamethonium compounds for the allosteric binding site,[41] the high-affinity compound **6a** was additionally evaluated in 5 mM Na^+/K^+-phosphate-buffered medium, resulting in a $pK_A = 9.06 \pm 0.02$, $p\alpha = -0.03 \pm 0.02$, $p(\alpha K_A) = 9.03 \pm 0.01$ (n = 3) and a $pEC_{50,diss} = 9.03 \pm 0.05$ (n = 4). Compared with **4a** ($pK_A = 8.03 \pm 0.12$, $p\alpha = -0.04 \pm 0.05$, $p(\alpha K_A) = 8.36 \pm 0.10$ (n = 3) and $pEC_{50,diss} = 8.46 \pm 0.09$ (n = 5)) as well as alcuronium ($pEC_{50,diss} = 8.40$ in Na^+/K^+/Pi),[10] the bisnaphthalimide compound **6a** is found to have the highest affinity reaching the subnanomolar range of concentrations.

The affinity of an allosteric agent for an orthosteric ligand-occupied receptor is influenced by the structure of the orthosteric ligand.[42] Therefore, cooperative interactions may depend in a specific fashion on the type of orthosteric ligand. In other words, the findings described here for the antagonist NMS cannot be generalized for other orthosteric ligands such as the endogenous agonist acetylcholine. The dependence of cooperativity on the type of orthosteric ligand is an interesting field for systematic investigations in order to understand the molecular basis of cooperativity.

Conclusion

Taken all results together it can be stated that the lateral imide moieties are able to modulate the affinities to free and NMS-liganded receptors within a rather wide range of 3 orders of magnitude. No relevant differences were found between symmetrical and nonsymmetrical compounds. Negative cooperativity with NMS could consistently be abolished or even switched into positive cooperativity by alkylation of the lateral propyl chains for all imide moieties studied in this regard. This effect results from a more pronounced gain in affinity in the NMS-occupied receptor than in the free receptor. As a consequence, the methylated compounds show higher allosteric potency in the nanomolar range of concentration. From these results the question arises whether a monoalkylation of the propyl chain would produce the same effects and whether monoalkylation yields enantioselectivity and whether ethylation, propylation, etc., are characterized by a "cutoff" phenomenon. This work is in progress.

Experimental Section

Melting points were determined with a Gallenkamp and a Dr. Tottoli melting point apparatus (Büchi, Switzerland) and were not corrected. ^1H and ^{13}C NMR spectra (Supporting Information) were recorded on a Varian XL 300 (^1H 299.956 MHz; ^{13}C 75.433 MHz) and on a Bruker AV 400 instrument (^1H 400.132 MHz; ^{13}C 100.613 MHz). Abbreviations for data quoted are: s, singlet; d, doublet; t, triplet; qu, quartet; quin, quintet; dd, doublet of doublets; m, multiplet; br, broad. The centers of the peaks of $CDCl_3$ and DMSO-d_6 were used as internal references. IR spectra were obtained using a Biorad PharmalyzIR FT-IR spectrometer and a Perkin-Elmer 298 spectrometer. Dry solvents were used throughout. Chemicals were of analytical grade and purchased from Lancaster (Mühlheim, Germany) and Aldrich (Steinheim, Germany).

1a (W84) was synthesized according to ref 18, **4a** (dimethyl-W84) according to ref 20, **1b**, **1d**, **2a**−**c**, and **4d** according to ref 28, and **3a**, **8**, and **10**−**13** according to ref 25. The intermediate compounds **22**, **25**, **31**, **32**, as well as **34**, **36**, and **37**, were obtained according to ref 28. Moreover the intermediate compounds **27** and **30** are known.[25]

