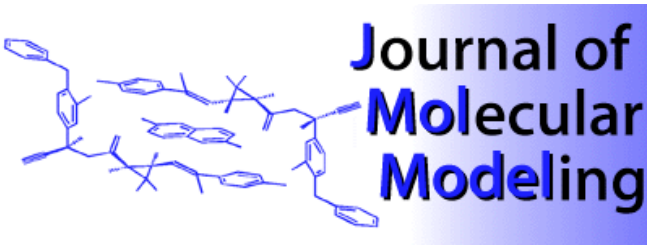
J. Mol. Model. **1998**, *4*, 113 – 131



FULL PAPER

Use of the Supermolecule Approach to Derive Molecular Similarity Descriptors for QSAR Analysis

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Abstract A relevant aspect in quantitative structure-activity (QSAR) and structure-selectivity (QSSR) relationships studies is the choice of the most appropriate molecular descriptors both with respect to the molecular series considered and the known or hypothetical mechanism of drug action. We have recently shown that *ad hoc* derived size and shape descriptors have been successful to derive QSAR and QSSR models for α_1-adrenergic antagonists, 5-HT_{1A} serotoninergic receptor ligands and M_1 muscarinic ligands, especially when dealing with non congeneric series of molecules. These descriptors describe the size-shape similarity with respect to a reference supermolecule which is obtained by superposition of the most active (selective) and structural different compounds, better if rigids. Molecular similarity indices based on molecular electrostatic potential (MEP) have found, as well, widespread use in the QSAR area.

In the present study we extend the use of the supermolecule as a reference structure also in the context of MEP similarity. We have defined an *ad hoc* MEP similarity index with respect to the supermolecule using the same formalism of Hodgkin and Richards. The MEP of the supermolecule is computed as the average MEP of the compounds defining the supermolecule.

A FORTRAN code is implemented to optimize the superposition of the ligands on the reference supermolecule in order to maximize the values of the ad hoc similarity descriptors defined in this study. The performance of the different matching criteria and the different *ad hoc* molecular similarity indices derived with the supermolecule approach are tested in QSAR modeling of the binding affinity and efficacy of a wide ranging series of M_1 muscarinic ligands previously studied.

Keywords QSAR, Molecular Similarity, *Ad hoc* size and shape descriptors, MEP, M_1 muscarinic ligands

Introduction

A relevant aspect in quantitative structure-activity (QSAR) and structure-selectivity (QSSR) relationships studies is the choice of the most appropriate molecular descriptors both with respect to the molecular series considered and the known or hypothetical mechanism of drug action.

The representation of molecular shape and its applications in the context of molecular similarity and modeling of chemical and biochemical process have been object of many studies [1, 2].

We have recently shown [3-5] that *ad hoc* derived size and shape descriptors defined on the ligand bioactive molecular form have been successful to derive QSAR and QSSR

models for α_1-adrenergic antagonists, 5-HT_{1A} serotoninergic receptor ligands and M_1 muscarinic ligands. These descriptors describe the size-shape similarity with respect to a reference supermolecule which is obtained by superposition of the most active (selective) and structural different compounds, better if rigids. They are defined through the following steps: a) each ligand is superimposed on the supermolecule by using dummy atoms defined on the basis of the pharmacophoric elements shared by all the ligands; b) the intersection (V_{in}), the outer (V_{out}) van der Waals volume of the ligand with respect to the volume of the supermolecule (V_{sup}), and the normalized indices $V_{Dnorm} = (V_{in} - V_{out})/V_{sup}$ and V_{in}/V_{mol} (V_{mol} is the van der Waals volume of the ligand) are computed.

The use of a supermolecule instead of a single ligand for molecular similarity comparison presents the main advantage of allowing the modeling of non congeneric series of compounds where the most active ligands share little topological and topographical structural similarity. The rationale behind the supermolecule is that it represents a map of the target active site or the extent to which the target can adapt (induced fit) to maximize the attractive intermolecular interactions with a ligand. In other words, the information content on the ligand-receptor complementarity encoded by the ensamble of the most active ligands, that constitute the reference supermolecule, is significantly greater than those encoded by a single reference molecule.

The *ad hoc* size and shape descriptors defined, assume that the volume of the most active ligands should be included as much as possible in that of the supermolecule but differ in the way they rank the ligands. In fact, when the intersection volume, V_{in}, takes its extreme values 0 and V_{mol}, the V_{in}/V_{mol} descriptor takes, respectively, the values of 0 and 1, and the V_{Dnorm} descriptor takes the values $-V_{mol}/V_{sup}$ and V_{mol}/V_{sup} (V_{Dnorm} will take its upper extreme value 1 only for an hypothetical ligand whose volume coincides with that of the supermolecule). The V_{in}/V_{mol} descriptor considers equally active, irrespective of their size, all the ligands whose V_{in} coincides with V_{mol} while the V_{Dnorm} descriptor ranks them according to their size.

Molecular similarity indices based on the overlap of molecular electron densities first defined by Carbó [6] and recently developped in the more general formalism of molecular quantum similarity measures [7] have been succesfully used in QSAR studies [8, 9]. Hodgkin and Richards introduced an alternative molecular similarity index which is more sensitive to the magnitude of the electron densities [10]. Molecular electrostatic potential (MEP), electric field and shape are commonly used alternatively to electron densities to calculate molecular similarity indices especially in drug design and in the QSAR area [11-15]. In the present study we extend the use of the supermolecule as a reference structure also in the context of MEP similarity [16]. We have defined a MEP similarity index with respect to the supermolecule using the same formalism of Hodgkin and Richards [10]. The MEP of the supermolecule is computed as the average MEP of the compounds generating the supermolecule. This adds to the *ad hoc* size and shape similarity descriptors an *ad hoc* MEP similarity index, which can better take into account the electronic contributions of the ligands.

In our previous studies the matching criteria were only based on the pharmacophoric elements shared by the molecules considered, with manual adjustments for those ligands which have a substructure in common with the reference molecule. This may render the superposition procedure to some extent subjective.

In the present paper, we have implemented a FORTRAN code to optimize the superposition of the ligands on the reference supermolecule, starting from the match achieved on the basis of the pharmacophore, with the following options: a) maximizing the *ad hoc* size and shape descriptor V_{in}/V_{mol}; b) maximizing the *ad hoc* MEP similarity index; c) maximizing the product of the two indices.

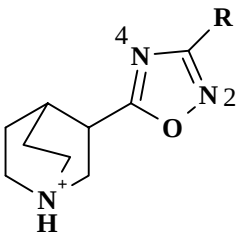
The performance of the different matching criteria and the different *ad hoc* molecular similarity descriptors derived with the supermolecule approach are tested in QSAR modeling of the binding affinity and efficacy of a wide ranging and structural heterogeneous molecular series of M_1 muscarinic ligands (Schemes 1 and 2), including antagonists, weak partial agonists, partial agonists and full agonists, previously studied by us [5].

Methods

Experimental binding affinity data used for QSAR analysis

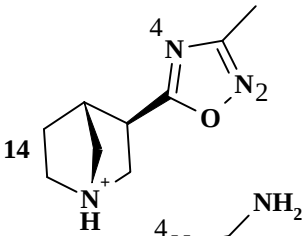
The binding affinity data, pNMS, are taken from Refs.[17, 18] and are expressed as the cologarithm of the binding affinity constants measured by displacement of the muscarinic antagonists [^{3}H]N-methylscopolamine (NMS) which labels both high affinity and low affinity states of the receptor. The binding affinity data, pOXO-M, are taken from Refs.[17, 18] and are expressed as the cologarithm of the binding affinity constants measured by displacement of the muscarinic agonist [^{3}H]oxotremorine-M (OXO-M) which labels the high affinity agonist state of the receptor. Since agonists recognize preferentially the high affinity state, displaying much higher affinity in the OXO-M assay with respect to the NMS assay, the ratio of the affinities of a given compound (NMS/OXO-M ratio) gives a measure of its cortical efficacy[17]. The log of this ratio, log(NMS/OXO-M), has been shown to correlate directly to the ability of the ligand to simulate the hydrolysis of cortical phosphoinositol [17]. Four broad categories of muscarinic ligands can be defined according to their efficacy as estimated from this ratio: antagonists show equal affinity in both assays and thus have ratio close to unity, weak partial agonists have low ratios of 10-200, partial agonists display intermediate ratios of 200-800 whereas, at the other end of this continuum, full agonists display a ratio in excess of 800 [17, 18].

Scheme 1 *Protonated molecular form of the M_1-muscarinic ligands considered in this study. The labels 2 and 4 indicate the heteroatoms used to define the pharmacophore*



R

1 $C(OH)Ph_2$
2 CH_2Ph
3 $N(CH_3)_2$
4 CH_2CH_3
5 $CONH_2$
6 OCH_3
7 $NHCH_3$
8 CN
9 Br
10 $NHCOCH_3$
11 Cl
12 CH_3
13 NH_2



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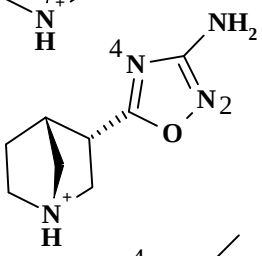

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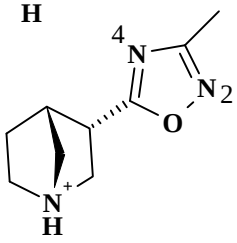

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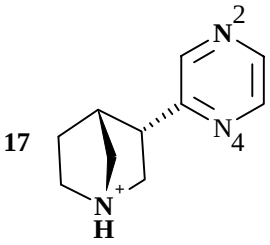

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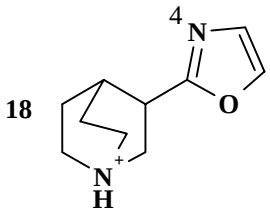

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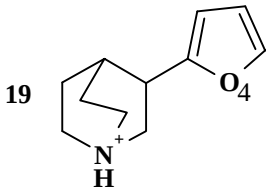

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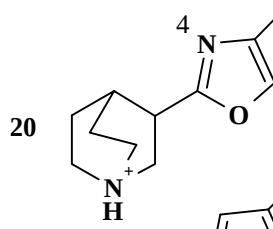

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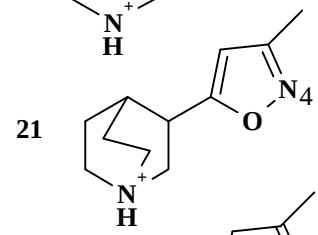

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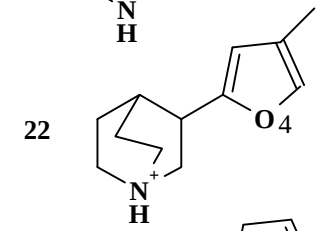

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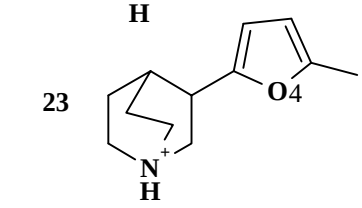

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ANTAGONISTS: 1-3
WEAK PARTIAL AGONISTS: 4-9, 18-23
PARTIAL AGONISTS: 10-12
FULL AGONISTS: 13-17

Calculations of molecular geometries

All calculation were performed by using the AM1 hamiltonian [19] included in the semiempirical MO program package AMPAC [20].

