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Comparison of structurally different allosteric modulators of muscarinic receptors by self-organizing neural networks

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Similarities in the molecular structure and surface properties of the allosteric modulators of muscarinic receptors, alcuronium, gallamine, tubocurarine, and the hexamethonium compound W84, a well-known pharmacological tool, are explored. The analysis of the molecular electrostatic potential (MEP) as well as of the shape of the molecular surface is performed by self-organizing neural networks. A distorted sandwich conformation of W84 is suggested to be the active form. The importance of the MEP for binding of these compounds could be established. © *1996 by Elsevier Science Inc.*

INTRODUCTION

Various substances have been found to retard the dissociation of antagonists, such as *N*-methylscopolamine (NMS), or atropine from the muscarinic receptors.[1] By means of radioligand-binding studies this mode of action has turned out to be an allosteric modulation of the receptors.[2] Most of the modulators belong to or are derived from the pharmacological group of neuromuscular blockers: On the one hand, there are well-known drugs, e.g., alcuronium, **1,** gallamine, **2,** and tubocurarine, **3,** whose allosteric potency was reported by Proska and Tucek,[3] Stockton et al.,[4] Ellis et al.,[5,6] and Waelbroeck et al.,[7] respectively. On the other hand, our group reported on newly synthesized hexamethonium derivatives such as W84, **4.**[8–10] A ranking of the allosteric modulators has been published[11,*]: Alcuronium was found to be the most potent drug (EC_{50} = 7 nmol/liter), followed by the well-known pharmacological tool W84, **4,** having an activity in the same concentration range. Gallamine is about 30 times and tubocurarine 130 times less active than alcuronium.

Poisoning by organphosphorus compounds such as insecticides (e.g., E605) and nerve agents (e.g., sarin) causes a huge excess of acetylcholine in the synaptic gaps of parasympathetic and sympathetic nerves by an irreversible blocking of acetylcholinesterase. To reduce the acetylcholine concentration and thus the overshooting parasympathetic nerve, reactivators of the esterase as well as high doses of the competitive antagonist atropine have been applied. Since the allosteric modulators are able to inhibit the dissociation of an antagonist from the receptor quantitatively, the therapy of the poisoning can take advantage of the allosteric modulation. In addition, the modulators might be applied in the receptor imaging using positron emission tomography (PET).

Inspection of the chemical structures (Figure 1) raises a question as to whether there are similar structural, electrostatic, or lipophilic features in all these compounds. The purpose of this study is to compare different conformations of the highly flexible W84, **4,** one of the most potent hexamethonium-type modulators,[11] with the rigid alcuronium and the other neuromuscular blockers in terms of their molecular electrostatic potential (MEP) and molecular lipophilic potential (MLP). W84 (**4**) is taken as a representative of a wider range of the hexamethonium- and bispyridinium-type compounds as a first step to a more detailed analysis of their structure–property relationships. The analysis is per-

Color Plates for this article are on pages 217 through 221.

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*The concentrations at which *N*-[^{3}H]methylscopolamine([^{3}H]NMS) dissociation was retarded by a factor of 2 are indicated as a measure of potency (EC_{50}). The EC_{50} values were determined using porcine cardiac membranes (4 mM Na_2HPO_4, 1 mM KH_2PO_4, pH 7.4, 23°C). The EC_{50} values described in Ref. 9 were measured in a buffer of 3 mM $MgHPO_4$, 50 mM Tris-HCl, pH 7.3, 37°C.

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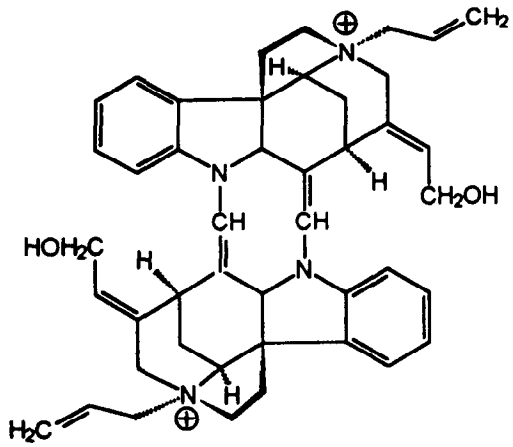
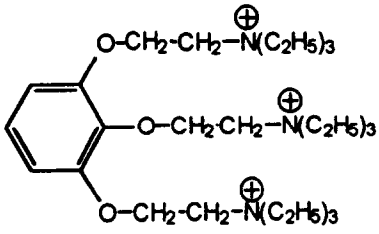
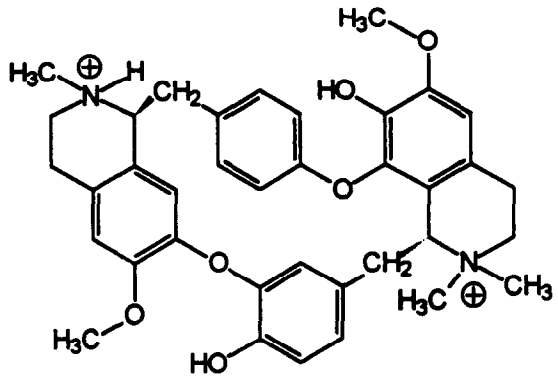