General Procedure for the Synthesis of 6-(3-Dimethylaminopropyl)pyrrolo[3,4-*b*]pyrazine-5,7-dione (21), 2-(3-Dimethylaminopropyl)-5,6-difluoroisoindole-1,3-dione (23), 2-(3-Dimethylaminopropyl)-5-trifluoromethylisoindole-1,3-dione (24), 5-*tert*-Butyl-2-(3-(dimethylamino-)propyl)isoindole-1,3-dione (26), 2-(3-Dimethylaminopropyl)-4,9[1'2']-benzeno-1*H*-benz[*f*]isoindole-1,3-dione (27), 2-(3-Dimethylaminopropyl)benzo[*f*]isoindole-1,3-dione (28), 5,6-Dichloro-2-(3-(dimethylamino)propyl)isoindole-1,3-dione (29), 2-(3-Dimethylaminopropyl)benzo-[*de*]isoquinoline-1,3-dione (30), and 2-(3-Dimethylamino-2,2-dimethylpropyl)benzo[*de*]isoquinoline-1,3-dione (33). A mixture of the appropriate anhydride (40 mmol), N^1,N^1-dimethylpropane-1,3-diamine or 2,2,N^1,N^1-tetramethylpropane-1,3-diamine (40 mmol) and catalytic amount of *p*-toluene-

Table 5. Reaction Conditions and Analytical Data of Compounds **2d**, **3a–d**, **5a–d**, **6a**, **6b**, **6d**, **7–21**, **23**, **24**, **26–30**, **33**, **35**, and **38**

