HYDROPATHICITY OF ALLOSTERIC MODULATORS OF THE M_2 RECEPTORS

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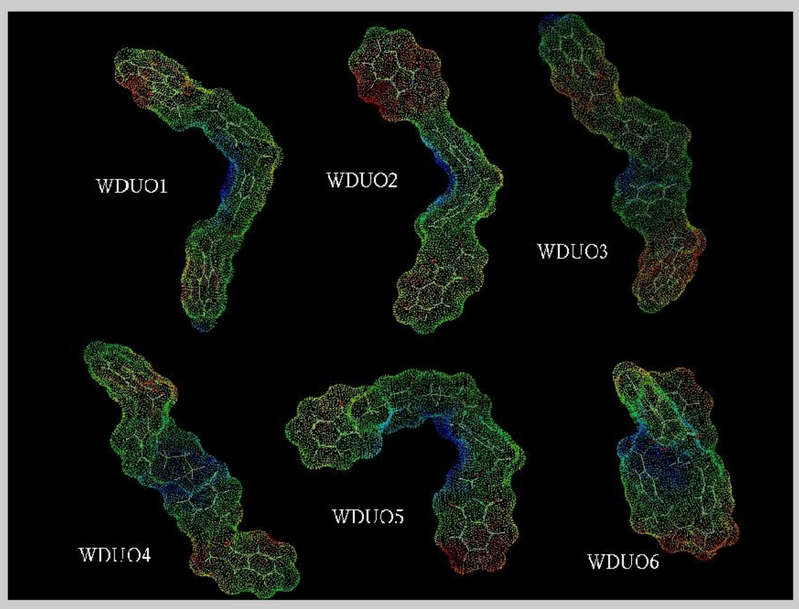
INTRODUCTION

Muscarinic M_2 acetylcholine receptors, bind allosteric modulators at a site apart from the orthosteric site used by conventional ligands, such as the antagonist N-methylscopolamine (NMS). As a consequence of the binding of the alloster to the receptor, the interaction between the antagonist and the orthosteric binding site is altered. Many of the compounds used to study the allosteric modulation belong to the group of neuromuscular blockers, such as alcuronium, tubocurarine or gallamine and the alkane bisammonium compound W84 (1). This finding gave the go-ahead of the synthesis of dozens of new compounds, which were derived from W84. Among others, derivatives of hexamethonium, DUO, WDUO and IWDUO were highly potent (2).

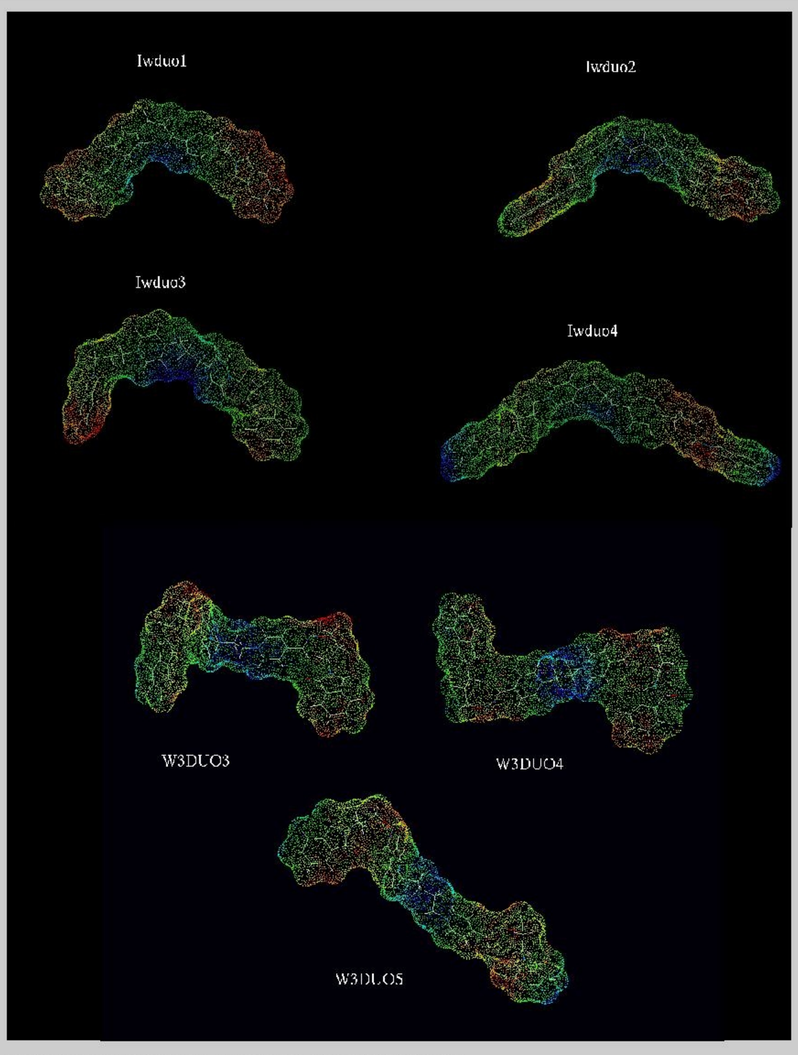
The purpose of this study was to explore the role of the lipophilicity of this compounds, which is unclear till now.