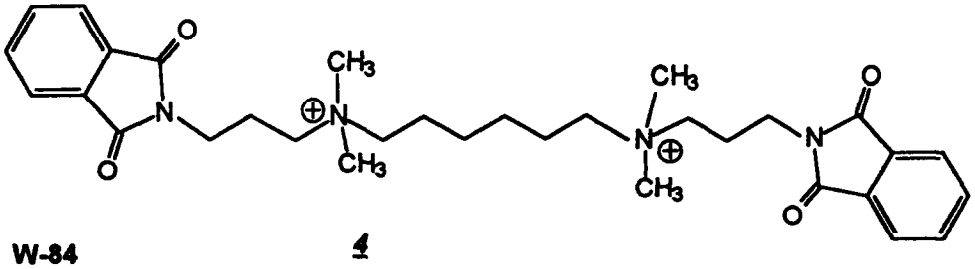
alcuronium **1**
EC_{50} = 7 nM



gallamine **2**
EC_{50} = 190 nM



D-tubocurarine **3**
EC_{50} = 920 nM



W-84 **4**
EC_{50} = 44 nM

Figure 1. Structural formulas of allosteric modulators of muscarinic receptors together with the concentration that reduces the rate of [^{3}H]NMS dissociation by 50% (EC_{50} value).

formed by using self-organizing neural networks as introduced by Kohonen.[12,13] This method has been successfully used for another class of allosteric modulators derived from the bispyridinium compound obidoxim.[14] These investigations should throw light onto the question concerning whether these compounds bind to the same active site of the muscarinic receptors in the same manner. In addition, the ranking of the different modulators might be explainable.

CONFORMATIONAL SEARCH AND SUPERPOSITION OF ALL MOLECULES

The three-dimensional structures of all compounds were generated using three-dimensional (3D) model builder CORINA.[15,16] These structures were additionally optimized by semiempirical quantum-mechanical methods (AM1) (Color Plate 1). In the case of the rigid structures no significant difference between the geometries obtained from both methods could be observed. This is in line with investigations that reported a comparison of X-ray- and CORINA-derived structures emphasizing the high quality of the models generated by CORINA.[17] From these calculations an extended structure, **4e,** was found for W84. Further force field calculations (MMX, PCMODEL; Serena Software, Bloomington, IN) and a molecular dynamics study (QUANTA version 4.0; Molecular Simulations Corp., Waltham, MA) revealed, in addition, a sandwich-like conformation, **4s.**

Stabilization of this geometry can come from hydrophobic interactions between the hexane chain in the center of the molecule and the aromatic rings of the two phthalimide skeletons. In addition, electrostatic interactions may account for this conformation: The arrangement of a partial negative charge (in the phthalimide ring) above and below the line connecting two positive charges (the quarternary nitrogen atoms) gives the strongest electrostatic interactions. Using this structure as a starting geometry for a geometry optimization by the semiempirical AM1 method produced a slightly distorted sandwich structure, **4d.** This conformation is characterized by ion-dipole interactions between the positively charged nitrogens of the quarternary centers and the oxygens of the C=O groups of the phthalimide (distance $N^+ \cdots O=C$ = 2.7Å). Both sandwich-like structures, **4s** and **4d,** are energetically slightly favored in comparison to the extended conformation (5 to 10 kcal/mol as calculated by AM1 and MMX methods). Clearly, because of its high flexibility, compound **4** has many low-energy conformations. The three conformations **4s, 4d,** and **4e** chosen here are to be considered as characteristic samples from this manifold. It is quite likely that these conformations calculated for the gas phase beside others are of comparable importance in the physiological medium.

To work out the essential structural elements responsible for the pharmacological activity, alcuronium was chosen as the template, because it is the most active compound and, additionally, the molecule is quite rigid. For an appropriate superposition of the different molecules the positively charged nitrogens were used first, because they are assumed to be the most important feature for the receptor recognition. As can be seen in Table 1 the distances between these atoms are rather similar (RMS in the range of 0.3 to 0.9 Å).

