Conformational Analysis, Molecular Shape Comparison, and Pharmacophore Identification of Different Allosteric Modulators of Muscarinic Receptors[§]

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Structurally dissimilar compounds such as alcuronium and the newly synthesized substances derived from the bisbenzyl ether TMB4 and from hexamethonium stabilize antagonist binding to M_2-cholinoceptors which is indicative of an allosteric action. In order to propose a hypothesis for the common pharmacophore and the corresponding active conformations, seven flexible compounds in a data set were individually aligned onto the most active and, additionally, rigid alcuronium molecule using a torsional angle flexible fit. An S-shape conformation was found to be a plausible general active conformation. In a subsequent molecular shape analysis the overlap and the nonoverlap steric volumes, RMS alignment as well as electrostatic field potentials were employed as possible structure−activity correlation descriptors. The corresponding 3D-QSAR formulation exhibits a correlation between allosteric modulation potency and the nonoverlap steric volume as well as the proton and oxygen anion probe electrostatic field potentials. Because of large structural diversity among the small number of compounds studied, the apparent 3D-QSAR is best thought of as a convenient representation of the common spatial pharmacophore hypothesis.

INTRODUCTION

Muscarinic receptors may be subject to allosteric modulation, especially the M_2-subtype.[1,2] The allosteric effect is indicated by a retarded dissociation of antagonist-receptor-complexes in the presence of allosteric substances; structurally dissimilar compounds such as alcuronium,[3] gallamine,[4] methoctramine,[5] and tubocurarine[6] have been described to exhibit modulator properties. In particular, phthalimidopropyl substituted hexamethonium derivatives (i.e., W84, see Figure 1) have to be pointed out, because the allosteric potency is high,[7] combinations with atropine displayed an overadditive muscarinic action in isolated cardiac preparations, and the combinations were found in animal experiments to have an overadditive antidote effect against organophosphate poisoning.[8] More recently, bispyridinium derivatives, bisbenzyl ethers of the AChE-reactivator TMB-4 (Figure 1) and corresponding imides, were found to exhibit a similar pharmacological profile (DUO-O and DUO-N, see Figure 1).[9,10] Additionally, hybrid molecules of the hexamethonium and the bispyridinium series were synthesized and turned out to be highly active (WDUO, DUOW see Figure 1).[10]

The dissimilarity of the structures among the aforementioned compounds raises the question whether structural elements or physicochemical properties can be defined which govern the allosteric potency. Recently, the comparison of alcuronium, gallamine, tubocurarine, and W84 by means of self-organizing neural networks has been published.[11] Since alcuronium is the most active compound in this series, and is additionally quite rigid, it was chosen as a template. All other compounds were aligned onto alcuronium based upon matching the positively charged nitrogens and the aromatic rings in each derivative. A slightly distorted "sandwich" conformation of W84 has been found to be the active conformation, and the molecular electrostatic potential (MEP) seems to be responsible for the high allosteric potency of W84 in comparison with gallamine and tubocurarine. Thus, the goals of this study are, firstly, to identify the active conformations of the synthesized compounds DUOW, WDUO, DUO-O, and DUO-N (see Figure 1) and, secondly, to calculate the corresponding molecular shapes and the physicochemical properties, such as the MEP or molecular lipophilic potential (MLP), which may explain the differences in the allosteric potency. Inverted WDUO derivatives (IWDUO)[12] are added to the series of compounds described above to have an appropriate set for comparison. The achievement of the goals provides a spatial pharmacophore hypothesis for the composite structure−activity relation (SAR).

METHODS

A. Biological Activity. The concentration of a modulator at which the rate of the radiolabeled antagonist [^{3}H]*N*-methylscopolamin ([^{3}H]NMS) dissociation is reduced by 50% (effective concentration = EC_{50} value) serves as a measure of the allosteric potency. Correspondingly, for this measure the half-life time is twofold increased.[8] The allosteric potencies were expressed in the common QSAR form:

$$\text{activity} = \log (1/EC_{50}) \qquad (1)$$

The activities are listed in Figure 1.

B. Building the Molecules. Crystallographic data are not available for any of the compounds. Additionally, the conformations of a derivative in solution cannot be identified,