Theoretical calculations according to the classical models of the lipophilicity were not successful due to the fact that the positively charged nitrogens in the molecules and the oxime ether functions, occurring in DUO and WDUO derivatives, are not parameterized (3).
A nearer characterization of the delicate balance that exists between hydrophilic and hydrophobic portions in these derivatives has been attempted by means of an original calculation method, which was developed in our laboratory (4).

This method is able to provide a global molecular hydropathicity index (ILM), but also a detailed tridimensional mapping of this property.



Hydropathicity surfaces of WDUO derivatives.
As explained in approach description, all hydropathicity surfaces (solid and dotted) are blue for hydrophilic areas and red for hydrophobic regions.
The interpyridinic spacer shows a more and more hydrophobic character, while the most hydrophilic portion is located in proximity of the charged moieties. One can see that in the folded derivatives the external convex part of the molecular surface is less hydrophilic than the concave portion. This is due to the U-shape of the surface, where the two symmetric moieties tend to trap some solvent molecules. In WDUO6 the spacer chain is long enough to allow the hydrophobic collapse and the expulsion of every solvent molecule from the space between the two symmetric portions.



Hydropathicity surfaces of IWDUO and W3DUO series.
In the W3DUO and the IWDUO series, the conformations are extended and the phthalimmidic portions results to be more hydropathic due to the simple fact that the solvent molecules are spatially less far from the outer boundaries of the solute.

FINAL TABLE OF ALL EXAMINED PROPERTIES

Compound	Activity EC_{50}	dΦ–Φ	dN-N	ILI Cutoff	RMS	3D Global Index
WDUO 1	N.A.	19.84	2.39	3.35	2.53	5.36
WDUO 2	N.A.	20.85	3.17	3.94	2.17	7.83
WDUO 3	0.5	23.20	4.35	4.04	2.50	7.41
WDUO 4	1.8	20.96	4.85	4.58	2.03	3.76
WDUO 5	N.A.	16.14	6.37	4.76	1.91	3.53
WDUO 6	1.3	6.43	5.33	4.63	1.73	3.60
IWDUO 1	51.6	16.68	8.90	3.72	2.11	9.49
IWDUO 2	3.6	19.32	9.55	3.99	2.38	7.58
IWDUO 3	5.1	21.88	10.05	4.29	2.13	5.47
IWDUO 4	0.7	24.74	10.15	4.33	2.69	6.30
W 84	2.8	16.22	8.83	4.55	2.21	4.39
W3DUO 3	0.53	18.34	4.821	4.14	2.11	6.53
W3DUO 4	0.50	20.05	6,171	4.43	1.93	4.44
W3DUO 5	0.50	22.98	7.310	4.94	2.02	6.79
W3DUO 6	0.70	29.04	8.914	N.A.*	N.A.*	N.A.*

The EC_{50} values are expressed in micromolar concentration.
The *cutofff* or *ILM* calculation is setted to 17 angstrom for all compounds.
The *RMS* and *3D global index* are calculated referred to alcuronium template.