Table 1. Distance between the positively charged nitrogens in each molecule

Compound	N^+–N^+ distance (Å)
Alcuronium	9.71
Gallamine	11.08
Turbocurarine	11.51
W84 linear	8.89
W84 sandwich	9.09
W84 distorted sandwich	8.87

We first addressed the most difficult problem of superimposing the highly flexible **4** onto **1.** If successful, it would attest to the feasibility of the approach and warrant further investigation.

The superposition of alcuronium and the linear conformation of W84, **4e,** with the fixpoint of the two quarternary nitrogen atoms exhibited protruding phthalimido substituents at both ends of alcuronium, **1** (Color Plate 2a).

It seems reasonable that the aromatic part of both molecules will take up a similar space on binding to the allosteric site of the receptor. Thus, the sandwich, **4s,** and distorted sandwich, **4d,** conformation of W84 (**4**) were also superimposed onto alcuronium (Color Plate 2b and c). The superposition of alcuronium and the distorted sandwich form **4d** revealed the best fit (Color Plate 2c): The central alkane part of W84 (**4**) occupies a space in alcuronium that is in both cases characterized by a hydrophobic part. The aromatic heterocycles of W84 (**4**) cover the space of the indole rings of alcuronium, but the phthalimido skeletons are skipped against the indole rings. The superposition of the sandwich form of W84, **4s** (Color Plate 2b), seems also quite acceptable, although the phthalimide rings are slightly more protruding from alcuronium than in the superposition with **4d.** This investigation already showed that alcuronium can be taken as a template for the superposition of the highly flexible W84 (**4**). An acceptable fit was found with an appropriate conformation of W84 (**4**), the distorted sandwich structure **4d.** This success provided stimulation to test also the superposition of other allosteric modulators in order to develop a pharmacophore model.

The conformation of D-tubocurarine, **3,** obtained from CORINA was taken as such and superimposed onto alcuronium, **1,** again taking both quarternary nitrogen atoms as anchor points. Color Plate 2d shows that both aromatic rings in D-tubocurarine nicely coincide with those of alcuronium; altogether the two molecules have many features in common and their 3D structures are quite similar.

Gallamine, **2,** presents special problems as it has *three* positively charged quarternary nitrogen atoms and has three highly *flexible* groups. Thus, it falls into a charge category quite different from the other three molecules **1, 3,** and **4,** which all bear only two positive charges. Clearly, this difference is largely artificial, a result of considering only the positive ions without accounting for the gegenion. Gallamine will not bind as a species bearing three complete positive charges to the receptor. It will quite likely have one or more gegenions quite close to one or more of the positive centers. Thus, it might have an overall charge pattern quite

similar to that of the other three molecules, which formally carry only two positive charges.

Next we must address the problem of conformational flexibility. There are two possibilities for fitting two of the three quarternary nitrogen atoms of gallamine to the two quarternary nitrogen atoms of alcuronium. The two nitrogen atoms can either be from adjacent, ortho, substituents, or from separated substituents, substituents in meta-position. Both cases were explored. The best correspondence between alcuronium and gallamine was found when the nitrogens from the meta-substituents were superimposed onto the two quarternary nitrogen atoms of alcuronium (in blue and magenta; see Color Plate 2). Even after fixing these two sites, gallamine has still substantial geometric freedom for the benzene ring, and, particularly, for the third substituent and its quarternary nitrogen atom (in green). Some exploration of this conformational flexibility was made in order to achieve a good geometric fit. The result shown in Color Plate 2e is quite acceptable but should not be taken as the final answer because there might still be a better overlap to be found. The phenyl ring of gallamine overlaps with one indole ring, and the other indole ring of alcuronium overlaps with the central (green) quarternary nitrogen atom of gallamine. In the absence of knowledge on the position of the gegenion of gallamine when binding to the muscarinic receptor we must refrain from a discussion of whether this correspondence of a quarternary alkyl substituted nitrogen atom of gallamine to the aromatic indole ring system of alcuronium is indeed plausible on electronic or hydrophobic or other effects. If, however, such corresponding effects do exist, the superposition of this conformation of gallamine, **2,** with alcuronium, **1,** is indeed quite extensive.

From these results a preliminary pharmacophore can be defined that is characterized by two positively charged nitrogens and either two aromatic systems or one aromatic system and a quarternary nitrogen atom altogether in distinct distances from each other (Color Plate 3).