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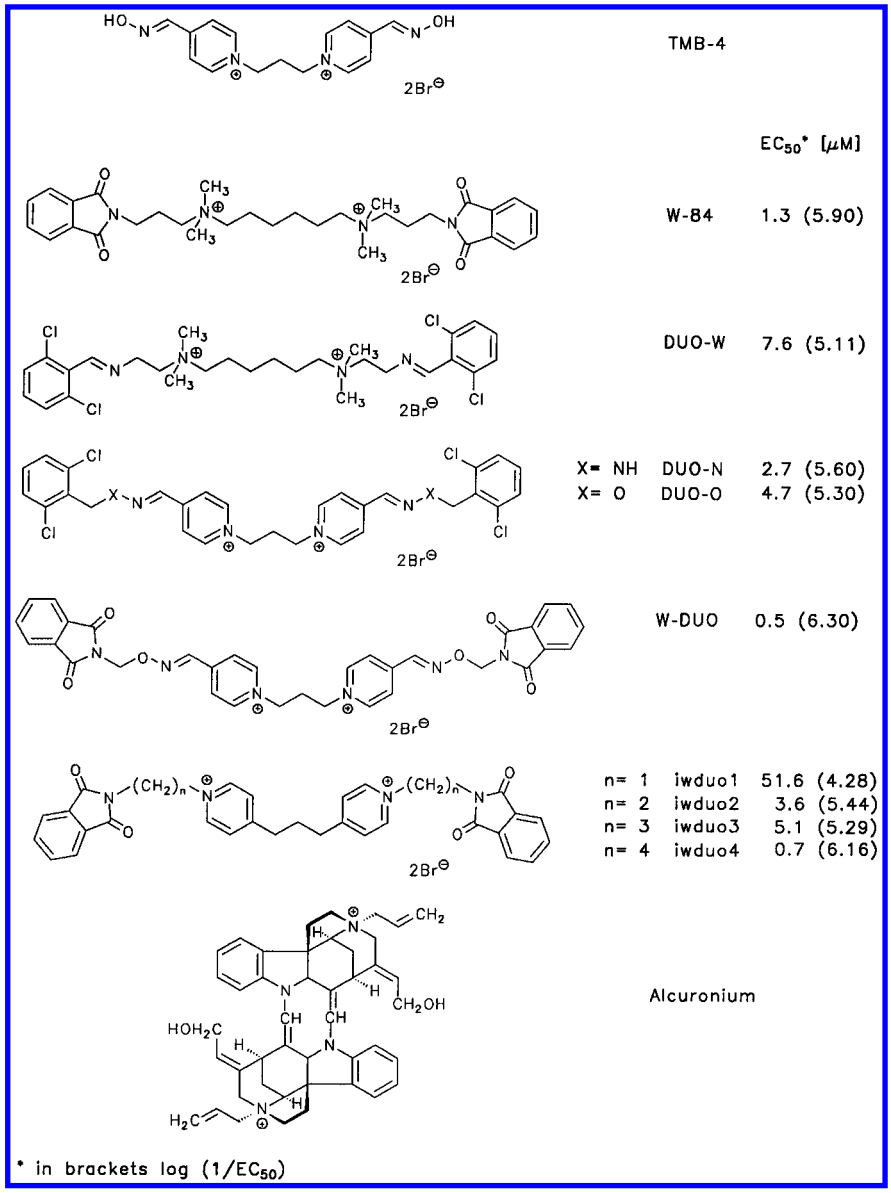

Figure 1. Structures of the allosteric modulators studied and TMB-4.

because the ^{1}H NMR spectra show neither characteristic coupling constants nor NOE effects. Hence, the three-dimensional structures of W84, DUO-N, DUO-O, DUO-W, WDUO, and IWDUO2/3/4 as well as alcuronium were built using the 2D-to-3D conversion program CORINA.[13] The resulting starting geometry of each compound was used in separate structure optimizations employing AM1[14a] and the MMFF option in Chemlab-II, an extended version of Allinger's MM2 program.[14b] The parameters of the oxime ether function ($-CH_2ON{=}CH-$) were not defined in this program. Therefore, after an initial search of the equilibrium conformations, the valence geometry force field parameters of the analogous imine function ($-CH_2CH_2N{=}CH-$) were used for the oxime ether function in the final structure optimizations.

Both methods, semiempirical MO and force field calculations, predict extended conformations for each newly synthesized derivative to be minimum energy structures.

C. Conformational Analysis. The Chemlab-II modeling package option SCAN was used to perform a fixed valence geometry conformational energy scan for fragments of the DUO-molecules DUO-O, DUO-N, and DUO-C (see Figure 2). ϕ_1 to ϕ_4 were scanned at 30° increments, while the other angles were hold fixed. The reference conformation is that for which the torsion angles, ϕ, are set to zero and correspond to an extended trans conformation for each molecule considered. The force field used for the conformational energy scan is composed of dispersion/steric, electrostatic, hydrogen bonding, and torsional potential contributions. The nonbonded potential set proposed by Hopfinger[15] was used to calculate the dispersion/steric interactions, and the electrostatic interactions were computed by means of the Coulomb potential with a molecular dielectric of 3.5 and MNDO-calculated charges. The hydrogen-bonding potential developed by Hopfinger[15] was also used to describe possible hydrogen-bonding interactions.

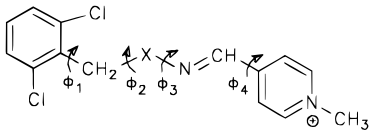

Figure 2. Definition of torsion angles for the compounds DUO-O (X = O), DUO-N (X = NH), and DUO-C (X = CH_2).