* The size of *W3DUO6* compound (total extended in best conformation) exceeds the water cluster dimension and so was neglected.

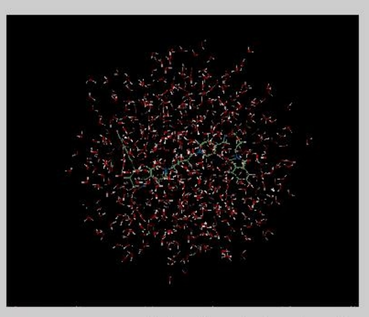
In this table one can observe that, in agreement with the expectations from the lipophilicity property of methylene group, a progressive increase of the $ILM_{....}$ with the increase of the length of the interpyridinic chain is obtained. For the WDUO6 derivative, which has a remarkably more folded conformation, the surface of the apolar moieties exposed to the solvent is reduced and a lower $ILM_{....}$ value is calculated. One can also observe a correlation between the N-N distances and the $ILM_{....}$ values and this is not true for the dF-F distances of the phthalimidic structures. For instance in the WDUO series, one can observe a progressive increase in the N-N interatomic distance, while the distance between the mass centers of the aromatic systems follows a parabolic trend, due to the progressive packing of the two aromatic subsystems.

THE ILM APPROACH

This method is based on the principle that at equilibrium the solvent molecules will be more probably found near the hydrophilic regions of the solute, while they will be repelled by the more hydrophobic moieties (10).
This allows the calculation of a global hydropathicity index (*ILM*) but this property can also be projected onto a molecular surface, giving rise to a very detailed local hydropathicity mapping color codified (blue for hydrophilic zones and red for hydrophobic regions). Because of the size of the molecular systems considered in the present work, a further step comprised the correction of the *ILM* values by exclusion of the contributions of the water molecules which, at the end of the simulation, were found at more than 17 Å from the solute atoms. This is allowed when one takes into account the fact that at such a distance the sum of the contributions of the solvent tends to average out the details in the local hydropathicity profile.

Computational protocol for the hydropathicity analysis of allosteric modulators

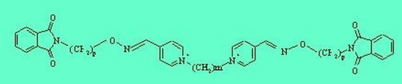
Building and preliminary minimization of the examined compounds
↓
Conformational analysis MonteCarlo and MD
↓
Insertion of best conformation in water cluster (radius = 17Å)
↓
Molecular Dynamic of the solute-solvent system and minimization of the best frame so obtained
↓
Calculation of the global index (ILM) and projection onto molecular surfaces both dotted and solid



$$ILM = \frac{\sum d_{ij}}{n_a n_s}$$

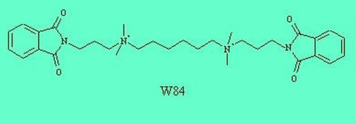
d_{ij} is the distance between the solute atom i and the center of mass of water molecule j.
N_a is the number of solute atoms and N_s is the number of water molecules.