For the congeneric series of quinuclidine analogues, compounds **1**-**13** and **18**-**23** of Scheme 1, complete geometry optimization of the neutral and protonated forms, was performed on the parent compound **1** whose starting geometry was constructed by taking the geometric parameters from standard compilations [21]. The minimized parent compound structures, neutral and protonated forms, were then used as starting geometries for the other derivatives which were submitted to an AM1 optimization where all the geometric parameters of the substituent R (compounds **1**-**13**) and of the five member heteroaromatic ring (compounds **18**-**23**) were allowed to vary (the r.m.s. deviations between the coordinates of a fully optimized structure and the coordinates of a structure where only the geometric parameters of the substituent were optimized, were computed for compounds **2**, **17** and **19** and were in the range of 0.3Å) The starting geometries of compounds **24**-**27** and **32** were taken from crystallographic data [22-26]. The starting geometry of the other ligands were constructed by taking the geometric parameters from standard compilations. The geometries of these ligands in their neutral and protonated or quaternary forms were fully optimized by the AM1 method.

Calculation of ad hoc *size and shape descriptors*

The van der Waals volume of the supermolecule, the volume of each ligand to be compared and their intersection volume (V_{in}) are computed numerically over a three-dimensional grid enclosing both molecules and extending 3Å from the larger coordinates value on each cartesian axis with grid points 0.5Å apart.

The number of grid points included within the volume of each molecule belonging to the supermolecule (i.e. the distance of the grid point from one of the atoms of the molecule is smaller than its van der Waals radius) multiplied by (grid-spacing)^3 gives the van der Waals volume of the supermolecule. The volume of a single ligand is computed analogously.

The intersection volume (V_{in}) is given by the number of grid points falling inside the volumes of both the supermolecule and the ligand to be compared multiplied by (grid-spacing)^3.

Calculation of ad hoc *MEP similarity indices*

Molecular electrostatic potential (MEP) values were calculated at the intersection of a rectilinear grid which has the same extension and spacing as defined for the volume calculations (see above section). The use of a larger grid, enclosing all the molecules of Schemes 1 and 2, did not change significantly the values of the molecular similarity index MEPsim (see below).

A probe atom was placed at each grid point and the MEP values were calculated classically by using atom centered point charges:

$$\sum_{i=1}^{n} \frac{q_p q_i}{d_i}$$

were q_p is the charge on the probe atom, which is set to 1.0, and q_i is the charge on the *i*th of the *n* atoms in the molecule and d_i. is the distance between the *i*th atom and the probe. In order to avoid singularities the evaluation is restricted to points outside the van der Waals volume of the molecule, this choice is subjective but is commonly used in MEP based molecular similarity calculations [10-14].

The Mulliken population charges from AM1 calculations were used. The use of atomic charges derived from the electrostatic potential (ESP charges) was as well tested. The ESP charges were computed by using the ESP option in the MOPAC 6.0 program[27]. Linear correlation (r^2=0.98) was found between the MEPsim index computed by using the ESP charges and the one computed by using the Mulliken charges.

The *ad hoc* MEP similarity index (MEPsim) with respect to the supermolecule is computed according to the Hodgkin and Richards molecular similarity index as:

$$MEP_{sim} = \frac{2\sum MEP_{sim} \cdot MEP_x}{\sum MEP_{\text{sup}}^2 + \sum MEP_x^2}$$

where MEP_{sup} and MEP_x are the MEP values of the supermolecule and of the molecule to be compared, respectively. The MEP_{sup} is calculated at each grid point as the average MEP of the molecules which belong to the supermolecule:

$$MEP_{\text{sup}} = \frac{\sum_{i}^{n\text{sup}} MEP_{xi}}{n_i}$$

where *nsup* is the number of molecules which define the supermolecule, MEP_{xi} are their MEP values at a given grid point and n_i is the number of molecules whose MEP_{xi} values was not set to zero. In other words, if a grid point falls inside the volume of one or more of the molecules, belonging to the supermolecule, MEP_{sup} is averaged only over the remaining molecules with the grid points outside their volumes.

Alternative ways of calculating the MEP_{sup}, tested by us, gave worst results when correlating the MEPsim index with the activity data. These alternatives consisted of:

(a) taking the sum of MEP_{xi} values instead of the average; (b) taking the sum of MEP_{xi} values and considering only grid points which fall outside the volume of all the molecules defining the supermolecule; (c) taking the average of MEP_{xi} values and considering only grid points which fall outside the volume of all the molecules defining the supermolecule.

Besides the MEPsim index we defined the MEPsur descriptor computed over a set of points distributed on the

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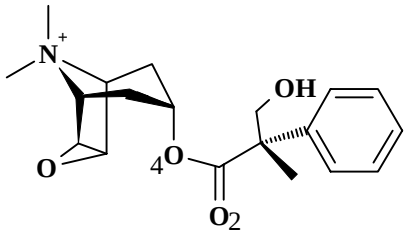

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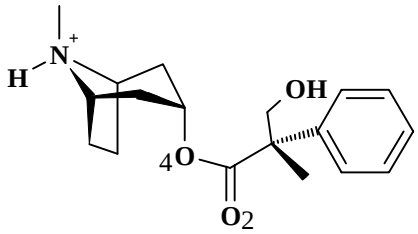

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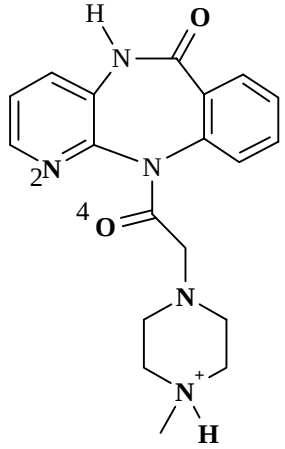

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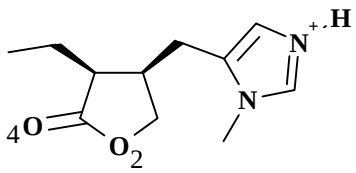

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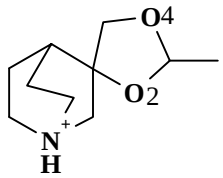

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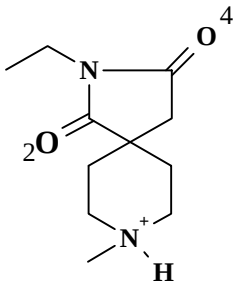

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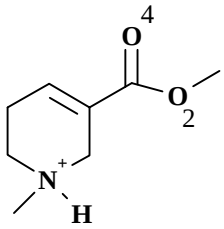

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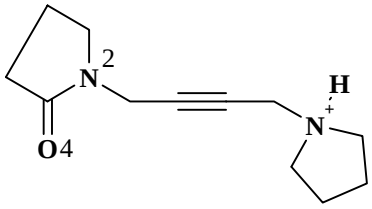

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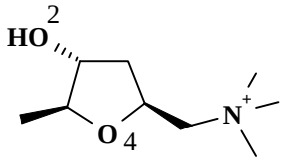

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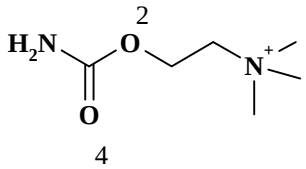

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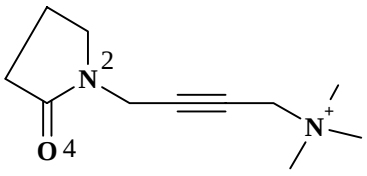


ANTAGONISTS: 24-26
WEAK PARTIAL AGONISTS: 27-29
PARTIAL AGONISTS: 30
FULL AGONISTS: 31-34

Scheme 2 *Protonated molecular form of the M_1-muscarinic ligands considered in this study. The labels 2 and 4 indicate the heteroatoms used to define the pharmacophore*