To further elucidate low-energy starting conformations for the subsequent molecular fitting procedures, molecular

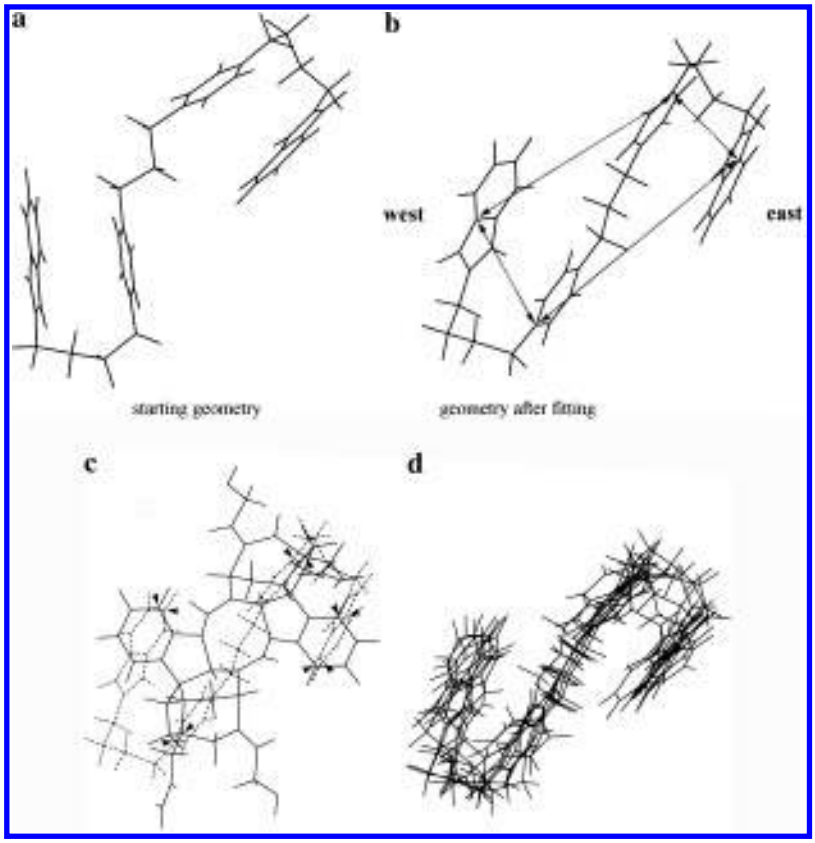

Figure 3. IWDUO4. (a) starting geometry obtained by MD simulations, (b) geometry obtained from the torsional flexible fitting procedure (arrows define the distances described in Table 1), (c) IWDUO4 fitted onto alcuronium using the positively charged nitrogens and aromatic carbon atoms in the indole and phthalimido skeleton, respectively, in each molecule (arrows define the matched atoms), and (d) self-consistent assembly of conformations found by the fitting procedure using different starting geometries.

dynamic, MD, simulations were carried out for each compound using the Molsim program package (version 3.0).[16] Each of the molecules was subjected to room temperature (300 K) MD simulation for 50 ps (50 000 steps; 0.001 ps/step) with trajectory structures being stored every 0.5 ps. Aqueous solvation energetics were included using a solvent hydration shell model[15] throughout the simulations. The interaction cutoff radius was set at 50 Å. The trajectory files were inspected using the Statview program (version 4.6), and low-energy conformations were chosen for further MMFF energy minimization.

D. Molecular Modeling. The Chemlab-II (version 11.0)[17] and Quanta (Version 3.3)[18] molecular modeling programs were used to perform the molecular modeling.

According to the concept developed by analysis using neural network and Kohonen maps[11] alcuronium was used as a template to map the pharmacophore of W84, DUO-O, DUO-N, WDUO, DUO-W, and IWDUO derivatives. Six atoms were chosen in each molecule to perform the pharmacophore fitting procedure: In alcuronium both positively charged nitrogens and two aromatic carbon atoms in each indole skeleton (both neighboring atoms (ortho) to the pyrrolidine ring of the indole and, thus, para to each other) were used as fitting sites (see Figure 3c). For the hexamethonium derivatives, the positively charged nitrogens were also chosen. Since the positive charges in the oxime ether compounds are shifted to the para-carbon atom in the pyridinium ring,[9] those atoms were matched to the nitrogens of alcuronium (see Figure 3). The pyridinium rings of the inverted WDUO derivatives are not as electronically influenced as the oxime ether substituted pyridines in the DUO derivatives. Thus, the nitrogens of the pyridinium rings were selected as centers of the positive charge. Whereas in the dichlorophenyl ring the ipso- and para-carbon atoms are the fitting sites, in the phthalimido moiety an ipso-carbon atom and the corresponding para-atom were used (see Figure 3).