compd	formula (mol wt)	reagents	reaction time: hours (h), days (d)	yield, %	mp, °C	IR (ν, cm^{-1})
2d[a]	$C_{37}H_{54}Br_2N_4O_4$ (778.7)	31 + 37	12 d	7	206–207	733, 1080, 1366, 1711, 1769, 2972
3a	$C_{36}H_{46}Br_2N_4O_4$ (758.6)	30 + 34	2 d	56	236	725, 780, 1660, 1710, 1770, 2670, 2950, 3010
3b[a]	$C_{38}H_{50}Br_2N_4O_4$ (786.7)	22 + 38	2 d	42	222–224	723, 780, 1236, 1339, 1587, 1657, 1707, 1768, 2940
3c	$C_{38}H_{50}Br_2N_4O_4$ (786.7)	30 + 36	1 d	51	198–200	730, 780, 1610, 1660, 1710, 1780, 2960
3d[a]	$C_{40}H_{54}Br_2N_4O_4$ (814.7)	31 + 38	15 d	14	201–202	729, 781, 1237, 1341, 1589, 1658, 1712, 1775, 2935
5a[a]	$C_{37}H_{48}Br_2N_4O_4$ (772.6)	30 + 35	1 d	78	256–257	744, 782, 1230, 1344, 1660, 1709, 1769, 2869, 2947
5b	$C_{39}H_{52}Br_2N_4O_4$ (800.7)	25 + 38	3 d	19	202–204	739, 781, 1236, 1338, 1587, 1657, 1699, 1765, 2945
5c	$C_{39}H_{52}Br_2N_4O_4$ (800.7)	30 + 37	4 d	59	204–207	740, 770, 1380, 1620, 1660, 1710, 1770, 2960
5d[a]	$C_{41}H_{56}Br_2N_4O_4$ (828.7)	32 + 38	12 d	8	198–199	750, 779, 1236, 1338, 1587, 1655, 1705, 1770, 2937
6a	$C_{40}H_{48}Br_2N_4O_4$ (808.7)	30	2 d	29	255	780, 1230, 1345, 1660, 1700, 2950, 3000
6b[a]	$C_{42}H_{52}Br_2N_4O_4$ (836.7)	30 + 38	2 d	73	219	775, 1238, 1336, 1382, 1587, 1649, 1693, 2960
6d[a]	$C_{44}H_{56}Br_2N_4O_4$ (864.8)	33	12 d	34	248	781, 1238, 1341, 1587, 1655, 1703, 2931
7	$C_{30}H_{42}Br_2N_6O_4$ (710.5)	21 + 34	2 d	26	165	730, 1405, 1710, 1770, 2950
8	$C_{32}H_{42}Br_2F_2N_4O_4$ (744.5)	23 + 34	2 d	44	226	725, 750, 1295, 1400, 1710, 1765, 2950, 3000
9[a]	$C_{33}H_{43}Br_2F_3N_4O_4$ (776.5)	24 + 34	2 d	35	228–231	692, 725, 1137, 1172, 1321, 1380, 1710, 3006
10	$C_{36}H_{52}Br_2N_4O_4$ (764.6)	26 + 34	2 d	17	241	725, 750, 1375, 1400, 1705, 1770, 2960, 3010
11	$C_{42}H_{54}Br_2N_4O_4$ (838.7)	27 + 34	2 d	43	258	730, 765, 1400, 1700, 1770, 2950, 3000
12	$C_{36}H_{46}Br_2N_4O_4$ (758.6)	28 + 34	2 d	51	253	725, 770, 1370, 1700, 1760, 2950, 3010
13	$C_{32}H_{42}Br_2Cl_2N_4O_4$ (777.3)	29 + 34	2 d	10	228	725, 1375, 1400, 1705, 1770, 2950
14	$C_{28}H_{40}Br_2N_8O_4$ (712.5)	21	2 d	17	114	700, 1195, 1445, 1535, 1665, 2950
15	$C_{32}H_{40}Br_2F_4N_4O_4$ (780.5)	23	2 d	46	241	745, 770, 1400, 1490, 1710, 1775, 2950, 3060
16[a]	$C_{34}H_{42}Br_2F_6N_4O_4$ (844.5)	24	2 d	49	246–247	691, 744, 1120, 1164, 1317, 1717, 1776, 3004
17	$C_{40}H_{60}Br_2N_4O_4$ (820.7)	26	2 d	34	176	750, 1375, 1400, 1705, 1770, 2960
18	$C_{52}H_{62}Br_2N_4O_4$ (966.9)	27	2 d	59	278	770, 1400, 1460, 1700, 1775, 2960, 3000
19	$C_{40}H_{48}Br_2N_4O_4$ (808.6)	28	2 d	26	261	770, 1375, 1700, 1760, 2950, 3010
20	$C_{32}H_{40}Br_2Cl_4N_4O_4$ (846.3)	29	2 d	21	227	745, 1380, 1400, 1705, 1770, 3100
21	$C_{11}H_{14}N_4O_2$ (234.3)	-	12 h	38	oil	730, 1470, 1530, 1660, 2950, 3300
23	$C_{13}H_{14}F_2N_2O_2$ (268.3)	-	12 h	76	93	745, 1200, 1405, 1690, 2770, 3050
24[a]	$C_{14}H_{15}F_3N_2O_2$ (300.3)	-	2 h	70	54	694, 750, 1126, 1320, 1699, 2767, 2949
26	$C_{17}H_{24}N_2O_2$ (288.4)	-	12 h	62	oil	750, 1370, 1710, 1770, 2930, 3470
27	$C_{23}H_{24}N_2O_2$ (360.5)	-	12 h	82	163	770, 1690, 1765, 2770, 2980, 3450
28	$C_{17}H_{18}N_2O_2$ (282.4)	-	2 d	34	97	770, 1385, 1690, 1760, 2770, 2940
29	$C_{13}H_{14}Cl_2N_2O_2$ (301.2)	-	2 d	60	146	750, 1640, 1710, 1775, 2770, 2950, 3300
30[a]	$C_{17}H_{18}N_2O_2$ (282.4)	-	3 h	88	119	775, 1232, 1340, 1587, 1651, 1695, 2762, 2942
33[a]	$C_{19}H_{22}N_2O_2$ (310.4)	-	9 h	61	112–114	780, 1236, 1330, 1588, 1662, 1699, 2763, 2960
35[a]	$C_{20}H_{30}Br_2N_2O_2$ (490.3)	25	2 d	84	145–146	738, 1391, 1698, 1762, 2858, 2941
38[a]	$C_{25}H_{34}Br_2N_2O_2$ (554.4)	33	11 d	55	178–180	781, 1237, 1338, 1586, 1659, 1705, 2950