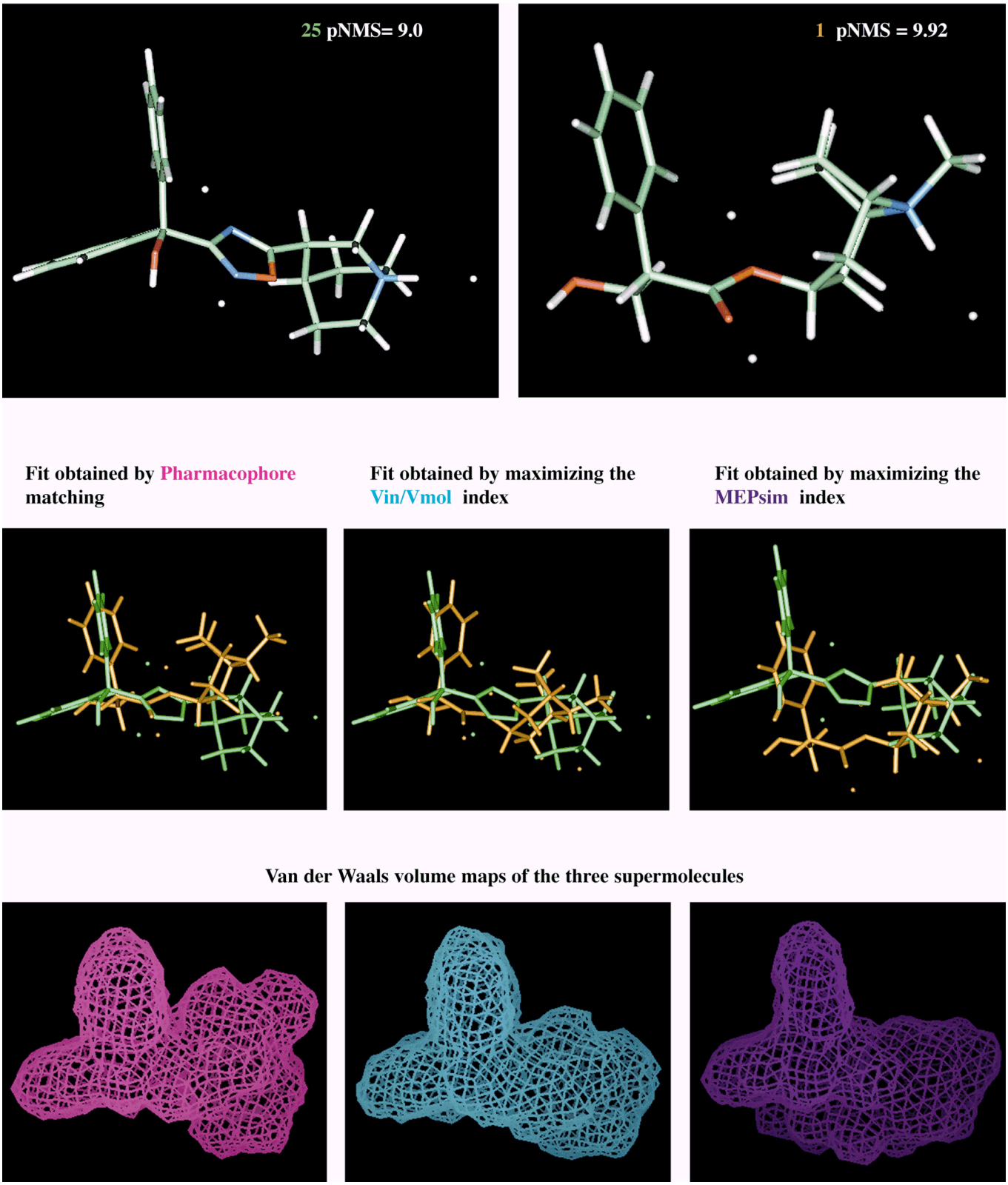


Figure 1 *Top: the molecular structures of the antagonists, compounds 25 (Scheme 2) and 1 (Scheme 1), which were used to define the reference supermolecule to model the pNMS binding affinity data; middle: compounds 1 and 25 superimposed according to the different matching criteria; bottom: the van der Waals volume maps of the corresponding supermolecules, the values of the supermolecule volumes are* V_{sup} *= 408 Å³ (pharmacophore matching),* V_{sup} *= 379 Å³ (*V_{in}*/*V_{mol} *matching criterion),* V_{sup} *= 414 Å³ (MEPsim matching criterion)*

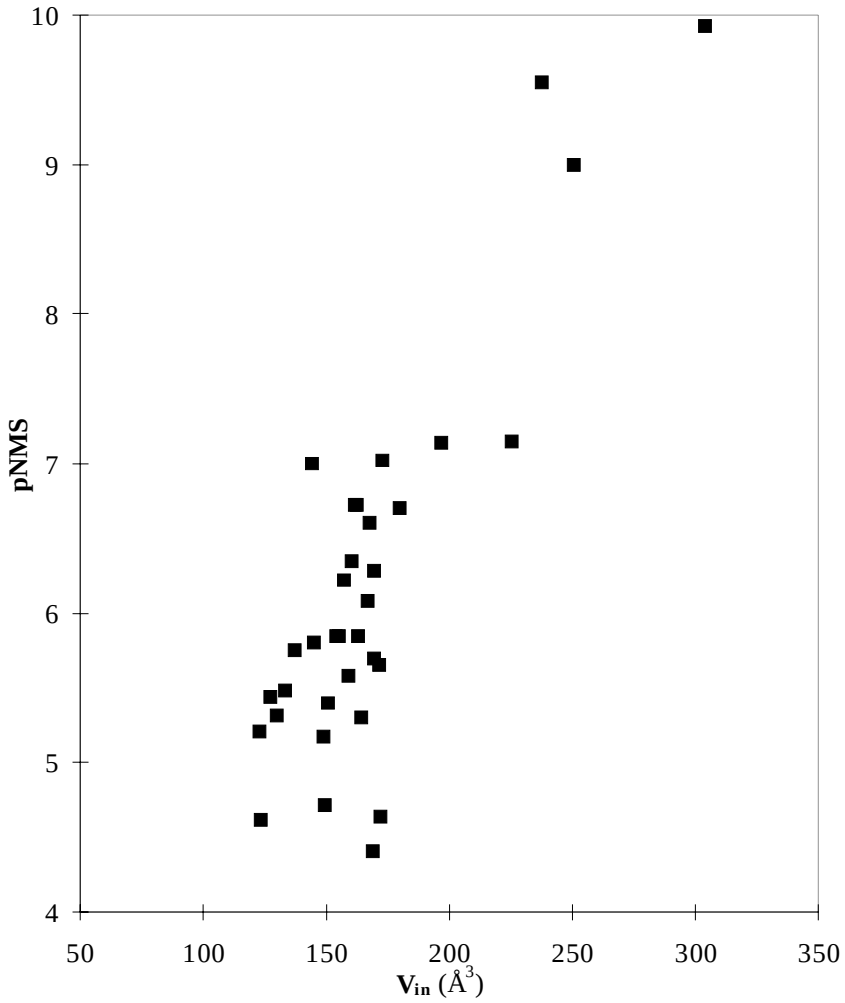


Figure 2 *Correlation between the pNMS binding affinity data and the values of the ad hoc size and shape descriptor Vin computed on the reference pNMS supermolecule obtained by the V_{in}/V_{mol} matching criterion. The linear regression equation is: pNMS = 0.029(±0.003)V_{in} + 1.3(±0.6), n = 34, r^2 = 0.702, r^2CV = 0.668, s = 0.710, F = 75.547. In this equation and equations of Figures 4 and 6, n is the number of compounds, r^2 and r^2CV are respectively the squared and the squared crossvalidated correlation coefficients, s is the standard deviation, and F is the value of the Fisher ratio; the numbers in parentheses are the 95% confidence intervals of the regression coefficient and the intercept*

van der Walls surface of the supermolecule. These points are allocated by generating around each atom of each molecule belonging to the supermolecule a gridded sphere corresponding to the atomic van der Waals radius. Each point falling at the intersection region of any two spheres is then discarded.

Superposition procedure

The protonated molecular form of each compound of Schemes 1 and 2, being the bioactive one [5, 28], was used in the superposition procedure.

Three different reference supermolecules were defined to model the binding affinity data values pNMS, pOXO-M and their ratio log(NMS/OXO-M), respectively.

The most active and structurally most different M_1-antagonists (compounds **1** of Scheme 1 and **25** of Scheme 2) were chosen to define and build the supermolecule which model the pNMS binding affinity; compound **1** was used as reference compound, i.e. kept fixed in the superposition procedure, for all compounds of Schemes 1 and 2 but for compound **24** which was superimposed on compound **25**.

The reference compound in the superposition procedure is generally chosen as the most active one or the one which is structurally more similar to the ligand to be superimposed. In few cases all the compounds belonging to the supermolecule were tested as reference compound and the one allowing the best superposition was used.

The most active and structurally most different full agonists (compounds **13** and **14** of Scheme 1 and **34** of Scheme 2) were chosen to define and build the supermolecule which model the pOXO-M binding affinity; compound **34** was used as reference compound for compounds **14**, **24-26**, **31** and **33**, compound **14** was used as reference compound for compounds **13**, **15-19**, **22-23**, **27-29** and **32**, compound **13** was used as reference compounds for compounds **1-12**, **20** and **21**, compound **31** was used as reference compound for compound **30**.

The full agonists, compounds **32**, **33** and **34**, showing the highest efficacy values, as estimated by the log(NMS/OXO-M) binding affinity ratio, were chosen to define and build the supermolecule which model the log(NMS/OXO-M) binding affinity ratio; compound **34** was used as reference compound for compounds **13**, **14-16**, **18-23**, **25**, **28**, and **30-33**, com-

Table 1 *Experimental binding affinities and selected ad hoc molecular similarity descriptors for compounds of Schemes 1 and 2*

No.	log (NMS/OXO-M)[a]	pNMS [a]	pOXO-M [a]	V_{in} [Å^3] [b]	MEPsim [c]	MEPsur [d]	MEPsim [e]	V_{in} [Å^3] [f]	MEPsim [g]
1	0.25	9.92	10.17	304.13	0.9633	0.9055	0.8629	175.00	0.9105
2	0.53	7.15	7.68	225.00	0.9637	0.9259	0.8662	165.63	0.9276
3	0.98	6.70	7.68	180.00	0.9715	0.9363	0.8595	156.00	0.9325
4	1.23	7.02	8.25	172.88	0.9696	0.9401	0.8492	149.88	0.9388
5	1.36	4.41	5.77	168.75	0.9676	0.9406	0.8602	153.13	0.9419
6	1.57	6.28	7.85	169.75	0.9729	0.9408	0.8517	143.38	0.9417
7	1.58	5.70	7.28	169.50	0.9709	0.9410	0.8567	145.50	0.9409
8	1.79	5.58	7.37	159.13	0.9681	0.9439	0.8895	144.13	0.9460
9	2.24	6.72	8.96	161.88	0.9660	0.9489	0.8787	144.00	0.9454
10	2.28	4.64	6.92	172.38	0.9621	0.9285	0.8765	159.13	0.9323
11	2.60	6.72	9.32	162.38	0.9690	0.9492	0.8726	141.25	0.9472
12	2.69	6.35	9.04	160.63	0.9706	0.9480	0.8882	146.13	0.9431
13	3.08	6.22	9.3	156.88	0.9712	0.9502	0.9909	145.75	0.9452
14	3.00	7.00	10.0	144.00	0.9549	0.9742	0.9938	136.38	0.9530
15	3.10	5.75	8.85	136.75	0.9594	0.9420	0.8880	121.38	0.9517
16	3.24	5.44	8.68	127.13	0.9549	0.9400	0.8775	138.00	0.9461
17	3.35	5.80	9.15	145.13	0.9589	0.9419	0.8572	137.00	0.9518
18	2.25	5.17	7.42	148.75	0.9679	0.9454	0.8588	136.38	0.9412
19	1.99	5.48	7.47	152.50	0.9654	0.9461	0.8747	137.00	0.9431
20	1.72	5.85	7.57	151.38	0.9627	0.9403	0.8622	139.25	0.9377
21	1.59	5.85	7.44	162.13	0.9682	0.9465	0.8612	144.88	0.9428
22	1.39	5.85	7.24	152.25	0.9605	0.9385	0.8652	138.75	0.9364
23	1.29	6.60	7.89	167.63	0.9689	0.9403	0.8448	148.75	0.9374
24	0.11	9.55	9.66	237.38	0.9498	0.8984	0.8105	158.75	0.9117
25	0.32	9.00	9.32	250.75	0.9658	0.9122	0.8269	157.75	0.9115
26	0.34	7.14	7.48	196.88	0.9336	0.8948	0.8770	148.13	0.9055
27	2.00	5.40	7.4	150.38	0.9294	0.8776	0.8658	131.00	0.9195
28	2.14	5.31	7.45	130.00	0.9542	0.9393	0.8356	122.75	0.9346
29	2.10	5.30	7.4	164.25	0.9538	0.9359	0.8518	140.75	0.9380
30	2.75	5.21	7.96	122.88	0.9423	0.9363	0.8586	123.13	0.9492
31	2.94	6.08	9.02	167.00	0.9462	0.9260	0.8397	161.13	0.9381
32	3.60	4.72	8.32	149.13	0.9632	0.9498	0.8952	157.38	0.9728
33	3.62	4.62	8.24	123.25	0.9632	0.9449	0.9110	129.13	0.9742
34	3.62	5.66	9.28	171.13	0.9546	0.9542	0.9884	178.50	0.9733