Since none of the synthesized derivatives adopts a conformation in the MD simulations in which the positive charges and the aromatic rings take an arrangement comparable to that in alcuronium, the torsional flexible fit option in QUANTA was applied. Six pairs of corresponding atoms in alcuronium and in each test compound were matched, and 100−400 cycles of the torsional flexible fit procedure were run to achieve as small as possible composite root-mean-square, RMS, fit values. The geometries realized were then optimized using MMFF (CHEMLAB-II). These geometries were again aligned onto alcuronium using the six pairs of corresponding atoms mentioned above, and the RMS values were determined by means of the rigid body fit of QUANTA. These procedures were repeated for several starting geometries of each compound to find the best overall alignment of the pairs of atoms being fit.

E. Quantitative Measures of Molecular Shape. For each compound the low-energy conformation which was found to fit best onto alcuronium was selected for the subsequent molecular shape analysis (MSA).[19] Alcuronium was chosen as the shape reference molecule. Thus, each of the entire analogs were pairwise oriented onto alcuronium using CHEMLAB-II. The common overlap steric volume, V_{OV},[19] between each synthesized derivative in the data set and alcuronium was computed as well as the nonoverlap volume V_{non},[19] which is a measure of the regions of space

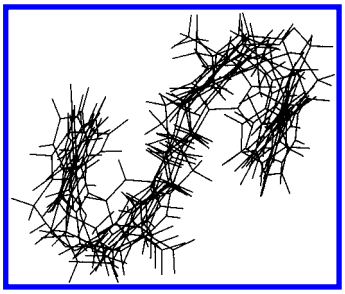

Figure 4. Superimposition of all compounds using the best alignment for each molecule.

not shared by the two molecules. Previous calculations demonstrated that the electrostatic potential can be an important determinant of activity.[11] Thus, three integrated potential field differences, ΔF,[20] corresponding to an oxygen anion, a methyl group, and a proton probe, were computed for each derivative. The integrated potential field difference of superimposed pairs of analogs yields a quantitative measure of how differently a receptor "sees" these two molecules for the region of space explored. Of course, this mimic of a receptor view depends on the choice of the probe. ΔF has been found to be a significant 3D-QSAR descriptor in previous studies and is a variation in the very successful CoMFA approach.[19]

The QSARs were constructed by performing multidimensional linear regression analysis using the BILIN software developed by Kubinyi[21] and the genetic function approximation, GFA,[22] for all combinations of descriptors, V_{OV}, V_{non}, and ΔF descriptors, and the RMS values. Both linear and square representations of the descriptors were used in the construction of trial QSARs.

RESULTS

A. Conformational Analysis. The systematical conformational search of each of the set of DUO-fragments (Figure 2), using the SCAN option, does not locate any apparent local energy minimum. Using a 1 kcal/mol energy cutoff more than 60 allowed conformations were found for each compound. These compounds are highly flexible and do not possess a preferred conformation. Even the torsion angle energy barrier of the aryl-CH=N moiety, normally stabilized by mesomerism, is small enough to be overcome at room temperature. Hence, the loss of conformational stability−loss of biological activity (LCS−LBA) approach[19] cannot be used to map out the active conformations of the derivatives studied. Another modeling approach has to be applied to identify the pharmacophore and its spatial arrangement.

MD simulations were used to generate an ensemble of conformations of each compound. These conformations were subsequently used in a torsional flexible fit procedure in order to see if an unique, common pharmacophore could be discerned. From the MD calculations up to ten low-energy structures were chosen for each molecule, and the corresponding geometries were optimized using the MMFF force field. The alignment criteria for the subsequent torsional flexible fit were derived from previous investigations,[11] where electrostatics were found to be decisive for the recognition between allosteric binding site and the ligands. Thus, the centers of both positive charges and the aromatic rings were attributed to the pharmacophore, and the phthalimido and dichlorobenzyl moiety were aligned onto the indole part of alcuronium in a way that they do not necessarily take the same plane but the same space. The phthalimido skeletons are skipped against the indole rings.

In the case of IWDUO-4 (see Figure 3a), and with some reservations for IWDUO-3, a conformation has been found by means of this MD simulation procedure which approximately fulfills the abovementioned criteria. In all cases the torsional flexible fit procedure, with subsequent geometry optimization (MMFF), has to be applied to find the confor-