This hypothesis is supported by the observations previously reported: First, the loss of one phthalimide substituent results in a rather strong decrease of biological activity.[9] Thus, two quarternary nitrogen centers and either two aromatic systems or one aromatic system and a quarternary nitrogen atom are necessary to inhibit the dissociation of antagonists from the muscarinic receptor. Second, the pharmacological potency sensitively depends on the distance between the positively charged nitrogen atoms; the optimum distance was found to be obtained with six to seven methylene groups.[10]

From the results discussed above, the different parts of the putative pharmacophore were colored in each molecule accordingly: The aromatic systems (i.e., indole, phthalimide, or benzene) red and cyan, respectively; the positively charged nitrogen and its first sphere of neighbor atoms magenta and blue, respectively, and the parts connecting these features yellow (for details see Color Plate 1).

PROJECTION OF MOLECULAR SURFACE PROPERTIES

The superpositions described above and shown in Color Plates 2 and 3 are a qualitative approach to the comparison of these molecules and to finding the active conformation of W84 **(4).** Neither the van der Waals volume of the molecules nor the electrostatic potential or the lipophilicity were taken into account. Both the electrostatic potential and the lipophilicity have previously been found to be important for the recognition of substrates by receptors. The electrostatic potential in particular governs the interaction of these two molecules. Therefore, the molecular electrostatic potentials (MEPs) on the van der Waals surfaces of the conformations of all molecules described above were calculated. The MEP was derived from partial atomic charges using a classical Coulomb model. The partial atomic charges were obtained by the empirical method of partial equalization of orbital electronegativities (PEOE).[18,19]

The question now concerns whether similarities in the molecular surfaces of molecules **1–4** and, particularly, in surface properties of these molecules can be detected. Molecular surfaces are three-dimensional objects and their comparison asks for inspection of these three-dimensional surfaces from many different observation points to find orientations with maximum common features in shape and surface properties such as the molecular electrostatic potential.

This is quite a difficult task with many possibilities. Before addressing this difficult optimization problem we have therefore attempted to reduce the complexity of the problem. Clearly, two-dimensional surfaces and maps of properties of such 2D planes are much easier to compare than three-dimensional surfaces and properties on such 3D surfaces. We therefore reduced the dimensionally of the problem by a nonlinear projection of the van der Waals surface of the molecules under investigation into a two-dimensional plane. We have previously shown that such a projection can be accomplished by the self-organizing features of the Kohonen neural network.[14,20–24]

In view of the central role that Kohonen networks play in the investigations reported here, a brief description of this method seems to be necessary. Neural networks are models for the information processing in the human brain. One very interesting feature of the brain is that it generates sensory maps of the environment in the visual, auditory, or somatosensory cortex. Thus, a two-dimensional map of the surface of the human body is generated in the somatosensory cortex in such a way that those parts of the body that have many sensory receptors have reserved an accordingly higher proportion of the somatosensory cortex. The Kohonen network attempts to model this feature of the human brain of generating two-dimensional maps of the three-dimensional surface of the human body.

A Kohonen network consists of a two-dimensional arrangement of basic processing elements, the so-called artificial neurons. Each neuron has as many weights as there are input variables for the objects under study. In our application we map points from three-dimensional molecular surfaces into a two-dimensional Kohonen network. Thus, each neuron has three weights, corresponding to the three Cartesian coordinates $\{x_1, x_2, x_3\}$ of the surface points (Figure 2).

Each surface point, s, characterized by the three coordinates x_{s1}, x_{s2}, x_{s3}, will be mapped into a specific neuron of the two-dimensional arrangement. That neuron, c, will be the winning neuron, will obtain a specific point, s, that has

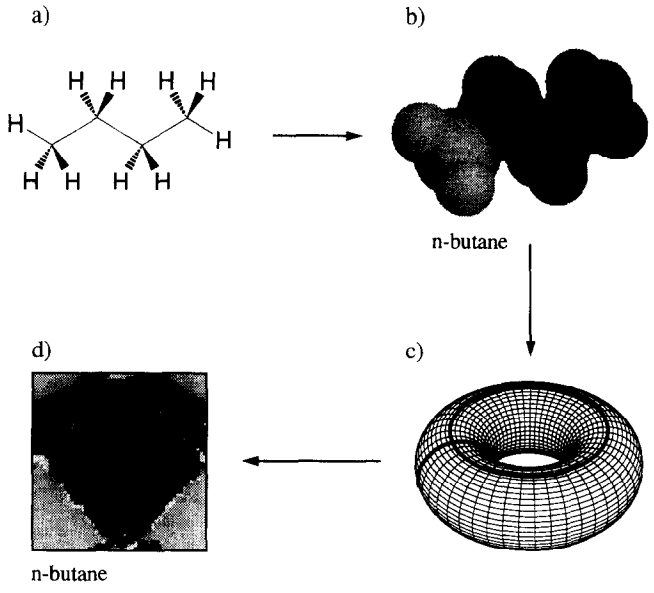


Figure 2. Basic method for the projection of a molecular surface into a Kohonen network of toroidal topology. Cutting the surface of this torus along two perpendicular lines leads to a two-dimensional Kohonen map.