EXAMINED COMPOUNDS

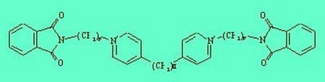


	p	m
WDUO1	1	1
WDUO2	1	2
WDUO3	1	3
WDUO4	1	4
WDUO5	1	5

	p	r
WDUO6	1	6
W3DUO3	3	3
W3DUO4	3	4
W3DUO5	3	5
W3DUO6	3	6



W84



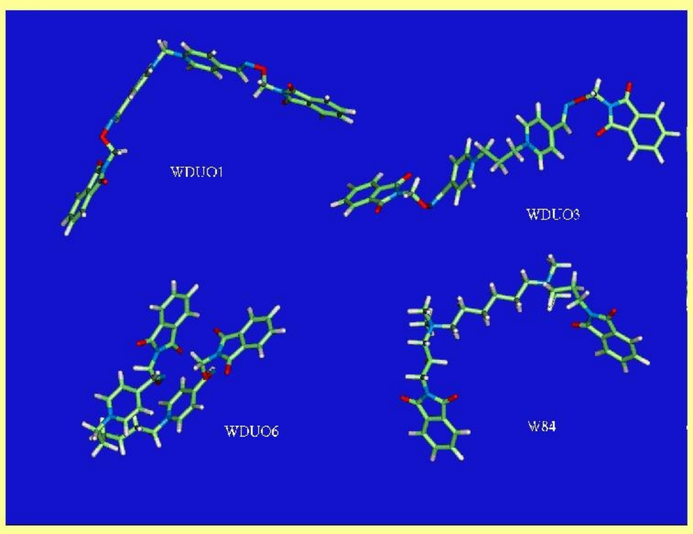
	p	r
IWDUO1	1	3
IWDUO2	2	3
IWDUO3	3	3
IWDUO4	4	3

CONFORMATIONAL PROPERTIES OF MUSCARINIC MODULATORS.

Three main factors affect the conformational properties of muscarinic modulators:
- The mutual electrostatic repulsion among the pyridinic rings (or ammonium groups in W84) positively charged.
- The $\pi-\pi$ interaction between the aromatic terminal moieties (phtalimido or diclorophenil).
- The length and flexibility of the interpyridinic chain.

In conclusion:
- If the interpyridinic bridge allows interactions among aromatic groups, the compound shows a FOLDED conformation
- If the poor flexibility of this chain inhibits p-p interactions, the conformation is EXTENDED type.

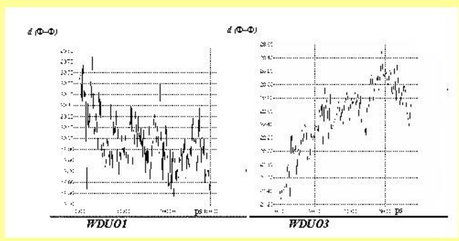
In order to evaluate the conformational behavior, two geometrical parametes were considered: the distance between the mass centers of the terminal aromatic rings (dΦ-Φ) and the distance between the two formally charged nitrogens (dN-N, see Table below).



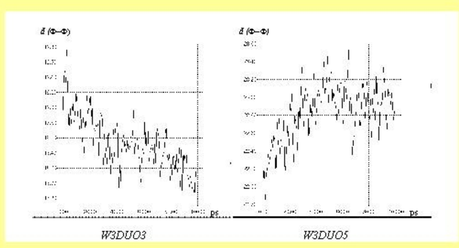
Best conformations of WDUO1, WDUO3, WDUO6 and W84 derivatives.

CONFORMATIONAL ANALYSIS IN SOLUTION

The molecular dynamics simulations performed in water in order to study the hydropathicity profile allowed also to establish that there is no significant modification of the conformational behaviour with respect to the results obtained in vacuo.
Also in this case, the distances between the mass centres of the aromatic systems (dΦ–Φ) and the distances between the two charged nitrogens (dN-N) were measured for each saved frame in the trajectories.
While the distance between the two formally charged nitrogens keeps oscillating within ranges which are too narrow to allow substantial conformational modifications, the variations of dΦ–Φ present some interesting features (9).
The results show that in water, the conformational tendencies already shown in vacuo are stressed. An example is the homologous series WDUO. During the simulation of the solvated WDUO1, the aromatic systems of which lie at approximately right angles at the start, the intramolecular distance dΦ–Φ progressively decreases. On the contrary, in the case of the WDUO3 derivative, the starting conformation of which is extended, the same distance is characterized by a progressive increase during the simulation in water.
Finally, the WDUO6 compound shows a geometry so folded that dΦ–Φ does not change but within a very narrow range.