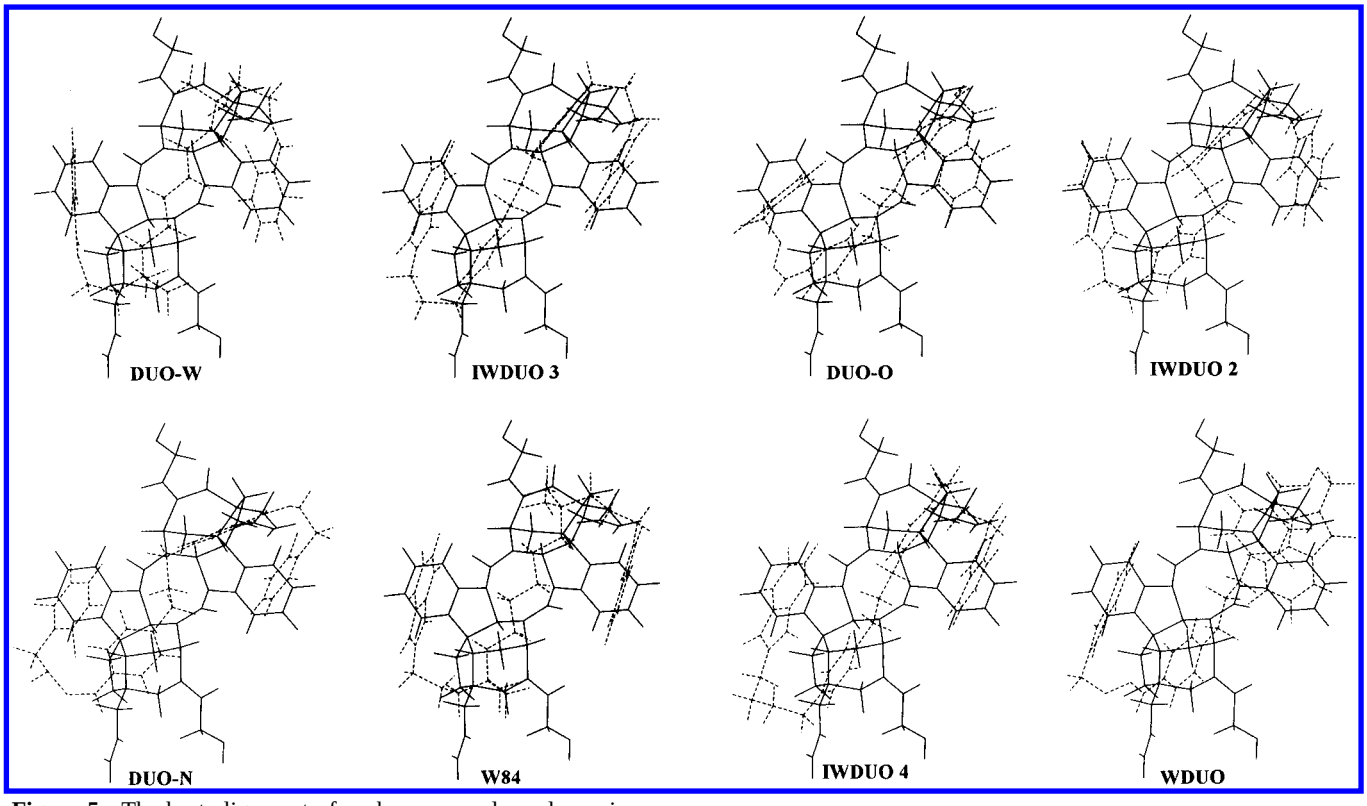

Figure 5. The best alignment of each compound on alcuronium.

Table 1. Conformational Energies and RMS Values of the Starting and Matched Geometries as well as Intra- and Intermolecular (Alignment) Distances

	starting geometry		matched geometry		intramolecular distances (Å)			intermolecular distances (Å)
compound	RMS[a] (Å)	conformational energy (kcal/mol)	RMS (Å)	conformational energy (kcal/mol)	Het−N$^+$(west)	N$^+$−N$^+$	Het−N$^+$(east)	N$^+_{cpd}$−N$^+_{alc}$
DUO-W	6.28	46.22	0.88	45.97	4.63//8.65	9.03	4.69//7.94	0.88//1.06
IWDUO-3	1.73	42.41	0.30	42.27	4.00//9.33	9.85	4.00//9.04	0.13//0.55
DUO-O	6.95	14.82	1.08	20.92	3.49//9.36	9.81	3.54//9.59	0.90//0.99
IWDUO-2	3.73	44.90	0.87	43.47	4.23//8.57	8.71	4.19//9.27	0.58//1.15
DUO-N	3.91	15.34	1.02	17.72	3.62//10.26	9.30	3.73//10.22	0.92//1.12
W84	2.96	69.76	0.82	70.62	4.81//8.64	9.01	4.84//8.48	0.78//1.06
IWDUO-4	1.06	47.65	0.44	51.21	4.51//8.60	10.13	3.84//8.87	0.50//0.57
WDUO	4.49	46.11	0.58	45.97	3.95//9.31	9.52	3.86//8.81	0.59//0.52

[a] Determined using the rigid body fit in QUANTA 3.3.

Table 2. Physicochemical Properties Used as 3D-QSAR Descriptors

compound	RMS (Å)	V_{OV} (Å^3)	V_{non} (Å^3)	ΔF_{CH_3} (kcal/mol)	ΔF_{O^-} (kcal/mol)	ΔF_{H^+} (kcal/mol)
DUOW	0.88	334	182	252	110	−93
IWDUO-3	0.30	310	169	−17	17	−11
DUO-O	1.08	266	198	228	122	−101
IWDUO-2	0.87	302	141	57	70	−58
DUO-N	1.02	260	215	141	124	−103
W84	0.82	327	182	150	83	−70
IWDUO-4	0.44	311	203	−79	65	−61
WDUO	0.58	259	201	88	38	−36

mations which exhibit the optimal alignment onto alcuronium (Figure3b). For each molecule a self-consistent assembly of conformations has been found, as indicated by RMS fitting values of the six pharmacophore atom pairs in a range of 0.57 to 1.00 Å for alignment of five to six adjusted fit conformations of each compound (also used for the fitting procedure onto alcuronium, see, for example, Figure 3d IWDUO4). Additionally, the RMS value in the match of all derivatives is only 1.09 Å (Figure 4). The best alignments of each compound on alcuronium are displayed in Figure 5, and the RMS values and conformational energies of the starting and final geometries as well as distances between essential pharmacophore groups are given in Table 1.