[a] Experimental binding affinities Refs. [17,18]
[b] computed by Vin/Vmol matching criterion, on pNMS reference supermolecule
[c] computed by MEPsim matching criterion, on pNMS reference supermolecule
[d] computed by Vin/Vmol matching criterion, on pOXO-M reference supermolecule
[e] computed by MEPsim matching criterion, on pOXO-M reference supermolecule
[f] computed by Vin/Vmol matching criterion, on log(NMS/OXO-M) reference supermolecule
[g] computed by MEPsim matching criterion, on log(NMS/OXO-M) reference supermolecule

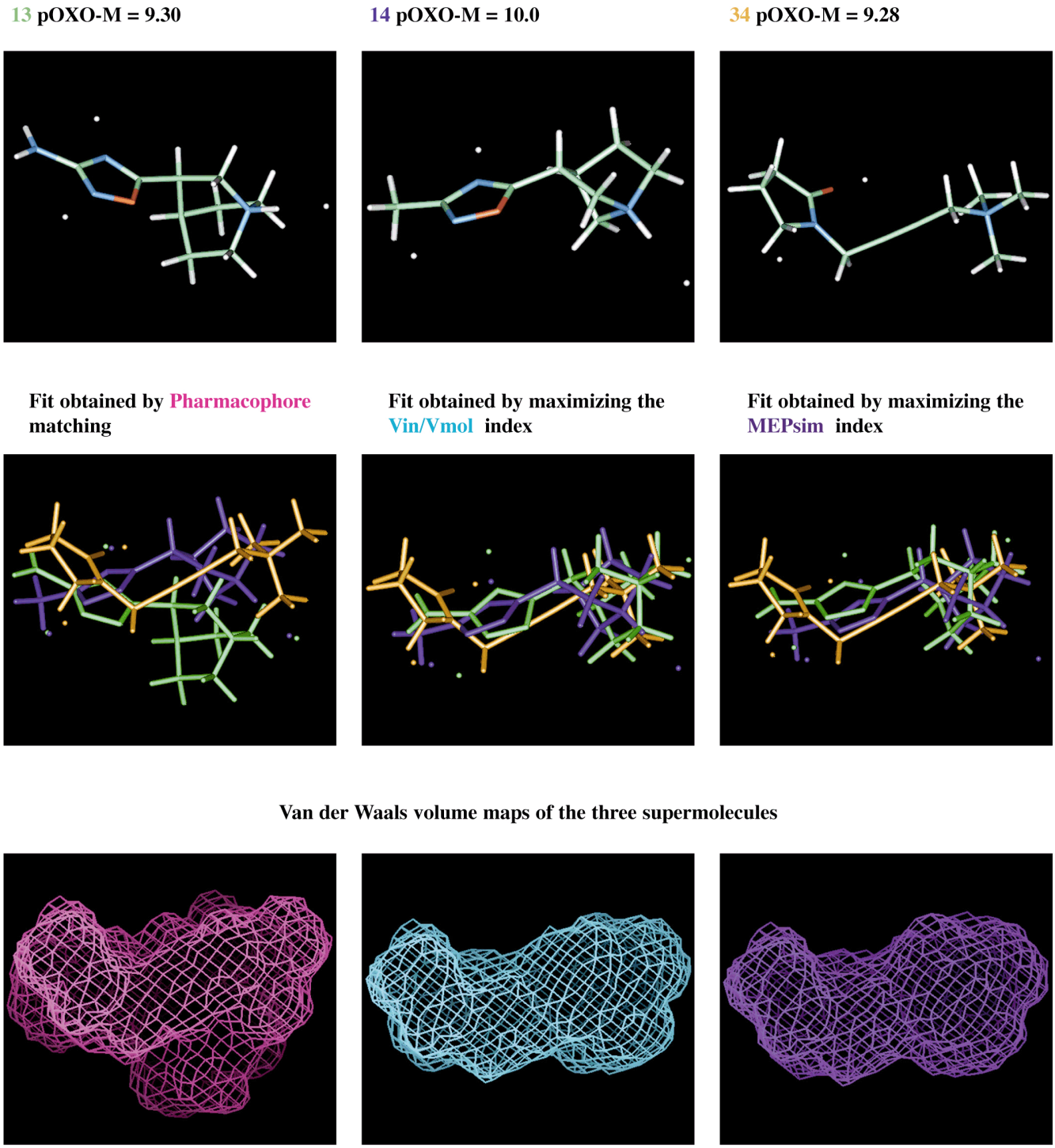


Figure 3 *Top: the molecular structures of the full agonists compounds 13, 14 (Scheme 1) and 34 (Scheme 2) which were used to define the reference supermolecule to model the pOXO-M binding affinity data; middle: compounds 13, 14 and 34 superimposed according to the different matching criteria; bottom: the van der Waals volume maps of the corresponding supermolecules, the values of the supermolecule volumes are Vsup= 350 Å³ (pharmacophore matching), Vsup= 245 Å³ (Vin/Vmol matching criterion), Vsup= 248 Å³ (MEPsim matching criterion)*

Table 2 *Correlation matrix of the ad hoc molecular similarity descriptors computed on the pNMS supermolecule and pNMS binding affinity data*

			fit obtained by pharmacophore matching					fit obtained by maximizing V_{in}/V_{mol}				
		pNMS	**MEPsim**	**MEPsur**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**	**MEPsim**	**MEPsur**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**
fit obtained by pharmacophore matching	**pNMS**	1.00										
	MEPsim	0.23	1.00									
	MEPsur	0.44	0.61	1.00								
	V_{in}	**0.69**	0.73	0.65	1.00							
	V_{Dnorm}	0.45	0.92	0.68	**0.92**	1.00						
	V_{in}/V_{mol}	0.20	**0.96**	0.56	0.73	0.93	1.00					
fit obtained by maximizing V_{in}/V_{mol}	**MEPsim**	0.37	**0.64**	0.33	0.62	0.65	0.62	1.00				
	MEPsur	0.39	0.21	0.54	0.39	0.31	0.14	0.42	1.00			
	V_{in}	**0.84**	0.22	0.43	**0.81**	0.53	0.21	0.45	0.47	1.00		
	V_{Dnorm}	0.76	0.50	0.57	0.89	**0.75**	0.48	0.62	0.53	**0.91**	1.00	
	V_{in}/V_{mol}	0.14	0.76	0.45	0.50	0.72	**0.74**	**0.70**	0.30	0.17	0.54	1.00
fit obtained by maximizing MEPsim	**MEPsim**	0.01	**0.82**	0.35	0.51	0.73	0.84	**0.65**	0.08	0.07	0.37	0.80
	MEPsur	0.32	0.37	0.56	0.46	0.48	0.39	0.56	0.71	0.41	0.58	0.59
	V_{in}	**0.81**	0.20	0.45	**0.80**	0.51	0.21	0.39	0.45	**0.99**	0.87	0.10
	V_{Dnorm}	0.73	0.47	0.61	0.90	**0.74**	0.48	0.53	0.52	0.91	**0.95**	0.44
	V_{in}/V_{mol}	0.09	0.77	0.53	0.54	0.75	**0.81**	0.55	0.23	0.14	0.47	**0.88**
fit obtained by maximizing MEPsim*(V_{in}/V_{mol})	**MEPsim**	0.03	**0.82**	0.38	0.52	0.73	0.84	**0.62**	0.10	0.09	0.37	0.78
	MEPsur	-0.49	0.14	-0.03	-0.26	0.00	0.19	0.14	0.00	-0.46	-0.20	0.50
	V_{in}	**0.81**	0.15	0.41	**0.77**	0.46	0.15	0.37	0.44	**0.99**	0.86	0.06
	V_{Dnorm}	0.77	0.39	0.57	0.88	**0.68**	0.39	0.50	0.51	0.95	**0.97**	0.38
	V_{in}/V_{mol}	0.06	0.73	0.51	0.49	0.71	**0.75**	0.53	0.22	0.12	0.48	**0.91**

pound **32** was used as reference compound for compounds **1-12**, **26**, **27** and **29**, compound **17** was superimposed on compound **14**.

For each supermolecule the same procedure, illustrated below, was used to calculate the *ad hoc* molecular similarity indices.