[a] Recorded on a Biorad PharmalyzIR FT-IR spectrometer.

sulfonic acid were refluxed in toluene (100 mL) using a water separator. After a reaction time of 2 h to 2 days the solvent was evaporated. Compound **33** was obtained from a solid residue that was recrystallized by a mixture of ethanol and a few drops of petroleum ether. Concerning compounds **21**, **23**, **24**, **26**, and **28–30**, their oily residues were purified by means of column chromatography (silica gel, eluent CH_2Cl_2:MeOH = 1:1). With exception of compounds **21** and **26** the obtained oils crystallized after a few hours at room temperature.

For analytical data see Table 5, for spectroscopic data see Table 8 (Supporting Information).

General Procedure for the Synthesis of (6-Bromohexyl)dimethyl-[3-(5-methyl-1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]ammonium Bromide (35) and (6-Bromohexyl)-[3-(1,3-dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)-2,2-dimethylpropyl]dimethylammonium Bromide (38). Precursor propylamines **25** and **33** (10 mmol) were stirred in a 10-fold excess of 1,6-dibromohexane (100 mmol) without any solvent. Compound **25** reacted at room temperature, whereas compound **33** was heated to 50 °C. After a reaction time of 2 and 11 days, respectively, a voluminous white precipitate was collected by filtration. To remove the 1,6-dibromohexane excess, the solid was suspended in petroleum ether, refluxed, cooled, filtered, and dried in vacuo.

For analytical data see Table 5, for spectroscopic data see Table 8 (Supporting Information).

General Procedure for the Synthesis of 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydrobenzo[*de*]isoquinolin-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]-propyl}benzo[*de*]isoquinoline-1,3-dione Dibromide (6a), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydrobenzo-[*de*]isoquinolin-2-yl)-2,2-dimethylpropyl]ammonium}-hexyl)-1,1-dimethylammonio]-2,2-dimethylpropyl}benzo-[*de*]isoquinoline-1,3-dione Dibromide (6d), 6-{3-[1-(6-{1,1-Dimethyl-1-[3-(5,7-dioxo-5,7-dihydropyrrolo[3,4-*b*]-pyrazin-6-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}pyrrolo[3,4-*b*]-pyrazine-5,7-dione Dibromide (14), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(5,6-difluoro-1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]ammo-nium}hexyl)-1,1-dimethylammonio]propyl}-5,6-difluoroisoindoline-1,3-dione Dibromide (15), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-5-trifluoro-methyl-isoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}-5-trifluoromethyl-isoindoline-1,3-dione Dibromide (16), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(5-*tert*-butyl-1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}-5-*tert*-butyl-isoindoline-1,3-dione Dibromide (17), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-4,9[1'2']benzeno-1*H*-benz[*f*]isoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}-(4,9[1'2']-benzeno-1*H*-benz[*f*])isoindoline-1,3-dione Dibromide (18), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydrobenzo[*f*]isoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}benzo[*f*]isoindoline-1,3-dione Dibromide (19), and 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(5,6-dichloro-1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}-5,6-dichloro-isoindolin-1,3-dione Dibromide (20). Two equivalents of **21**, **23**, **24**, **26–30**, and **33** (2 mmol) and 1 equiv of 1,6-dibromohexane (1 mmol) were dissolved in acetonitrile (50 mL), and a catalytic amount of KI and K_2CO_3 (1:1) was added. The reaction solution was refluxed for 2 to 12 days. After the reaction was completed (TLC-monitoring: silica gel, mobile phase = CH_3OH:0.2 M NH_4NO_3 solution (aqueous) = 3:2), the solution was allowed to cool to room temperature and a white to light yellow precipitate was collected by filtration. It was washed several times with acetonitrile and pentane and dried in vacuo.