Even the template molecule, alcuronium, exhibits some geometric symmetry although its conformations achieved from the fitting procedure are not perfectly symmetrical as can be seen from the different distances in the "eastern" and "western" parts of the molecules. This loss in overall symmetry may be due to the fact that the phthalimido and dichlorobenzyl moieties only occupy the space of the aromatic indole ring and in most alignments not the plane of the indole ring of alcuronium. Not all molecules are able to adopt the optimal distance between the centers of the positive charge which is found to be equivalent to seven trans-methylene groups[23] (about 10 Å). The conformational energies for the starting and "matched" conformations, obtained from the MMFF calculations, are in same range (see Table 1). Only in the case of DUO-O is the energy difference greater than 6 kcal/mol.

In the molecular shape analysis (MSA) alcuronium was considered as the shape reference compound. The values of molecular shape descriptors, V_{OV} and V_{non}, as well as the field shape descriptors and the RMS values are reported in Table 2. Using all compounds, and all descriptors, in stepwise multiple regression analysis did not lead to a significant correlation, that is the 3D-QSAR equation. This prompted performing a genetic function approximation, GFA, analysis.[22] The GFA is a nonlinear optimization procedure based upon evolving models from parts of other models which show promise. The best correlations obtained from the GFA study are

$$\log(1/EC_{50}) = 1.424\text{RMS} + 0.009V_{non} - 0.094\Delta F_{H^+} - 0.093\Delta F_{O^-} + 3.856 \quad (2)$$

$$N = 8; \quad r^2 = 0.86; \quad \text{LSE} = 0.02$$

$$\log(1/EC_{50}) = -0.000\,008\Delta F_{CH_3} - 0.0678\Delta F_{O^-} - 0.0801\Delta F_{H^+} + 0.000\,02V_{non} + 5.2253 \quad (3)$$

$$N = 8; \quad r^2 = 0.78; \quad \text{LSE} = 0.03$$

where N is the number of compounds, LSE is the least-squares error, and r^2 is the square of the correlation coefficient. These two equations represent two different models with similar r^2 values. Both equations contain ΔF_{H^+}, ΔF_{O^-}, and V_{non} with about the same regression coefficients. Thus, it would seem that a combination of the V_{non}, ΔF_{H^+}, and ΔF_{O^-} descriptors provide a reasonable structure−activity representation of the data set but cannot be validated for the entire set of compounds. Omitting IWDUO3, which fits onto alcuronium unexpectedly well, two significant 3D-QSAR equations are found using the BILIN program:[21]

$$\log(1/EC_{50}) = +0.0108(\pm 0.0088)V_{non} - 0.0123(\pm 0.0065)\Delta F_{O^-} + 4.726(\pm 1.64) \quad (4)$$

$$N = 7; \quad r^2 = 0.89; \quad s = 0.18; \quad F = 16$$

$$\log(1/EC_{50}) = +0.0117(\pm 0.0087)V_{non} + 0.0162(\pm 0.0084)\Delta F_{H^+} + 4.669(\pm 1.60) \quad (5)$$

$$N = 7; \quad r^2 = 0.89; \quad s = 0.18; \quad F = 17$$

where s is the standard deviation of fit and F is the total variance measure. Equations 4 and 5 suggest ΔF_{H^+} and ΔF_{O^-} are providing the same information over the set of seven compounds and, therefore, are interchangeable. The molecular lipophilicity potential (MLP) is not found to be a significant correlation descriptor.

The development of equations 2−5 is presented within the context of 3D-QSAR analysis. However, the small number of compounds ($N = 8$) and the large structural diversity limit the usage of equations 2−5 in any predictive

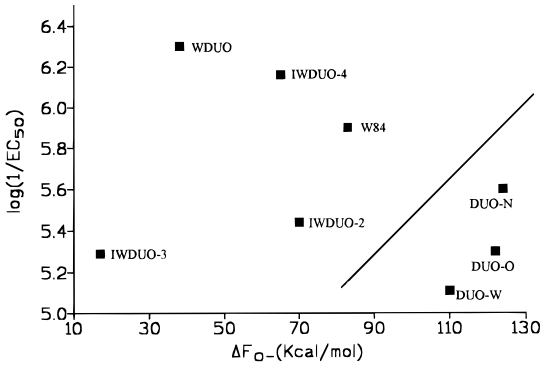

Figure 6. Plot of the electrostatic field parameter ΔF_{O^-} versus [log(1/EC_{50})]. Possible clustering is indicated.

sense. In this study a 3D-QSAR methodology serves as a convenient analysis and representation tool in the development of a general spatial pharmacophore for allosteric modulators of muscarinic receptors which behave in a fashion similar to alcuronium. The "3D-QSAR" approach employed here is not for the development of a predictive model but rather a general structure−activity hypothesis.