weights, w_{ji}, that are most similar to the input coordinates [Eq. (1)].

$$\text{out}_c \Leftarrow \min \left\{ \sum_{i=1}^{3} (x_{si} - w_{ji})^2 \right\} \quad (1)$$

The weights of all neurons will be adjusted so as to make them more similar to the input coordinates. However, this adjustment is highest for the winning neuron and decreases with increasing distance from the winning neuron. Although the model and the learning method of a Kohonen network is very simple it is, nevertheless, quite good in reproducing the mapping capability of the human brain and has powerful mapping capabilities.[20–22]

As molecular surfaces have no beginning and no ending, we have also decided to choose as plane of projection a plane without beginning and without ending. The surface of a torus is such a plane; in Figure 2 two perpendicular cuts are made through this torus and its surface is then flattened into a genuine two-dimensional plane.[21]

Clearly, the projection of a three-dimensional surface (that of a molecule) into two dimensions must lead to distortions and some loss of information. Thus, the topological distortion resulting from the projection of a molecular surface onto a torus shows up in Color Plates 4–6 as white patterns indicating empty neurons.[24] However, we have shown that such 2D maps of molecular surface properties such as the molecular electrostatic potential can be used to identify similarities in substrates that bind to the same receptor.[21,22] Thus, use of this projection method for the comparison of surface properties of molecules **1–4** was deemed a worthy exploration.

With each of these molecules in some of their conformations, specifically, with the 3D structures **1, 2, 3, 4e, 4s,** and **4d,** individual Kohonen networks were trained. Since the molecular surfaces are of comparable size, the network consisted of an array of 100 × 100 neurons in each case. Points from the van der Waals surface of each molecule were selected at random with a density of 100 points/Å^2 and their three Cartesian coordinates taken to train the Kohonen network.

The results of such a projection can be visualized by identifying each point in the projection plane, the 2D array of neurons, by the property that existed at the location of this point on the molecular surface. It must be stressed that learning in a self-organizing network such as in the Kohonen model is an unsupervised process: Only the three Cartesian coordinates of the points on the molecular surface are used for training, effectively projecting each single point into a specific neuron of the two-dimensional arrangement of neurons. The property existing at the location of this point on the molecular surface is *not* used in the training of the network. Thus, the projection of a molecular surface can be highlighted with any molecular surface property.

First, we have assigned the molecular surface into different parts corresponding to the dissection of the molecules indicated on their skeletons in Color Plate 1. Thus, points from the surface of one phthalimido ring of W84 (**4**) are colored red, whereas points from the other phthalimido rings are colored in light blue (cf. Color Plate 1). These maps are therefore called atom surface assignment (ASA) maps. Color Plate 4 shows these ASA maps of **1, 2,** and **3** and the three conformations of **4.** A first glance already shows that the map of the extended conformation of W84, **4e** (Color Plate 4d), is quite different from all the other maps shown in Color Plate 4. This again proves that this conformation is quite different from all the other molecules and cannot therefore be the conformation active as an allosteric modulator. The other maps show more or less similar general features, attesting that these molecules indeed might have similar characteristics. In fact, from all the maps that of the sandwich and the distorted sandwich form of W84, **4s** (Color Plate 4e) and **4d** (Color Plate 4f), are those most similar to that of alcuronium, **1** (Color Plate 4a). It should be recalled that W84 (**4**) comes closest to **1** in allosteric activity.