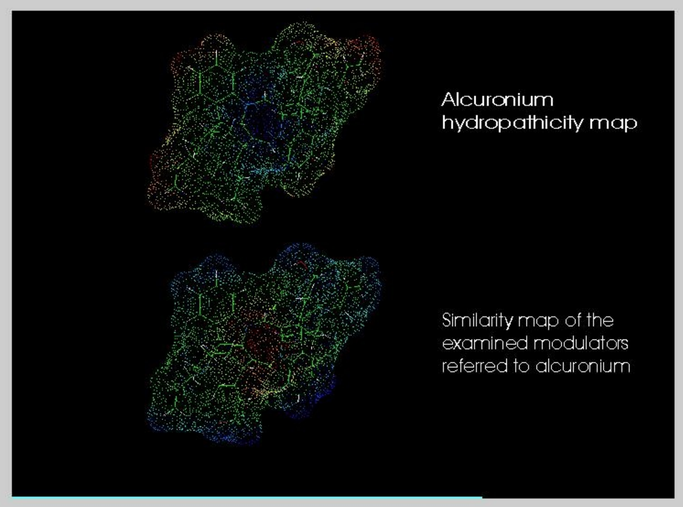
Analogously, the derivatives of the W3DUO series show a very similar behaviour. For the W3DUO3 derivatives, which shows a folded, sigmoid conformation at the beginning of the simulation, dΦ–Φ is progressively reduced during the simulation, while the extended W3DUO5 shows the opposite behaviour, i.e. an increase of dΦ–Φ.



SIMILARITY ANALYSIS OF THE HYDROPATHICITY SURFACES

In order to compare the hydropathicity profile of some of the bisammonic derivatives with alcuronium we used an originally developed method to perform superposition and similarity analysis of the calculated surfaces (5).
One can apply this method using both dotted surfaces, using points coordinates, and solid surfaces, using the coordinates of triangle centers.
This chart highlights the main steps of this new method:

Superposition of the surfaces of all examined compounds with the surface of rigid analogue, minimizing this score function:
$SCORE = \sum_{i=1}^{i=1} \sum_{j=1}^{j=2} Dist_{ij} \cdot (ILM_i - ILM_j)$
→ Overlap Index = **Best SCORE**
↓
3D Grid construction containing all overlapping surfaces and projection of the local similarity (ΔILM) onto grid points
→ 3D **Local** Similarity Indexes: Average on each point
→ 3D **Global** Similarity Indexes: Average on all points
↓
Projection of average Indexes calculated for each grid point onto the surface of a rigid analogue and search for sites with constant (**red**) and different (**blue**) hydropathicity



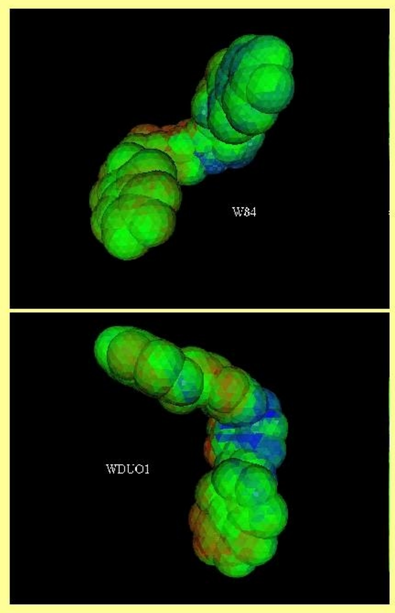
SIMILARITY SURFACE REFERRED TO ALCURONIUM TEMPLATE.
The color coding corresponds to the degree of similarity of the hydropathicity values (red for maximum, blue for minimum similarity). This representation allows to see that the highest overlap of the hydropathicity property can be found in the regions near the charged moieties, whereas it diverges for all external molecular regions.

VRML LANGUAGE AND SOLID SURFACES

In order to overcome the difficulty in visualizing property-colored solid surfaces we here propose a new method to display and manipulate these surfaces based on VRML language (6).
VRML language is used to insert 3D scenes in Web hypertext and so all internet browsers can visualize objects described using VRML. Though the language was designed for the construction of virtual reality, it seemed to be useful for computational chemistry applications. For these reasons you can display these solid surfaces without other graphic software, but using the normal Web browsers (e.g. Netscape, Internet Explorer, Webspace, etc.) both on workstation and on P.C.
VRML is a general graphics language which contains more possibilities than are usually desired in a chemical environment. To view surfaces some elementary rendering techniques (for example the light sources definition) are included which allows a more realistic appearance of the model.
This outline describes the new way to calculate and visualize solid hydropathicity surfaces.