DISCUSSION

The goals of this study were (1) to find the active conformation of each of the allosteric modulators and (2) to identify the corresponding key physicochemical properties of the bioactive form for the ligand, i.e., the form in which the modulators bind to the allosteric site of the receptor. Overall, the achievement of these goals provides a spatial pharmacophore hypothesis as opposed to a predictive SAR model.

The molecular shape analysis 3D-QSAR techniques used in this study do not yield highly significant correlation equations for several reasons.

1. The number of compounds available for the training set is small, and, additionally, the compounds exhibit considerable structural diversity. The diversity is demonstrated by the fact that a limited clustering of the phthalimido and dichlorobenzyl substituted derivatives can be discerned in Figure 6, where the biological activity is plotted against the electrostatic field potential descriptor ΔF_{H^+} (the same can be shown for the field descriptor ΔF_{O^-} [data not shown]).

2. The choice of alcuronium as a template is somewhat arbitrary. Even though alcuronium is the most active compound and, in addition, quite rigid, it is not known for sure if this large molecule is a precise negative of the allosteric binding site of the muscarinic receptor protein. In addition, the ligands studied may bind to the receptor in slightly different ways. Both of these concerns argue for some inaccuracy in the alignment modeling, which can only be overcome by additional SAR information.

3. The potential energy field probes H^+ and O^- are used to monitor positive and negative charge response characteristics.[20] Clearly, the fields of these probes depend on the chemical structure and the conformation of each of the derivatives as well as the relative alignment used in the molecular comparison. Thus, the field descriptors are subject to the same limitations as V_{OV} and V_{non}. Additionally, the field descriptors did not converge when a reference compound other than alcuronium was chosen.

Although the spatial pharmacophore model, represented as a 3D-QSAR, developed here is limited, the following conclusions can be made: Firstly, all compounds studied are able to adopt the "distorted sandwich" conformation which was previously found to be the active conformation of W84.[11] Thus, the distorted sandwich appears to be the conformation that the symmetric compounds take up upon binding to the receptor protein. Secondly, the steric volumes (size) of the compounds investigated do not appear to govern the biological activity. This is in accord with the experimental findings for highly substituted compounds, such as CHIN3/6,[24] consisting of terminal heterocycles substituted with protruding phenyl rings and tetra- and hexaphthalimidopropyl substituted hexamethonium derivatives[25] both of which turn out to be highly active. Thus, the hypothesis of a binding site at the extracellular part of the muscarinic receptor protein with rather small spatial restrictions is supported by these findings.[2] Thirdly, electrostatic interactions (as portrayed in the 3D-QSAR descriptors) have been found to be primarily responsible for molecular recognition between the allosteric binding site and the ligands. Both positive charges seem to be as important as the terminal aromatic rings in molecular recognition. This is consistent, on the one hand, with the previous modeling results obtained with Kohonen maps[11] and, on the other hand, with experimental findings: The dependence of allosteric potency on the distance between the centers of the positive charges[9,24] supports the necessity of two charges separated by a distinct distance. Cutting off the benzene rings at the ends of molecules, i.e., hexamethonium[7] or TMB-4,[26] leads to less active derivatives and, in turn, demonstrates the importance of the aromatic groups.

The spatial pharmacophore model developed in this study and represented by a 3D-QSAR is to be used to select new compounds for synthesis which will, hopefully, fill in the "holes" of the current SAR and lead to more potent modulators.

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REFERENCES AND NOTES

(1) Lee, N. H.; El-Fakahany, E. E. Allosteric Antagonists of the Muscarinic Acetylcholine Receptor. *Biochem. Pharmacol.* **1991**, *42*, 199−205.

(2) Tucek, S.; Proska, J. Allosteric Modulation of Muscarinic Acetylcholine Receptors *TiPS* **1995**, *16*, 205−212.

(3) Proska, J.; Tucek, S. Mechanism of Steric and Cooperative Actions of Alcuronium on Cardiac Muscarinic Acetylcholine Receptors. *Mol. Pharmacol.* **1994,** *45*, 709−717.

(4) Ellis, J.; Seidenberg, M.; Brann, M. R. Use of Chimeric Muscarinic Receptors to Investigate Epitopes involved in Allosteric Interactions. *Mol. Pharmacol.* **1993**, *44*, 583−588.

(5) Lee, N. H.; El-Fakahany, E. E. Allosteric Interactions at the m1, m2 and m3 Muscarinic Receptor Subtypes. *J. Pharmacol. Exp. Therapeut.* **1991**, *256*, 468.