Pharmacophore matching

Each ligand of Schemes 1 and 2 was superimposed on the corresponding reference compound (see previous session) by a rigid fit procedure minimizing the r.m.s deviation with respect to the following dummy atoms pairs: (a) a dummy atom positioned 3Å from the protonated nitrogen atom (see Schemes 1 and 2) on the vector defined by the N-H$^+$ bond; for the compounds bearing a quaternary nitrogen atom (compounds **24** and **32-34** of Scheme 2), the distance between this atom and the protonated nitrogen of the reference compounds used in the superposition procedure was minimized; (b) two dummy atoms positioned 2Å from the heteroatoms labeled as 2 and 4 in Schemes 1 and 2 on the direction of the heteroatoms lone pairs; (c) for compounds **18-23** only the dummy atom corresponding to the heteroatom in position 4 was used; (d) the protonated nitrogen atom was used as an additional matching point for compounds **18-23** and **31**; (e)

Table 2a *Correlation matrix of the ad hoc molecular similarity descriptors computed on the pNMS supermolecule and pNMS binding affinity data*

		fit obtained by maximizing MEPsim					fit obtained by maximizing MEPsim*(V_{in}/V_{mol})				
		MEPsim	**MEPsur**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**	**MEPsim**	**MEPsur**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**
fit obtained by maximizing MEPsim	**MEPsim**	1.00									
	MEPsur	0.40	1.00								
	V_{in}	0.07	0.41	1.00							
	V_{Dnorm}	0.38	0.61	**0.92**	1.00						
	V_{in}/V_{mol}	**0.86**	0.61	0.15	0.51	1.00					
fit obtained by maximizing MEPsim*(V_{in}/V_{mol})	**MEPsim**	**0.98**	0.41	0.08	0.38	0.83	1.00				
	MEPsur	0.42	0.39	-0.46	-0.20	0.50	0.42	1.00			
	V_{in}	0.02	0.38	**1.00**	0.89	0.09	0.03	-0.49	1.00		
	V_{Dnorm}	0.28	0.56	0.95	**0.99**	0.41	0.29	-0.27	**0.94**	1.00	
	V_{in}/V_{mol}	0.83	0.59	0.11	0.48	**0.97**	**0.81**	0.52	0.07	0.40	1.00

the carbon atom of the pentatomic ring attached to the quinuclidine ring was used as an additional matching point for compounds **18, 19** and **22, 23**.

Matching by optimization of the ad hoc *similarity descriptors*

The V_{in}/V_{mol}, the MEPsim and the product of the two indices were used as criteria for superimposing molecules on the supermolecules. These indices were maximized by minimizing respectively the functions 1-V_{in}/V_{mol}, 1 - MEPsim and 1 - (V_{in}/V_{mol})×MEPsim by using a routine taken from Ref. [29] employing the Simplex method of Nelder and Mead[30]. The Simplex parameters were chosen as follow: the maximum number of iterations was set to 500, the fractional convergence tolerance to be achieved in the function (the similarity descriptor) value, ftol, was set to 10^{-9} and the minimization routine was restarted (taking the found minimum as one of the simplex vertex) unless the values of the function between two minima differ by less than 10^{-4}.

The first step, when using this optimization routine, is to obtain a supermolecule according to each superposition criterion. This is done by superimposing, one at time, each compound belonging to the supermolecule on the corresponding reference compound (see *pharmacophore matching* section). The reference compound is kept fixed while the other compound is allowed to move (rotation and translation in three dimensions) toward the position of maximum similarity with the reference one. The initial relative orientation of the two compounds is that obtained by pharmacophore matching, except in the case of optimization of the (V_{in}/V_{mol})×MEPsim product, where the initial relative orientation was the one obtained by optimization of the MEPsim index.

Each "new" supermolecule is then used as reference structure for the superposition, according to the corresponding maximum similarity criteria, of all other ligands of Schemes 1 and 2. The initial relative orientation of each compound with respect to the supermolecule is always that obtained by the pharmacophore matching criteria.

For each type of supermolecule were computed the following descriptors MEPsim, MEPsur, V_{in}, V_{Dnorm}, and V_{in}/V_{mol}. The calculation of the volume descriptors (V_{in}, V_{Dnorm}, and V_{in}/V_{mol}) was done by using a grid enclosing all the molecules of Schemes 1and 2 in their orientation achieved after matching, in order to avoid slightly different values of V_{sup} because of different grid dimensions.

The codes were written on fortran 77 and all similarity and superposition calculations were run on a SG Indigo 2 workstation.

Results and discussion

The molecular series of muscarinic ligands considered in this study is shown in Schemes 1 and 2. Table 1 reports the binding affinities (pNMS and pOXO-M) and the binding affinity ratio (log(NMS/OXO-M) for the M_1 muscarinic receptor, together with the values of some of the computed *ad hoc* molecular similarity descriptors. These molecular descriptors were all computed on the protonated molecular forms of com-

Table 3 *Correlation matrix of the ad hoc molecular similarity descriptors computed on the pOXO-M supermolecule and pOXO-M binding affinity data*

			fit obtained by pharmacophore matching					fit obtained by maximizing V_{in}/V_{mol}				
		pOXO-M	MEPsur	MEPsim	V_{in}	V_{Dnorm}	V_{in}/V_{mol}	MEPsur	MEPsim	V_{in}	V_{Dnorm}	V_{in}/V_{mol}
fit obtained by pharmacophore matching	**pOXO-M**	1.00										
	MEPsur	0.26	1.00									
	MEPsim	0.03	0.66	1.00								
	V_{in}	0.17	0.04	0.29	1.00							
	V_{Dnorm}	-0.02	0.54	**0.81**	0.59	1.00						
	V_{in}/V_{mol}	0.00	0.60	**0.86**	0.44	**0.97**	1.00					
fit obtained by maximizing V_{in}/V_{mol}	**MEPsur**	0.28	0.71	0.46	-0.05	0.47	0.55	1.00				
	MEPsim	0.06	0.71	**0.89**	0.31	0.89	0.94	0.59	1.00			
	V_{in}	0.18	-0.16	-0.04	**0.88**	0.29	0.10	-0.26	0.00	1.00		
	V_{Dnorm}	-0.03	0.51	0.71	0.54	**0.93**	0.88	0.42	**0.81**	0.38	1.00	
	V_{in}/V_{mol}	-0.05	0.60	0.80	0.30	0.92	**0.95**	0.57	**0.91**	0.06	**0.93**	1.00
fit obtained by maximizing MEPsim	**MEPsur**	0.32	0.65	0.38	0.18	0.41	0.45	0.84	0.50	0.00	0.34	0.42
	MEPsim	0.05	0.71	**0.86**	0.26	0.88	0.92	0.59	**0.94**	-0.02	0.84	0.93
	V_{in}	0.14	0.00	0.17	**0.95**	0.52	0.35	-0.13	0.22	**0.95**	0.57	0.28
	V_{Dnorm}	-0.08	0.57	0.76	0.44	**0.96**	0.94	0.48	0.86	0.22	**0.97**	0.96
	V_{in}/V_{mol}	-0.06	0.64	0.82	0.28	0.93	**0.97**	0.59	0.92	-0.01	0.89	**0.98**
fit obtained by maximizing MEPsim*(V_{in}/V_{mol})	**MEPsur**	0.30	0.66	0.46	0.13	0.50	0.54	0.68	0.62	-0.07	0.45	0.52
	MEPsim	0.04	0.62	**0.84**	0.32	0.87	0.90	0.55	**0.92**	0.00	0.80	0.87
	V_{in}	0.19	-0.19	-0.08	**0.80**	0.24	0.06	-0.26	-0.04	**0.88**	0.30	-0.01
	V_{Dnorm}	-0.05	0.47	0.64	0.44	**0.86**	0.82	0.41	0.74	0.24	**0.90**	0.83
	V_{in}/V_{mol}	-0.05	0.57	0.74	0.21	0.85	**0.88**	0.56	0.85	-0.07	0.83	**0.90**

pounds of Schemes 1 and 2. In fact, structure-function relationships of muscarinic drugs established that a cationic head group is essential for strong muscarinic or antimuscarinic activity. Furthermore, mutagenesis studies of M_1 muscarinic receptor suggest that the binding process is initiated by an ion-ion interaction between the protonated amine moiety of the ligands and an aspartic residue of the receptor [28, 31].

However, as far as the *ad hoc* MEP similarity descriptor is concerned, it has been evaluated both on the neutral and the protonated molecular forms. It is likely that the interaction between the protonated amine function of the ligands and the anionic site of the receptor (Asp) renders the electronic characteristics of the protonated form similar to those of the neutral one [32]. The use of neutral species prevents to consider in the correlations compounds **24** and **32-34** which bear a quaternary ammonium ion, this narrows the range of variation of the binding affinity ratio log(NMS/OXO-M) data being compounds **32-34** the most active full agonists and implies a different choice of the pOXO-M and log(NMS/OXO-M) reference supermolecules (see sections below).

The choice of the pharmacophoric elements, always used to determine the initial orientation of each ligand with respect to the reference supermolecules, was based both on the qualitative binding model for muscarinic ligands proposed by Saunders et al. [18] and on the theoretical models recently obtained by us [33]. According to these models the cationic

Table 3a *Correlation matrix of the ad hoc molecular similarity descriptors computed on the pOXO-M supermolecule and pOXO-M binding affinity data*

		fit obtained by maximizing MEPsim					fit obtained by maximizing MEP*(V_{in}/V_{mol})				
		MEPsur	**MEPsim**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**	**MEPsur**	**MEPsim**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**
fit obtained by maximizing MEPsim	**MEPsur**	1.00									
	MEPsim	0.50	1.00								
	V_{in}	0.09	0.21	1.00							
	V_{Dnorm}	0.36	**0.89**	0.45	1.00						
	V_{in}/V_{mol}	0.43	**0.94**	0.24	**0.96**	1.00					
fit obtained by maximizing MEPsim·(V_{in}/V_{mol})	**MEPsur**	0.67	0.63	0.08	0.49	0.55	1.00				
	MEPsim	0.49	**0.94**	0.24	0.85	0.89	0.72	1.00			
	V_{in}	-0.04	-0.07	**0.88**	0.18	-0.04	0.02	0.08	1.00		
	V_{Dnorm}	0.29	0.77	0.47	**0.91**	0.84	0.52	**0.84**	0.39	1.00	
	V_{in}/V_{mol}	0.38	0.88	0.19	0.90	**0.92**	0.61	**0.93**	0.08	**0.93**	1.00

head group is an essential pharmacophoric element (represented in our pharmacophore model by the dummy atom defined on the N^+-H bond vector, see Schemes 1 and 2 and Methods section) for all ligands, agonists as well as antagonists. Full agonists are, in general, small hydrophilic molecules and utilize at least two hydrogen bonding interactions (represented in our pharmacophore model by the dummy atoms defined with respect to heteroatoms 2 and 4, see Schemes 1and 2 and Methods section), to bind the receptor. Antagonists require maximally one, and possibly no hydrogen bonding site and instead utilize hydrophobic and dispersion forces in order to stabilize the complex with receptor.