As discussed, the topological feature maps of the Kohonen networks of molecules **1–4** were colored in Color Plate 4 by collecting entire parts of the molecular surface into common areas. We have already mentioned that these networks can be identified with any molecular surface property and this was done with the molecular electrostatic potential (MEP) in Color Plate 5. As mentioned previously, gallamine, **2,** presents a special problem as it has three formal positive charges compared to all the other molecules **1, 3,** and **4** with only two formal positive charges. It is reasonable to assume that the overall charge class of all allosteric modulators of muscarinic receptors is the same, that the extra charge in gallamine is annihilated by the presence of a gegenion. To convert gallamine, **2,** into a species having only two formal positive charges we substituted the positively charted third nitrogen that was not superimposed onto one of the two quarternary nitrogen atoms by the isoelectronic carbon atom and performed the calculation of partial atomic charges and MEP with this modified molecule gallamine-C, **2-C.**

Also in this case, the map of the extended form of W84, **4e** (Color Plate 5d), is distinctly different from all the other

maps, again emphasizing that this conformation is quite likely not the active form. The MEP maps of alcuronium, **1** (Color Plate 5a), gallamine, **2-C** (Color Plate 5b), tubocurarine, **3** (Color Plate 5c), the sandwich form of W84, **4s** (Color Plate 5e), and the distorted sandwich form of W84, **4d** (Color Plate 5f), all show similar global characteristics: two areas with strongly positive potential (in magenta), resulting from the areas around the quarternary nitrogen atoms, and two areas with only a slightly positive potential (in light green, yellow, and orange). Note that the entire range of the MEP is shifted into the positive domain by the two formal positive charges in all these structures. The MEP maps of **1, 4s,** and **4d** (Color Plates 5a, e, and f) are the most similar ones,† a fact that is reflected in the high activity of **1** and **4.** The MEP map of gallamine-C, **2-C** (Color Plate 5b), is somehow more different from that of **1** (Color Plate 5a), but this might also be due to the substitution of one quarternary nitrogen atom, N^+, by a carbon atom in the charge calculation. The map of tubocurarine, **3** (Color Plate 5c), shows a strong pronounciation of the strongly positive and slightly positive MEP. Other molecular surface properties can also be visualized by this technique. The lipophilicity was calculated according to methods developed by Ghose and Crippen.[25] Positively charged quarternary nitrogen atoms are not parameterized in this scheme and were substituted by the isoelectronic carbon atom in the calculation of the lipophilicity potential. The molecular lipophilicity potential (MLP) was then taken as an additional property to color the maps obtained by the Kohonen networks. These maps are quite different from each other and are therefore not reproduced here.

In summary, the self-organizing feature maps obtained from the Kohonen neural network indicate the similarity of **1, 2, 3, 4s,** and **4d** in terms of three-dimensional molecular surface fragments (ASA maps, Color Plate 5), and molecular electrostatic potential (MEP maps, Color Plate 5). The extended form of W84, **4e,** quite likely is not the active conformation. Furthermore, the molecular lipophilicity potential is not the major factor for the binding of these molecules to the allosteric binding site of the muscarinic receptor. Interestingly, no correlation was found between the experimental partition coefficient (octanol/water) and the pharmacological activity.[8,9,26]

COMPARISON OF THE SHAPE OF MOLECULES

Having established similarities in the 2D maps of the molecular surface and the MEP of the allosteric modulators **1–4** we addressed the more difficult task of looking for similarities in the three-dimensional molecular surfaces directly. We have developed a method for the qualitative and quantitative comparison of the 3D shape of molecular surfaces.[27] The method is again building on the learning properties of Kohonen neural networks. The method used in the previous section basically stores the Cartesian coordinates of a molecular surface in a Kohonen network (cf. Figure 2). The weights correspond to the coordinates of the points on the molecular surface. Taking this fact one step further, the weights in the Kohonen network of one molecule can be taken as reference for the comparison of the geometry of the surface of a second molecule. If the coordinates of points from the surface of the second molecule correspond to points with quite similar coordinates in the first molecule they will find quite similar weights in the Kohonen network, taken as reference structure or template of the first molecule. If, however, there are regions in the surface of the first molecule that have no matching coordinates in the second molecule (beyond a certain threshold 1 Å), these regions will not be activated by points from the surface of the second molecule and thus stay empty. This leads to regions of empty neurons in the Kohonen map of the second molecule if sent through the Kohonen network of the first molecule (cf. Figure 3).

In studies aimed at elucidating the spatial requirements for the surface of molecules binding to the same receptor and thus coming up with a pharmacophore mapping it makes sense to take the compound with highest biological