(6) Waelbroeck, M.; Robberecht, P.; De-Neef, P.; Christophe, J. Effects of d-Tubocurarine on Rat Muscarinic Receptors. *J. Recept. Res.* **1988**, *8*, 787−808.

(7) Kostenis, E.; Holzgrabe, U.; Mohr, K. Allosteric Effect on Muscarinic M_2-Receptors of Derivatives of the Alkane-bis-ammonium Compound W84. Comparison with Bispyridinium-type Allosteric Modulators. *Eur. J. Med. Chem*. **1994,** *29***,** 947.

(8) Jepsen, K.; Lüllmann, H.; Mohr, K.; Pfeffer, J. Allosteric Stabilization of ^{3}H-*N*-Methylscopolamine Binding in Guinea-Pig Myocardium by an Antidote against Organophosphate Intoxication. *Pharmacol. Toxicol.* **1988**, *63*, 163−168.

(9) Gasteiger, J.; Holzgrabe, U.; Kostenis, E.; Mohr, K.; Sürig, U.; Wagener, M. Variation of the Oxime Function of Bispyridinium-type Allosteric Modulators of M_2-Cholinoceptors. *Pharmazie* **1995**, *50*, 99−105.

(10) Bejeuhr, G.; Holzgrabe, U.; Mohr, K.; Sürig, U.; v. Petersenn, A. Molecular Modelling and Synthesis of Potent Stabilizers of Antagonist Binding to M-Cholinoceptors. *Pharm. Pharmcol. Lett.* **1992**, *2*, 100−103.

(11) Holzgrabe, U.; Wagener, M.; Gasteiger, J. Comparison of Structurally Different Allosteric Modulators of Muscarinic Receptors by Self-Organizing Neural Networks. *J. Mol. Graph.* In press.

(12) Holzgrabe, U. Personal communication.

(13) Sadowski, J.; Gasteiger, J. From Atoms and Bonds to Three-dimensional Atomic Coordinates: Automatic Model Builders. *Chem. Rev.* **1993**, *93*, 2567−2581.

(14) (a) MOPAC version 6.0, QCPE Program 581, Bloomington, IN, U.S.A. (b) Allinger, N. L. Conformational Analysis. 130. MM2. A Hydrocarbon Force Field Utilizing V_1 and V_2 Torsional Terms. *J. Am. Chem. Soc.* **1977**, *99*, 8127.

(15) Hopfinger, A. J. *Conformational Properties of Macromolecules.* Academic Press: New York, 1973.

(16) Doherty, D. C. *Molsim User Guide*, Version 3.0; Molsim, The Chem 21 Group: 1780 Wilson Drive, Lake Forest, IL 60045, 1995.

(17) Pearlstein, R. A. *CHEMLAB-II User Guide*; Chemlab. Inc.: Lake Forest, IL, 1988.

(18) QUANTA: Version 3.3; Molecular Simulations Inc.: 200 Fifth Ave, Waltham, MA 02154.

(19) Burke, B. J.; Hopfinger, A. J. Advances in Molecular Shape Analysis. In *3-D QSAR in Drug Design - Theory Methods and Applications*; Kubinyi, H., Ed.; ESCOM Science: Leiden, Netherlands, 1993; pp 276−306.

(20) Hopfinger, A. J. Theory and Application of Molecular Potential Energy Fields in Molecular Shape Analysis: A Quantitative Structure−Activity Relationship Study of 2,4-Diamino-5-pyrimidines as Dihydrofolate Reductase Inhibitors. *J. Med. Chem*. **1983**, *26*, 990−996.

(21) Hugo Kubinyi, BASF AG, 67056 Ludwigshafen, FRG.

(22) Rogers, D.; Hopfinger, A. J. Application of Genetic Function Approximation to Quantitative Structure-Activity Relationships and Quantitative Structure−Property Relationships. *J. Chem. Inf. Comput. Sci*. **1994**, *34*, 854−866.

(23) Nassif-Makki T.; Kostenis, E.; Bejeuhr, G.; Mohr K. Dependence of the Potency of Bisquaternary Allosteric Modulators of M_2-Cholinoceptors on the Length of the Central Alkyl Chain.. *Eur. J. Pharm. Sci*. **1994**, *2*, 117.

(24) Bejeuhr, G.; Blaschke, G.; Holzgrabe, U.; Mohr, K.; Sürig, U.; Terfloth, G. A. Stable and Highly Potent Hexamethonium Derivative Which Modulates Muscarinic Receptors Allosterically in Guinea-pig Hearts. *J. Pharm. Pharmacol*. **1994**, *46*, 108−112.

(25) Nassif-Makki T. Thesis, Bonn, FRG, 1995.

(26) Botero Cid, M. H.; Holzgrabe, U.; Kostenis, E.; Mohr, K.; Tränkle, C. Search for the Pharmacophore of Bispyridinium-Type Allosteric Modulators of Muscarinic Receptors. *J. Med. Chem*. **1994**, *37*, 1439−1445.

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