For analytical data see Table 5, for spectroscopic data see Tables 6 and 7 (Supporting Information).

General Procedure for the Synthesis of 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-5-methylisoindol-2-yl)-2,2-dimethylpropyl]ammonium}hexyl)-1,1-dimethylammonio]-2,2-dimethylpropyl}-5-methylisoindole-1,3-dione Dibromide (2d), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]-2,2-dimethylpropyl}benzo-[*de*]isoquinoline-1,3-dione Dibromide (3b), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-2,2-dimethylpropyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}benzo[*de*]isoquinoline-1,3-dione Dibromide (3c), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-2,2-dimethylpropyl]ammonium}hexyl)-1,1-dimethylammonio]-2,2-dimethylpropyl}- benzo[*de*]isoquinoline-1,3-dione Dibromide (3d), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-5-methyl-isoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylammonio]propyl}benzo[*de*]isoquinoline-1,3-dione Dibromide (5a), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-5-methyl-isoindol-2-yl)propyl]-ammonium}hexyl)-1,1-dimethylammonio]-2,2-dimethylpropyl}benzo[*de*]isoquinoline-1,3-dione Dibromide (5b), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-5-methyl-isoindol-2-yl)-2,2-dimethylpropyl]-ammonium}hexyl)-1,1-dimethylammonio]propyl}benzo[*de*]isoquinoline-1,3-dione Dibromide (5c), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydro-5-methyl-isoindol-2-yl)-2,2-dimethylpropyl]ammonium}hexyl)-1,1-dimethylammonio]-2,2-dimethylpropyl}benzo-[*de*]isoquinoline-1,3-dione Dibromide (5d), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydrobenzo[*de*]-isoquinolin-2-yl)-2,2-dimethylpropyl]am-monium}hexyl)-1,1-dimethylammonio]propyl}-benzo[*de*]isoquinoline-1,3-dione Dibromide (6b), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]ammonium}hexyl)-1,1-dimethylam-monio]propyl}pyrrolo[3,4-*b*]-pyrazine-5,7-dione Dibro-mide (7), 2-{3-[1-(6-{1,1-Dimethyl-1-[3-(1,3-dioxo-1,3-di-hydroisoindol-2-yl)propyl]-ammonium}hexyl)-1,1-dimethylammonio]propyl}-5-trifluoromethylisoindole-1,3-dione Dibromide (9). Equimolar amounts (2 mmol) of **21**−**33** and **34**−**38** were dissolved in acetonitrile (50 mL), and a catalytic amount of KI and K_2CO_3 (1:1) was added. The reaction solution was refluxed for 1 to 15 days. After the reaction was completed (TLC-monitoring: silica gel, mobile phase = CH_3OH:0.2 M NH_4NO_3 solution (aqueous) = 3:2), the solution was allowed to cool to room temperature. If there was no precipitate the solution was cooled to 4 °C for several days. The obtained precipitates were collected by filtration and washed several times with little amounts of acetonitrile and with pentane and dried in vacuo.

For analytical data see Table 5, for spectroscopic data see Tables 6 and 7 (Supporting Information).