Modeling of the pNMS binding affinity

The protonated forms of the most active and structurally most diverse antagonists **1** of Scheme 1 and **25** of Scheme 2 were used to construct the reference supermolecule to model the pNMS binding affinities. Figure 1 shows the supermolecules obtained according to the different matching criteria. It can be observed that the size and the overall shape of the three supermolecules are similar but a better fit of the two compounds **1** and **25** is realized with the V_{in}/V_{mol} criterion. Tables 2 and 2a show the correlation matrix between the ad hoc similarity descriptors and the pNMS affinity data values, reported in Table 1. The V_{in} descriptor, independently of the matching criterion, shows the highest correlation coefficient with pNMS. The best correlation is achieved when the matching criterion is the optimization of the V_{in}/V_{mol} descriptor (r^2=0.702, the correlation is plotted in Figure 2). These results agree with the proposed interaction mechanism between the receptor and the antagonists, in fact the V_{in}/V_{mol} descriptor mainly mimics dispersion-hydrophobic interactions.

The intercorrelation between the various similarity descriptors can be summarized as follow (the correlation coefficients corresponding to the points discussed below are reported in bold character on Tables 2 and 2a):

a) V_{in} and V_{Dnorm} descriptors computed with the same matching criterion and V_{in}, V_{Dnorm} and V_{in}/V_{mol} descriptors computed with different matching criteria are always intercorrelated;

b) the MEPsim descriptors computed with different matching criteria show the same trend;

c) the MEPsim and the V_{in}/V_{mol} descriptors computed with the same matching criterion show the same trend.

For comparative purpose the MEPsim index has been computed also on the neutral molecular form of compounds of Schemes 1 and 2. The superposition obtained by pharmacophore matching of the protonated forms was used as starting orientation, then each ligand was allowed to move in order to optimize the MEPsim index computed by using the charge distribution of the neutral molecular forms. The supermolecule obtained in this case does not differ from that obtained

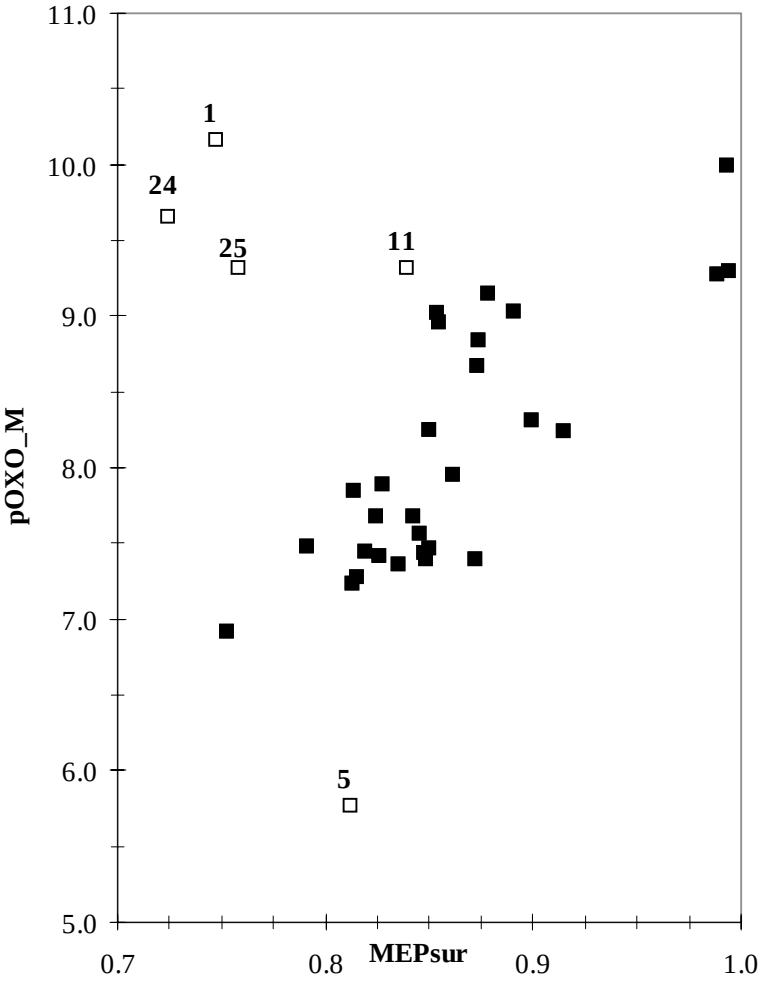


Figure 4 *Correlation between the pOXO-M binding affinity data and the values of the ad hoc similarity descriptor MEPsur computed on the reference pOXO-M supermolecule obtained by the Vin/Vmol matching criterion. The linear regression equation is: pOXO-M = 12(±1.6)MEPsur -2(±1.4), n = 29, r2 = 0.642, r2CV = 0.602, s = 0.491, F = 48.42, where compounds 1, 5, 11, 24 and 25 of scheme 1 have been omitted as showing a large deviation from the regression. See caption to Figure 2 for a description of the parameters*

by pharmacophore matching. No improvement in the correlation statistics was obtained by plotting the V_{in} descriptor versus pNMS. However, a linear trend was observed between the MEPsim index computed considering only the negative values of the MEP computed on the neutral molecular forms and pNMS (r^2= 0.60, i.e compounds **24** and **32-34** omitted).

Modeling of the pOXO-M binding affinity

The protonated forms of the most active and structurally diverse full agonists **13** and **14** of Scheme 1 and **34** of Scheme 2 were used to construct the pOXO-M binding affinities reference supermolecule. Fig. 3 shows the supermolecules obtained according to the different matching criteria. The supermolecules obtained according to the V_{in}/V_{mol} and MEPsim optimization matching criteria are almost equal, while the supermolecule obtained by pharmacophore matching has a higher van der Waals volume of about 100Å^3 and a different shape especially in the proximity of the protonated nitrogen atom. Tables 3 and 3a show the correlation matrix between the *ad hoc* similarity descriptors and the pOXO-M affinity data values, reported in Table 1. The intercorrelations between the various descriptors are similar to those observed when modeling the pNMS binding affinities as far as points *b* and *c* of the previous section are concerned, while the V_{Dnorm} descriptor, in this case, is correlated with the V_{in}/V_{mol} instead of V_{in} (see point *a* above section). Furthermore the MEPsim and the V_{Dnorm} descriptors computed with the same matching criterion are correlated.

The only descriptor satisfactorily correlated (r^2 = 0.64, Fig. 4) with the pOXO-M binding affinity data values is the MEPsur similarity index computed on the ligands superimposed according to the V_{in}/V_{mol} matching criterion and omitting from the correlations the antagonists **1**, **24** and **25** and the partial agonists **5** and **11**. This correlation represents an improvement with respect to those previously obtained by using as descriptors either the V_{Dnorm} computed with a pharmacophore matching criterion [5] or the calculated interaction energies of the minimized ligand-M_1-receptor complexes [33]. The MEPsur descriptor takes into account both electrostatic and dispersion interactions. Therefore the hypothesis on the intermolecular interactions operative in the receptor-ligand complex, which discriminate between hydrophilic full agonists and hydrophobic antagonists, is supported.

Table 4 *Correlation matrix of the ad hoc molecular similarity descriptors computed on the log(NMS/OXO-M) supermolecule and log(NMS/OXO-M) binding affinity ratio*

		fit obtained by maximizing V_{in}/V_{mol}					fit obtained by maximizing MEPsim				
	log(NMS/OXO-M)	**MEPsim**	**MEPsur**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**	**MEPsim**	**MEPsur**	**V_{in}**	**V_{Dnorm}**	**V_{in}/V_{mol}**
log(NMS/OXO-M)	1.00										
MEPsim	0.80	1.00									
MEPsur	0.82	0.79	1.00								
V_{in}	-0.35	-0.39	-0.13	1.00							
V_{Dnorm}	0.70	**0.82**	0.72	0.03	1.00						
V_{in}/V_{mol}	**0.83**	**0.93**	0.81	-0.25	**0.94**	1.00					
MEPsim	**0.85**	**0.91**	0.90	-0.22	0.84	0.93	1.00				
MEPsur	0.61	0.41	0.80	-0.12	0.26	0.40	0.59	1.00			
V_{in}	-0.04	0.09	0.20	**0.77**	0.44	0.21	0.23	-0.01	1.00		
V_{Dnorm}	0.67	**0.86**	0.69	-0.10	**0.95**	0.93	**0.86**	0.25	0.46	1.00	
V_{in}/V_{mol}	0.75	**0.92**	0.77	-0.26	0.90	**0.97**	**0.93**	0.37	0.31	**0.97**	1.00

In order to test the performance of the MEPsim descriptor computed on the neutral molecular forms we considered the more homogeneous series of compounds **1-23** of Scheme 1. In this case the reference supermolecule was obtained by superposition of compounds **13** and **14** (compound **34** was not considered bearing a quaternary nitrogen atom). The MEPsim descriptor computed on the neutral molecular forms was used as well as matching optimization criterion. A linear trend was observed between the MEPsim index computed considering only the negative values of MEP and pOXO-M (r^2= 0.62, i.e compounds **1** and **17** omitted).

Modeling of the log(NMS/OXO-M) binding affinity ratio

Two methods have been used to model the efficacy as expressed by the log(NMS/OXO-M) binding affinity ratio. The first one consisted of defining a reference supermolecule with the full agonists, compounds **32**, **33** and **34**, showing the highest log(NMS/OXO-M) binding affinity ratio values, the second one consisted of defining a new set of molecular descriptors ΔV_{in}, ΔV_{Dnorm}, ΔMEPsim and ΔMEPsur as differences between the corresponding descriptors computed by using the pOXO-M and pNMS supermolecules, respectively.

The supermolecules obtained according to the V_{in}/V_{mol} and MEPsim matching criteria do not differ significantly and are shown in Fig. 5.

Tables 4 and 4a show the correlation matrices between the ad hoc similarity descriptors computed with the two methods and the log(NMS/OXO-M) binding affinity ratio, reported in Table 1. The MEPsim, computed on the log(NMS/OXO-M) reference supermolecule obtained by the MEPsim matching criterion, the V_{in}/V_{mol}, computed on the log(NMS/OXO-M) reference supermolecule obtained by the V_{in}/V_{mol} matching criterion, and the ΔMEPsim descriptors show good linear correlations, r^2=0.72, r^2=0.68 and r^2=0.69, respectively (Fig. 6a-6c), with the log(NMS/OXO-M) binding affinity data. These results are similar to those previously obtained by us [5], where good linear regressions were obtained between the log(NMS/OXO-M) binding affinity ratio and the S^P_{2+4}(HOMO) descriptor (defined as the sum of the electrophilic superdelocalizability, AM1 calculations, of the heteroatoms 2 and 4, computed on the protonated molecular forms of compounds of Schemes 1 and 2), which describes the hydrogen bond propensity of the heteroatoms 2 and 4 (Schemes 1 and 2), as well as between the log(NMS/OXO-M) binding affinity ratio and the size and shape descriptor ΔV_{Dnorm} (computed with a pharmacophore matching criterion).