- Calculation of triangles vertexes for a examined structure with a modified version of GEPOL93 (8) for obtaining the solvent accessible surface (SAS).
- Calculation of local ILM values for each triangle centre.
- Editing of the VRML code for the calculated triangles, translating the ILM indexes in RGB ternary values to describe the triangle color.

All three steps were performed by one Fortran program *ILMSOL*, which uses the PDB file of the solvated compound to produce the ILM solid surface.
Once the VRML-ASCII file has been created and loaded into the viewer the solid surface can be rotated, translated and zoomed in and out by means of simple mouse clicks.
One obvious shortcoming is the fact that for every new 3D image a new file has to be created: any modification can not be done online.
This program can be easily modified to build a solid surface coloured with others properties (MEP, Energy Interaction with probes, Steric Effect, Lipophilicity, etc.)
The possibility of translating the VRML code into a ray-tracing code, using for example *vrml2pov* software, which translate VRML into PovRay language, allows to obtain also high-quality images.
In the future the combination of VRML and Java3D (7) languages allows a visualization and interactive processing tool in computational chemistry cost free and platform independent.



Solid surfaces of W84 and WDUO1 compounds

CONCLUSION

The results obtained point to a range of qualitatively differences as far as the conformational behaviour, both in vacuo and in solution, and the hydropathicity profile among the bisammonic compounds considered. This suggests that they may bind in quite different ways to the biological target, although sharing some common substructures, as its highlighted from the analysis of the similarity surface . However, no quantitative correlation with the allosteric activity was possible. This is a hint to the fact that the role of the hydropathicity profile is important, but complementary to that of other molecular descriptors in the description of the complex mechanism of allosteric modulation.
On the other hand the method here proposed for solid surface visualization can replace third party graphics program and ensures platform indipendence and portability of surface visualization as a whole.

REFERENCES

1. J. Proska, S. Tucek. "Mechanisms of steric and cooperative actions of alcuronium on cardiac muscarinic acetylcholine receptors". Mol. Pharmacol. 45, 709 - 717 (1994).
2. U. Holzgrabe, K. Mohr. "Allosteric modulators of ligand binding to muscarinic acetylcholine receptors". Drug Discovery Today 3, 214 - 222 (1998) and references cited herein.
3. A. J. Leo in C. Hansch , P.G Sammes and J. B. Taylor, Eds., Comprehensive Medicinal Chemistry: "Theirational design , mechanistic study & therapeutic applications of chemical compounds", Vol. 4, Pergamon Press, Oxford , England, 1990, Chapter 18.7, pp. 295-319
4. P. Fantucci, A. M. Villa, L. Villa, G. Vistoli. "A new dynamic approach to the calculation and representation of the local hydrophilicity-hydrophobicity", Trends in QSAR and Molecular Modelling, F. Sanz ed. Prous Barcelona (1995).
5. G. Vistoli, "A new three-dimensional parameter of molecular hydropathicity", Ph. D. Thesis (1998).
6. VRML 1.0 language specification. http://www.vrml.org/VRML1.0/vrml10c.html. 1996.
7. Introduction to VRML and Java3D. CourseSlides. http://www.sdsc.edu/~nadeau/Courses/Visualization97/Slides.
8. J. L. Pascual-Ahuir, E. Silla and I. Tuñon. "GEPOL: An improved description of molecular surfaces." J. Comput. Chem. 15, 1127-1138 (1994).
9. G. Vistoli, A. Pedretti, A.M. Villa, L. Villa, U. Holzgrabe, A. Cambareri Molecular Hydropathicity Index: application to a series of allosteric modulators of the M_2 receptors. An alusys (1998) in press.
10. All calculations are performed with Quanta/CHARMm package implemented on a workstation SGI Indigo^2.

As can be noticed here, the most recent documentation about VRML can be found on the Internet.