Pharmacology. Radioligand binding experiments were carried out with domestic pig heart ventricle homogenates in a buffer composed of 2.7 mM $MgHPO_4$, 45 mM Tris-HCl, pH 7.3 at 37 °C. As indicated in the text, selected compounds were additionally applied in a $Na^+/K^+/P_i$ buffer which allows a high allosteric potency (4 mM Na_2HPO_4, 1mM KH_2PO_4; 23 °C; pH 7.4). The procedure to prepare the homogenates has been described previously.[10,43] Protein content was determined according to Lowry et al.[44] (1951) and amounted to 4.89 ± 0.49 mg/mL (mean value ± SEM; $n = 8$). The radioligand used was [^{3}H]*N*-methylscopolamine ([^{3}H]NMS, PerkinElmer Life Sciences, Boston, MA) with a specific activity of 70−83.5 Ci/mmol and at a concentration of 0.20 nM. Nonspecific [^{3}H]NMS-binding was determined in the presence of 1 μM atropine and was smaller than 10% of the total binding. [^{3}H]NMS binding affinity amounted to $pK_D = 9.50 \pm 0.04$ (mean value ± SEM; $n = 8$), and the concentration of receptors in the cardiac homogenates was $B_{max} = 76.47 \pm 10.6$ fmol/mg membrane protein (means ± SEM; $n = 8$). Membranes were separated by a rapid vacuum filtration procedure (glass filters No. 6; Schleicher and Schüll, Dassel, Germany). Filters were washed twice with 5 mL of distilled water. After adding 5 mL of scintillation fluid (Ready protein, Beckman, Palo Alto, CA) the radioactivity was measured by liquid scintillation counting in a Beckman LS 6000 counter.

Two types of experiments were applied to evaluate the effect of the test compounds on the dissociation of [^{3}H]NMS. In the case of "complete dissociation experiments" membranes were preincubated with 0.2 nM [^{3}H]NMS over a period of 30 min in a volume of about 23 mL before 1 μM atropine was added alone or together with a test compound to initiate the reaction. Aliquots of 1 mL were drawn from the incubation medium at appropriate intervals over a period of 120 min and processed as described above. Data were analyzed by means of nonlinear regression analysis; [^{3}H]NMS dissociation was monophasic in the absence and in the presence of test compound and was characterized by the apparent rate constant of dissociation k_{-1}. In "two-point kinetic experiments" (cf. ref 36) membranes were incubated with the radioligand for 30 min to obtain the binding equilibrium. Specific [^{3}H]NMS binding was measured before ($t = 0$ min) and after ($t = 10$ min) addition of 1 μM atropine alone or in combination with various concentrations of 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}. The half time of dissociation amounted to $t_{1/2} = 3.9 \pm 0.9$ min (mean value ± SEM, 118 experiments). To obtain concentration-effect-curves for the allosteric delay of [^{3}H]NMS dissociation, the k_{-1}-values were plotted over the log drug concentration. Curve fitting was based on a four parameter logistic function (GraphPad Prism Version 3.00; GraphPad, San Diego, CA). Applying a partial F-test ($p < 0.05$ taken to indicate a significant difference), we checked whether it was possible to set the parameters "top" to a value $k_{-1} = 100\%$, "bottom" to a value $k_{-1} = 0\%$ and "slope factor" to $n_H = -1$; this was always the case.

Allosteric effects of the test compound on [^{3}H]NMS equilibrium binding was measured with 0.2 nM [^{3}H]NMS and increasing concentrations of a test compound. The incubation time required to obtain binding equilibrium was calculated based on the eq 31 in the study of Lazareno and Birdsall[3] and amounted to 180−530 min. Data for test compound effects on specific [^{3}H]NMS equilibrium binding were analyzed by nonlinear regression according to Ehlert[13] using eq 3 in ref 34.

Statistical comparisons between affinity parameters were performed applying a two-tailed unpaired *t*-test with Welch correction when necessary ($p < 0.05$ taken to indicate a significant difference).

QSAR Analysis. The lateral varied *N*-methylimides were built up using the model builder in HyperChem 5.1 (Hypercube Inc. Waterloo, Ont. Canada 1997). Geometry optimization was achieved using the MM+ program implemented in HyperChem. By means of ChemPlus (Hypercube Inc.) the following physicochemical properties were calculated: the octanol/water partition coefficient (log *P*), the steric parameters volume and surface area (grid) as well as polarizibility and refractivity, a chameleon parameter comprising both steric bulk and polarizibility. The allosteric potencies (EC_{50}) were transformed in the QSAR form $pEC_{50,diss}$. The QSARs were constructed by performing a multidimensional linear regression analysis using the BILIN software developed by Kubinyi.[45]

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Christopoulos, A.; Kenakin, T. G-Protein-coupled receptor allosterism and complexing. *Pharmacol. Rev.* **2002**, *54*, 323−374.
(2) Christopoulos, A. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. *Nature Rev. Drug Discuss.* **2002**, *1*, 198−210.