Table 4a *Correlation matrix of the descriptors defined as the difference of the corresponding ad hoc molecular similarity descriptors computed for the pNMS and pOXO-M supermolecules and log(NMS/OXO-M) binding affinity ratio*

		fit obtained by maximizing V_{in}/V_{mol}					**fit obtained by maximizing MEPsim**				
	log(NMS/OXO-M)	**ΔMEPsim**	**ΔMEPsur**	**ΔV_{in}**	**ΔV_{Dnorm}**	**$\Delta V_{in}/V_{mol}$**	**ΔMEPsim**	**ΔMEPsur**	**ΔV_{in}**	**ΔV_{Dnorm}**	**$\Delta V_{in}/V_{mol}$**
log(NMS/OXO-M)	1.00										
ΔMEPsim	**0.74**	1.00									
ΔMEPsur	0.68	0.67	1.00								
ΔV_{in}	0.67	0.83	0.70	1.00							
ΔV_{Dnorm}	0.64	0.79	0.67	**0.98**	1.00						
$\Delta V_{in}/V_{mol}$	0.64	**0.83**	0.68	**0.98**	**0.97**	1.00					
ΔMEPsim	**0.83**	**0.86**	0.71	0.75	0.72	0.75	1.00				
ΔMEPsur	0.55	0.54	0.77	0.51	0.46	0.52	0.61	1.00			
ΔV_{in}	0.70	0.80	0.71	**0.98**	0.97	0.95	0.79	0.52	1.00		
ΔV_{Dnorm}	0.66	0.77	0.67	0.95	**0.98**	0.94	0.75	0.45	**0.98**	1.00	
$\Delta V_{in}/V_{mol}$	0.69	**0.79**	0.69	0.94	0.94	**0.95**	**0.81**	0.56	**0.97**	**0.97**	1.00

The intercorrelations among the different descriptors are similar to those observed in the pOXO-M case.

Also in this case the MEPsim descriptor has been computed also on the neutral molecular forms of compounds **1-23** of Scheme 1, which represent a more structural homogeneous molecular series. The reference supermolecule was obtained by superposition of compounds **16** and **17** which show, in this series, the highest log(NMS/OXO-M) binding affinity ratio. The MEPsim descriptor computed on the neutral molecular forms was used as well as matching optimization criterion. The MEPsim descriptor computed on the neutral form gives a satisfactory correlation, r^2=0.65, with log(NMS/OXO-M) only if compound **3** (antagonist), **4, 5**, **7** and **9** (weak partial agonists) are omitted from the correlation. Saunders et al.[18] reported a very good linear regression between the MEP minima (computed on the neutral molecular forms of the model molecules obtained by replacing the quinuclidine nucleus with a methyl group) near the heteroatoms 2 and 4 of the pentatomic ring of compounds **11-13** and **20-23** of Scheme 1 and the log(NMS/OXO-M) binding affinity ratio. If we consider the same subset of compounds the correlation coefficient for the above liner regression with the MEPsim descriptor goes from r^2=0.65 to r^2=0.81 and to r^2=0.89 when MEPsim is computed only with the negative MEP values. This shows that the neutral MEP distribution can be a useful molecular descriptor when dealing with very congeneric series of compounds of similar size.

Conclusions

The use of the supermolecule approach to derive *ad hoc* molecular similarity descriptors has been successful in QSAR modeling procedures. In fact, it represents a flexible tool which can be used to describe both congeneric and non-congeneric series of compounds and can be continuously updated with new coming experimental data. Furthermore, when the three-dimensional (3D) structure of the target and its binding site are available, the supermolecule can be defined as the van der Waals volume of the binding cavity. This allows the use of 3D information in indirect, i.e ligands based, QSAR modeling.

The results obtained on the very structural heterogeneous series of muscarin ligands considered in this study suggest the following concluding remarks:

a) the V_{in}/V_{mol} descriptor seems particularly suitable as matching criterion to optimize the superposition of the lig-

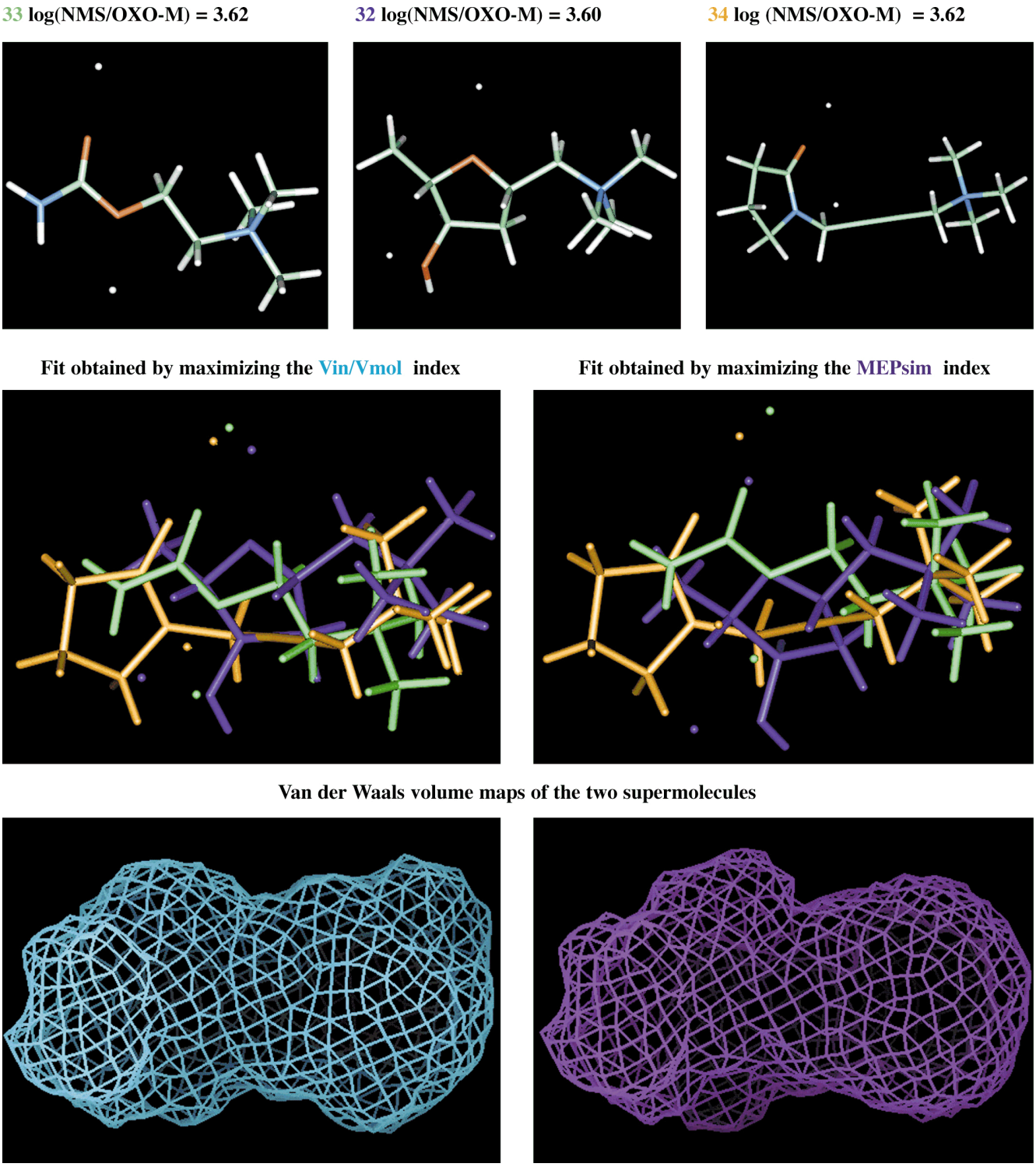
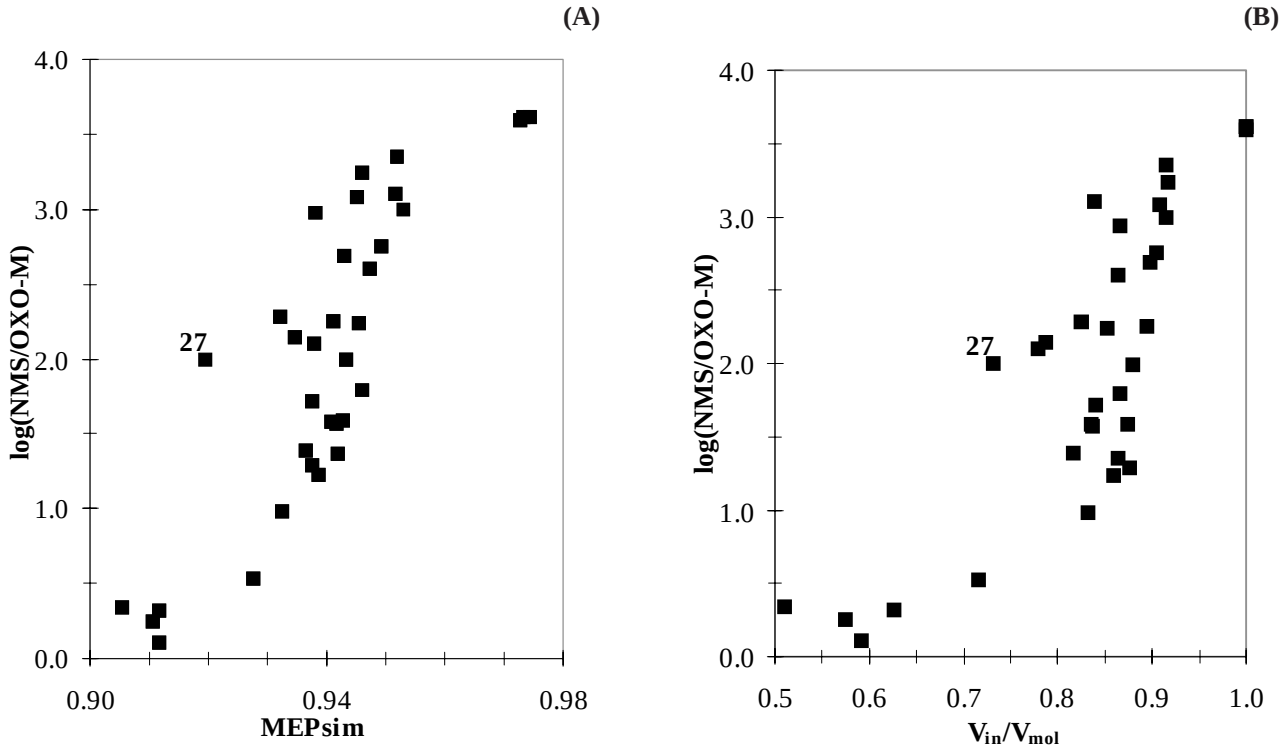