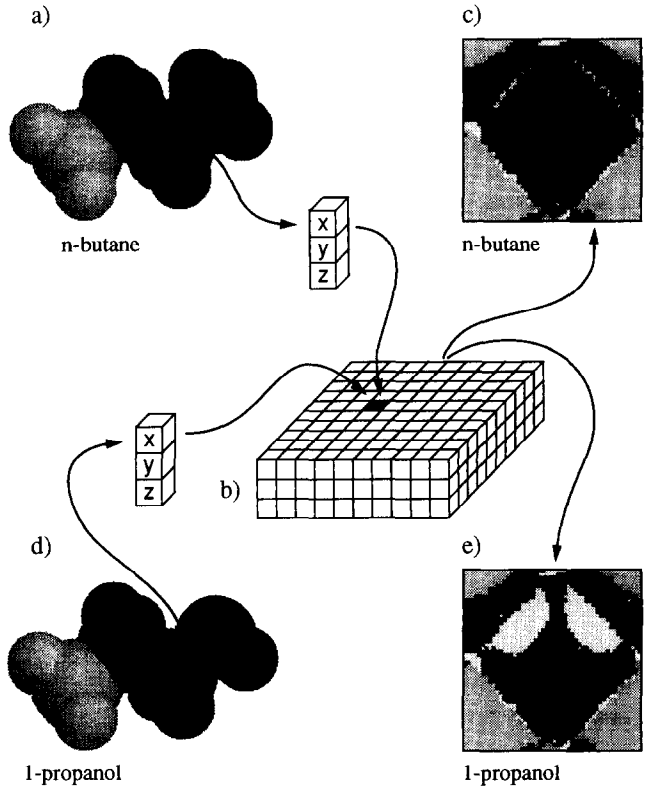


Figure 3. The Kohonen network of the surface of one molecule as reference for a comparison with the surface of a second molecule. The Kohonen network of toroidal topology (cf. Figure 2) is shown here (b) in the form where the torus has been cut along two perpendicular lines and spread in a plane. In addition, for each neuron the three weights are indicated as small boxes. The coordinates of the molecule surface to be investigated [of 1-propanol (d)] are sent through this network (b) trained with the reference surface of n-*butane (a) leading to the map of (e).*

†Note that the perception of similarity in these maps must take account of the pattern of empty neurons and of features of different size, both global and local, having the same color. The human is one of the best pattern recognizers for this task, while automation of this process is rather difficult.

activity as reference structure. For, it can be assumed that the most active compound has the most ideal shape for the respective receptor (clearly, this is only true when neglecting conformational flexibility). In our case, we therefore took alcuronium, **1,** as the reference structure. In this case we have the additional benefit that alcuronium is quite a rigid molecule and therefore the assumption that its shape comes closest to the ideal shape must be the more true.

We therefore trained a Kohonen network with points from the surface of alcuronium, **1.** The other allosteric modulators in the conformations **2, 3, 4e, 4s,** and **4d,** shown in Color Plate 1, were then superimposed onto **1** as shown in Color Plates 2 and 3. Points from the surface of molecules **2, 3, 4e, 4s,** or **4d** were individually sent through this network of alcuronium as reference molecular surface. This leads to Kohonen maps of the other molecules **2, 3, 4e, 4s,** and **4d** with empty neurons indicated by white spaces (Color Plate 6b–f) at those places where the geometry of the surface of alcuronium has no corresponding points on the surface of the second molecule. Atom surface assignment (ASA) coloring was chosen for the visualization of those parts of the surface of the two molecules that agree with each other (see Color Plate 1). Color Plate 6a shows the map of the reference surface of alcuronium, **1,** Color Plate 6b–f give the maps obtained by sending points of the surface of **2, 3, 4e, 4s,** and **4d,** respectively, through the reference network of **1.**

The maps obtained for the surfaces of the structures **2, 3, 4e, 4s,** and **4d** when taking the surface of **1** as a reference shows a large number of empty neurons. This indicates that the shapes of these molecules are fairly different from the shape of the reference structure alcuronium, **1.** However, as the ASA coloring indicates, for the compounds **3, 4s,** and **4d** (Color Plate 6c, e, and f) these differences are largely concentrated in the yellow-colored areas, corresponding to those molecular parts that connect the aromatic rings and the quarternary nitrogen atoms. The areas colored in red, light blue, dark blue, and magenta show only small white areas. This indicates that the shape of the structures **3, 4s,** and **4d** have good correspondence at the two aromatic rings and the two quarternary nitrogen atoms. This points out that these sites appear to be important for biological activity; in order that molecules can act as allosteric modulators their shapes must be similar at those sites.

It has been shown that the number of empty neurons can be taken as a quantitative measure for the similarity of the shape of two molecular surfaces.[27] The number of empty neurons in the maps shown in Color Plate 6a–f and consisting of 10,000 neurons, are given in Table 2.