(3) 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.
(4) Tucek, S.; Proška, J. Allosteric modulation of muscarinic acetylcholine receptors. *Trends Pharmacol. Sci.* **1995**, *16*, 205−212.
(5) Ellis, J. Allosteric binding site on muscarinic receptors. *Drug Dev. Res.* **1997**, *40*, 193−204.
(6) Christopoulos, A.; Lanzafame, A.; Mitchelson, F. Allosteric interactions at muscarinic cholinoceptors. *Clin. Exp. Pharmacol. Phys.* **1998**, *25*, 185−194.
(7) Holzgrabe, U.; Mohr, K. Allosteric modulation of antagonist binding to muscarinic receptors. *Drugs Discovery Today* **1998**, *3*, 214−222.
(8) Soudijn, W.; van Wijngaarden, I.; Ijzerman, A. P. Allosteric modulation of G-protein-coupled receptors. *Expert Opin. Ther. Patents* **2001**, *11*, 1889−1904.
(9) Christopoulos, A.; Mitchelson, F. Assessment of the allosteric interactions of the bisquaternary heptane-1,7-bis(dimethyl-3'-phthalimidopropyl) ammonium bromide at M_1 and M_2 muscarine receptors. *Mol. Pharmacol.* **1994**, *46*, 105−114.
(10) 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.
(11) Ellis, J.; Seidenberg, M. Two allosteric modulators interact at a common site on cardiac muscarinic receptors. *Mol. Pharmacol.* **1992**, *42*, 638−641.
(12) 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.
(13) Ehlert, F. J. Estimation of the affinities of allosteric ligands using radioligand and pharmacological null methods. *Mol. Pharmacol.* **1988**, *83*, 187−194.
(14) Proska, J.; Tucek, S. Mechanism of steric and cooperative actions of alcuronium on cardiac muscarinic acetylcholine receptors. *Mol. Pharmacol.* **1994**, *45*, 709−717.
(15) 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 derrivatives and alkane-bisammonium ligands. *Mol. Pharmacol.* **2002**, *61*, 160−168.
(16) Waelbroeck, M.; Robberecht, P.; De-Neef, P.; Christophe, J. Effects of d-tubocurarine on rat muscarinic receptors. *J. Recept. Res.* **1988**, *8*, 787−808.
(17) Waelbroeck, M. Identification of drugs competing with d-tubocurarine for an allosteric site on cardiac muscarinic receptors. *Mol. Pharmacol.* **1994**, *46*, 685−692.
(18) Giraldo, E.; Micheletti, R.; Montagna, E.; Giachetti, A.; Vigano, M. A.; Ladinsky, M.; Melchiorre, C. Binding and functional characterization of the cardioselective muscarinic antagonist methoctramine. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 1016−1020.
(19) Jepsen, K.; Lüllmann, H.; Mohr, K.; Pfeffer, J. Allosteric stabilization of ^{3}H−*N*-methylscopolamine binding in guinea-pig myocardium by an antidote against organophosphorus intoxication. *Pharmacol. Toxicol.* **1988**, *63*, 163−168.
(20) 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.
(21) 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.
(22) Nassif-Makki, T.; Tränkle, C.; 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.
(23) Staudt, M.; Tränkle, C.; Mohr, K.; Holzgrabe, U. Contributions of lateral substituents of heptane-bisammonium derivatives to the allosteric modulation of antagonist binding to muscarinic M_2-receptors. *Life Sci.* **1998**, *62*, 423−429.
(24) Botero Cid, H. M.; Tränkle, C.; 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.
(25) Bender, W.; Staudt, M.; Mohr, K.; Holzgrabe, U. Probing the size of a hydrophobic binding pocket within the allosteric site of muscarinic acetlylcholine M_2-receptors. *Life Sci.* **2000**, *66*, 1675−1682.