Figure 5 *Top: the molecular structures of the full agonists compounds 33, 32 and 34 which were used to define the reference supermolecule to model the log(NMS/OXO-M) binding affinity ratio; middle: compounds 32, 33 and 34 superimposed according to the different matching criteria; on the bottom the van der Waals volume maps of the corresponding supermolecules are shown. the values of the supermolecule volumes are Vsup= 243 Å³ (Vin/Vmol matching criterion), Vsup= 247 Å³ (MEPsim matching criterion)*



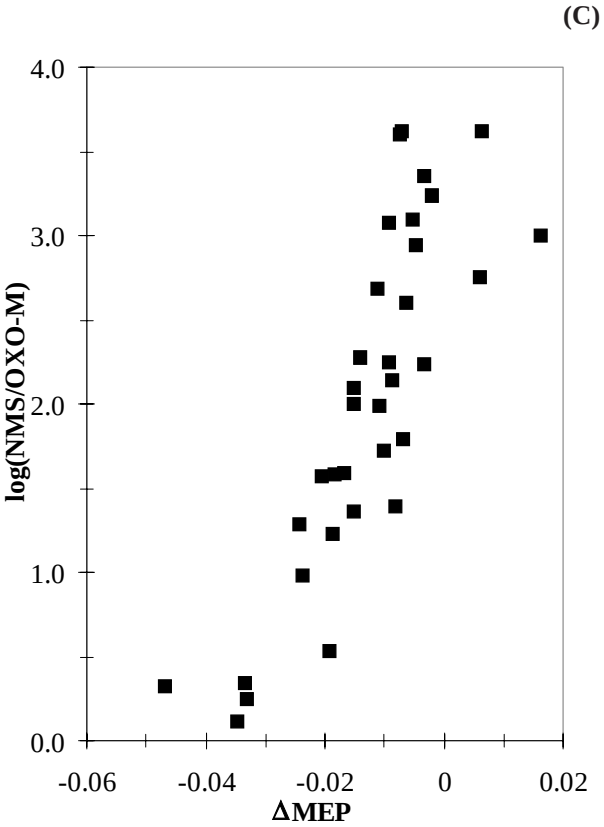


Figure 6 *(***a***) Correlation between the cortical muscarinic efficacy log(NMS/OXO-M) and the values of the ad hoc similarity descriptor MEPsim computed on the reference log(NMS/OXO-M) supermolecule obtained by the MEPsim matching criterion. The linear regression equation is: log(NMS/OXO-M) = 54(±6.0)MEPsim -49(±5.6), n = 34, r* ^{2} *= 0.719,* r^2*CV = 0.692, s = 0.555, F = 81.83. If compound 27 of Scheme 2 is omitted from the regression The linear regression equation is: log(NMS/OXO-M) = 57(±5.8)MEPsim -52(±5.5), n = 33,* r^2 *= 0.756,* r^2*CV = 0.734, s = 0.525, F = 96.20. (***b***) Correlation between the cortical muscarinic efficacy log(NMS/OXO-M) and the values of the ad hoc size and shape descriptor* V_{in}/V_{mol} *computed on the reference log(NMS/OXO-M) supermolecule obtained by the Vin/Vmol matching criterion. The linear regression equation is: log(NMS/OXO-M) = 7.22(±0.9)*V_{in}/V_{mol} *-4.0(±0.8), n = 34, r2 = 0.647, r2CV = 0.610, s = 0.622, F = 58.56. If compound 27 of Scheme 2 is omitted from the regression. The linear regression equation is: log(NMS/OXO-M) = 7.4(±0.9)*V_{in}/V_{mol} *-4.1(±0.8), n = 33,* r^2 *= 0.662,* r^2*CV = 0.624, s = 0.619, F = 60.60. (***c***) Correlation between the cortical muscarinic efficacy log(NMS/OXO-M) and the values of the ad hoc similarity descriptor ΔMEPsim (see text for its definition). The linear regression equation is: log(NMS/OXO-M) = 68(±8) ΔMEPsim +2.9(±0.14), n = 34,* r^2 *= 0.689,* r^2*CV = 0.642, s = 0.583, F = 71.01. See caption to Figure 2 for a description of the parameters*

ands on the reference supermolecule. In fact, improved correlations were obtained with respect to those obtained by pharmacophore matching. Furthermore this descriptor is simple and fast to compute and a further development could be its use for database searching.

b) The analysis of the intercorrelations between the various ad hoc molecular similarity descriptors suggests that *ad hoc* normalized size and shape descriptors as well as MEP similarity descriptors codify both electronic and size-shape features of the molecule.

c) The ad hoc MEP similarity descriptors give satisfactory correlations with the pOXO-M and log(NMS/OXO-M) binding affinity data values. This agrees with the hypothesis that agonists mainly interact with the receptor *via* hydrogen bonding interactions. The MEPsim descriptor computed on the neutral molecular forms give less satisfactory correlations.

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Supplementary material available Table containing the values of the experimental binding affinities and the values of all the computed *ad hoc* molecular similarity descriptors for compounds **1**-**34** of Schemes 1 and 2.

References

1. *Concepts and Applications of Molecular Similarity*; Johnson, M.A.; Maggiora, G.M. Eds.; Wiley: New York, **1990**.
2. *Molecular Similarity in Drug Design*; P.M. Dean Ed.; Chapman Hall: London, **1995**.
3. Cocchi, M.; Menziani, M.C.; Fanelli, F.; De Benedetti, P.G. *J. Mol. Struct. (Theochem)* **1995**, *331*, 79.
4. De Benedetti, P.G.; Cocchi, M; Menziani, M.C.; Fanelli, F. *J. Mol. Struct. (Theochem)* **1994**, *305*, 101.
5. De Benedetti, P.G.; Cocchi, M; Menziani, M.C.; Fanelli, F. *J. Mol. Struct. (Theochem)* **1995**, *333*, 1.
6. Carbó, R.; Leyda, L.; Arnau, M. *Int. J. Quantum Chem.* **1980**, *17* , 1185.
7. Besalu E.; Carbó, R.; Mestres, J.; Solà, M. *Top. Curr. Chem.*, **1995**, *173*, pp. 32-60.
8. Fradera X.; Amat L.; Besalu E.; Carbó Dorca R. *Quant. Struct.-Act. Relat.*, **1997**, *16* , 25.
9. Carbó Dorca R.; Besalu E.; Amat L.; Fradera X. In *Advances in Molecular Similarity*, vol. 1, Carbó Dorca, R. and Mezey, P.G. Eds.; JAI PRESS Inc.: 55 Old post Road, GREENIWICH, **1996**, pp. 1-42.
10. Hodgkin, E.E.; Richards, W.G. *Int. J. Quantum Chem.: QuantumBiol.Symp.* **1987**, *14*, 105.
11. Good, C.A.; Peterson, J. S.; Richards, W. G. *J. Med. Chem.* **1993**, *36*, 2929.
12. Sanz, F.; Manaut, F.; Rodriguez, J.; Lozoya, E.; Lopez de Brinas, E. *J. Comp.-Aided Mol. Des* **1993,** *7*, 337.
13. Náray-Szabó, G.; Ferenczy, G.C. *Chemical Review* **1995**, *95*, 829 and references therein.
14. Thorner, D. A.; Willett, P.; Wright, P.M.; Taylor, R.; *J. Comp.-Aided Mol. Des.* **1997**, *11*, 163.
15. Mestres, J; Roherer D. C.; Maggiora, G. M. *J. Comp. Chem.* **1997**, *18*, 934.
16. Cocchi, M.; Selmi, M.; De Benedetti, P. G. In *Abstracts Book of the Second European conference on Computational Chemistry*, EUCO-CC2, 2-6 September 1997, Lisbon, Portugal; **1997**, 21.
17. Freedman, S. B.; Harley, E.; Iversen, L. L. *Br. J. Pharmacol.* **1988**, *93*, 437 and references therein.
18. Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, L. L.; Kneen, C.; MacLeod, A. M.; Merchant, K. J.; Snow, R. J.; Baker, R. *J. Med. Chem*. **1990**, *33*, 1128.
19. Dewar, M. J. S.; Zoebisch, E. G.; Healey E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
20. Dewar, M. J. S.; Stewart, J. J. P. *QCPE Bull.* **1986**, *6*, 24.
21. Landolt-Bornstein *Structure data of free polyatomic molecules;* Vol. 7; Hellwege K. H. Ed.; Springer-Verlag: Berlin, **1976**.
22. Glaser, R.; Charland J.- P.; Michel, A. *J. Chem. Soc Perkin Trans. II* **1989**, 1875.
23. Kussaether, E.; Haase, J. *Acta Crystallogr., Sect. B* **1972**, *28*, 2896.
24. Jellinek, F. *Acta Crystallogr.* **1957**, *10*, 277.
25. Codding, P. W.; James, M. N. G. *Acta Crystallogr., Sect. B: Struct. Sci.* **1984**, *40*, 429.
26. Trummlitz, G.; Schmidt, G.; Wagner, H.-U.; Luger, P. *Arzneim.-Forsch./Drug Res.* **1984**, *34*, 849.
27. MOPAC-version 6.0, QCPE 455.
28. Wess, J. *Life Sci.* **1993**, *53*, 1447 and references therein.
29. Press, W.H.; Flannery, B.P.; Teukolsky, S.A.; Vetterling, W.T. In *Numerical Recipes (Fortran);* Cambridge University Press: UK, **1989**, cap. 10.
30. Nelder, J.A.; Mead, R. *Computer Journal* **1965**, *7*, 308.
31. Savarrese, T. M.; Fraser, C. M. *Biochem. J.* **1992**, *283*, 1 and references therein.
32. Weinstein, H.; Chou, D.; Kang, S.; Johnson, C. L.; Green, J. P. *Int. J. Quantum Chem. (Quantum Biology Simp.)* **1976**, *3*, 135.
33. Fanelli, F.; Menziani, M.C.; Carotti, A.; De Benedetti P.G. *Bioorg. & Med. Chem.* **1994**, *2*, 195.