It has already been said that the projection of a molecular surface onto a torus necessarily leads to topological distortions resulting in empty neurons.[24] The 601 empty neurons in the map of alcuronium are the result of this effect and a similar number of empty neurons must be attributed to this effect in the maps of the other molecules. The additional empty neurons, however, result from the different shapes of the respective molecules. Taking this number as a quantitative measure for the similarity of molecular surfaces leads to the conclusion that the extended conformation of W84, **4e,** is, from all molecules investigated here, the one most different from alcuronium. The distorted sandwich form of W84, **4d,** and gallamine, **2,** on the other hand, have the fewest empty neurons and therefore their shape approaches, of all molecules studied here, closest to that of alcuronium. Interestingly, alcuronium, W84 (**4**), and gallamine have a higher allosteric activity than D-tubocurarine. Apparently, this is due both to the influence of the electrostatic potential (see Color Plate 5) and the shape of the molecules (Color Plate 6 and number of empty neurons).

Table 2. Number of empty neurons in Kohonen maps shown in Color Plate 6a–f

Compound	Color Plate 7	No. of empty neurons
Alcuronium, **1**	a	601
Gallamine, **2**	b	5657
Turbocurarine, **3**	c	6284
W84-e, **4e**	d	6846
W84-s, **4s**	e	6297
W84-d, **4d**	f	5980

There is an even more illustrative method for showing the correspondence of two molecular surfaces. The map of the second molecule obtained by sending it through the Kohonen network of the first molecule can be projected back onto the three-dimensional surface of the first, the reference structure. Color Plate 7a–f show the 3D models of surface of alcuronium **1** with a back-projection of the Kohonen map of **1, 2, 3, 4e, 4s,** and **4d,** respectively. Those places that have empty neurons in Color Plate 6a–f are indicated by a black open mesh on the surface of alcuronium.

This form of representation impressively shows the similarities. Whereas the extended conformation, **4e,** of W84 fills only the center of the alcuronium surface the sandwich and the distorted sandwich form of W84, **4s** and **4d,** cover a much larger area of the surface of alcuronium. In addition, the essential features, both heterocycles and both positively charged nitrogens, color the surface exactly at the same places for the sandwich and distorted form, **4s** and **4d,** and alcuronium, **1.** As these structural elements govern the electrostatic potential the distribution of colors is comparable in both the ASA and the MEP maps. From these results it can be stated, first, that W84 (**4**) will likely occupy the allosteric binding site of the muscarinic receptor in the distorted sandwich conformation, denoted as the active conformation. Second, W84 (**4**) and alcuronium will interact with this binding site in the same manner. The electrostatic potential is likely to be the driving force for the interaction with the allosteric binding site.

As expected, the 3D projection of gallamine and tubocurarine fill similar spaces on the alcuronium surface but the distribution of colors is somehow different, especially in the case of gallamine. This findings might explain the differences in the EC_{50} values between the most active alcuronium and gallamine and tubocurarine.

Interestingly, alcuronium, gallamine, and tubocurarine are found to be neuromuscular blockers whereas W84 (**4**) does not show this activity. Either the pharmacophore responsible for binding to the neuromuscular receptor or the

properties responsible for the binding might be different from the one focused in this study.

CONCLUSIONS

The technique of visualization of MEP, MLP, and ASA using Kohonen maps in two and three dimensions is a valuable method for finding similarities in biologically active molecules. These similarities were used to define the active conformation of W84 (**4**), the distorted sandwich conformation, **4d**, and the pharmacophore of the derivatives. Two positively charged nitrogens, which are held at a certain distance by a hexane chain, and two heterocyclic, aromatic rings, which are closely located to the hexane chain. It is tempting to speculate that the binding site at the muscarinic receptor is characterized by two negative charges, e.g., acid amino acids, and polar areas that are able to interact with the heterocycles.

EXPERIMENTAL

Three-dimensional models of the molecules were built with the automatic 3D-structure generator CORINA.[15–17] The conformations were optimized by semiempirical calculations with AM1 (J.J.P. Stewart, MOPAC 6.0, QCPE Program No. 455, University of Indiana, Bloomington, IN 47405). The sandwich conformation of W84 (**4**) was obtained by force field calculations (MMX, PCMODEL; Serena Software, Bloomington, IN). The Kohonen maps of the MEP, MLP, and ASA were generated with the program KMAP developed in the research group of the Computer-Chemie-Centrum, Universität Erlangen-Nürnberg using SUN or Silicon Graphics workstations. The molecular electrostatic potential used in the Kohonen maps were derived from a classical Coulomb potential using partial atomic charges. These charges were calculated by partial equalization of orbital electronegativity (PEOE).[18,19] The parameters for the calculation of the MLP were taken from Ghose and Crippen.[25]

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