(26) Bejeuhr, G.; Holzgrabe, U.; Mohr, K.; Sürig, U.; v. Petersenn, A. Molecular modelling and synthesis of potent stabilizers of antagonist binding to M_2-cholinoceptors. *Pharm. Pharmacol. Lett.* **1992**, *2*, 100−103.
(27) Kostenis, E.; Botero Cid, H. M.; Holzgrabe, U.; Mohr, K. Evidence for a multiple binding mode of bispyridinium-type allosteric modulators of muscarinic receptors. *Eur. J. Pharmacol.* **1996**, *314*, 385−392.
(28) 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.
(29) Daiss, J. O.; Duda-Johner, S.; Burschka, C.; Holzgrabe, U.; Mohr, K.; Tacke, R. N+/Si replacement as a tool for probing the pharmacophore of allosteric modulators of muscarinic M_2 receptors: synthesis, allosteric potency, and positive cooperativity of silicon-based W84 derivatives. *Organometallics* **2002**, *21*, 803−811.
(30) Contractor, R. B.; Peters, A. T. New intermediates and dyes. Part I. 4-Tert.-butylphthalic acid and its anhydrides *J. Chem. Soc.*, Perkin Trans. 2 **1949**, 1314.
(31) Larner, B. W.; Peters, A. T. New intermediates and dyes. Part II. Preparation and properties of 4-*tert.*-butylphthalic anhydride. Orientation of its condensation products with benzoid hydrocarbons. *J. Chem. Soc.*, Perkin Trans. 1 **1952**, 680−686.
(32) Lombardino, J. G. Unusual dimeric salts of 2-(3-pyridyl)-1,3-indandione. *J. Org. Chem.* **1967**, *32*, 1988−1992.
(33) Pawlowski, G.; Hanack, M. A. A convenient synthesis of trifluormethyl substituted phthalocyanines. *Synth. Commun.* **1981**, *11*, 351−363.
(34) Tränkle, C.; Andresen, I.; Lambrecht, G.; Mohr, K. M_2 receptor binding of the selective antagonist AF-DX 384: possible involvement of the common allosteric site. *Mol. Pharmacol.* **1998**, *53*, 304−312.
(35) Christopoulos, A. Assessing the distribution of parameters in models of ligand−receptor interaction: to log or not to log. *Trends Pharmacol. Sci.* **1998**, *19*, 351−357.
(36) Kostenis, E.; Mohr, K. Composite action of allosteric modulators on ligand binding. *Trends Pharmacol. Sci.* **1996**, *17*, 443−444.
(37) Kubinyi, H. QSAR: Hansch analysis and related approaches; VCH: Weinheim, 1993.
(38) 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.
(39) 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.
(40) Stiefl, N.; Baumann, K.; MaP: A novel 3D QSAR technique based on the distribution of molecular surface properties. *J. Med. Chem.* **2002**, accepted.
(41) Burgmer, U.; Schulz, U.; Tränkle, C.; Mohr, K. Interaction of Mg^{2+} with the allosteric site of the muscarinic M_2 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *357*, 363−370.
(42) Lysíková, M.; Havlas, Z.; Turek, S. Interaction between allosteric modulators and 4-DAMP and other antagonists at muscarinic receptors: potential significance of the distance between the N and carboxyl C atoms in the molecules of antagonists. *Neurochem. Res.* **2001**, *26*, 383−394.
(43) Tränkle, C.; Mohr, K. Divergent modes of action among cationic allosteric modulators of muscarinic receptors. *Mol. Pharmacol.* **1997**, *51*, 674−682.
(44) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(45) Kubinyi, H., BASF AG, 67056 Ludwigshafen, Germany, 1997.

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