Journal of Computer-Aided Molecular Design **18:** 361–374, 2004.

GRIND-derived pharmacophore model for a series of α-tropanyl derivative ligands of the sigma-2 receptor*

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Received 23 July 2003; accepted in revised form 27 July 2004

Key words: alignment free 3D descriptors, GRIND descriptors, pharmacophore model, sigma ligands, sigma receptor

Summary

A pharmacophore model for the sigma-2 receptor was derived using GRIND (GRid INdependent Descriptors) descriptors arising from a 3D-level procedure whose main prerogative is that it does not require ligand alignment. PLS models for sigma-2 affinity (sigma-2 model: r^2=0.83, q^2=0.63) and sigma-1/sigma-2 selectivity (r^2=0.72, q^2=0.46) were derived using a series of α-tropanyl derivatives. The models provide pictures of the virtual receptor site (VRS) significant enough to attain a qualitative pharmacophoric representation of the sigma receptor. They give the internal geometrical relationships within two hydrophobic areas (hydrophobic-*1* and -*2*) and a H-bond donor receptor region with which ligands establish non-covalent bonds.

Introduction

Sigma receptors are a well-defined receptor class, distinct from opioid and phencyclidine binding site, that are present in the central nervous system as well as in various peripheral tissues [2]. They are involved in several physiological effects [3, 4], including modulation of NMDA receptor functions [5], neurotransmitter release [6, 7], neuroprotection [8] and learning and memory [9]. Moreover, several human tumoral cell lines express sigma receptors [10], thus suggesting a potential diagnostic utility of sigma ligands as tumor imaging agents [11].

Despite the great therapeutic potential of sigma modulators, sigma receptors remain somehow enigmatic. In fact, their preferential localization (in the membrane of the endoplasmic reticulum) is different from that of neurotransmitter receptors; no endogenous substance has been conclusively identified as the physiological agonist, although progesterone and certain neurosteroids [12–14] show affinity for this receptor, and the signal transduction pathway is not yet clear either [4, 15, 16].

Based on their enantioselectivity for benzomorphans and on their pharmacological profile, two different subtypes have been proposed, termed σ_1 and σ_2 [17], whereas no significant differences have been recognized between a putative third sigma-subtype, σ_3 [18–20], and the histamine H_1 receptors [21]. The type-1 sigma receptor has been recently cloned and its primary structure has been determined [22]. The predicted topology shows only one transmembrane (TM) domain and two additional hydrophobic stretches, but recently a model with two TM stretches has been proposed [15]. There is no significant homology to other known mammalian proteins [23], although a certain degree of homology (30%) has been found with the fungal Δ^{8-7}-sterol isomerase [22], and there is

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*Preliminary results have been presented at the XVIIth International Symposium on Medicinal Chemistry, September 1–5 2002, Barcelona, Spain [1].

evidence that sigma receptor is involved in sterol biosynthesis [23, 24]. The type-2 sigma receptor has not yet been cloned.

The chemical structures of sigma receptor ligands are heterogeneous, but many of them possess one nitrogen atom and two aromatic rings, at a certain distance from each other; however, also non-basic ligands, such as steroids, can interact with sigma receptor [13]. Many high affinity and selective sigma-1 ligands have been synthesized [25–35], and a model for the binding of phenylalkylamines to the sigma-1 receptor has been proposed [25–28]. Some sigma-2 selective compounds have also been discovered [36–40], but the scarcity of selective ligands has prevented the derivation of similar models for the sigma-2 receptor subtype.

At the moment, there is no evidence that it will be possible in a short time to obtain 3D structure information about the sigma receptor, due both to the lack of significant homology of the σ1-binding protein to other known mammalian proteins and to the intrinsic difficulty in X-ray structure derivation of membrane proteins. Nevertheless, considering the many phatophysiological functions where sigma receptors are involved [3], any effort in defining the spatial arrangement of relevant receptor-ligand interaction points can be positively regarded as an essential aid for the rational design of sigma ligands. However, the task is made difficult owing to the wide number of chemically diverse ligands that sigma receptor is able to accommodate.

In this scenario, the present work aims to provide a qualitative pharmacophore model for a series of α-tropanyl derivatives [41–43] (Table 1) acting as sigma ligands through the application of a chemometric methodology and the characterisation of the compounds by GRIND descriptors [44] in order to obtain hints for the synthesis of new analogs with an improved pharmacological profile.

Materials and methods

All molecular modeling studies were performed on an SGI O2 R10000 workstation (operating system: IRIX 6.5). 3D structures of the sigma ligands in Table 1 were generated using fragment libraries and/or the builder module of the InsightII 2000 package [45]. The energies of the molecules were minimized with the conjugate gradient procedure using the AMBER forcefield (FF) parameterized in vacuum and the Discover module of InsightII 2000. Compounds were generated in the non-protonated form according to Bowen [3], whose results indicate the uncharged lipophilic form as the active one.

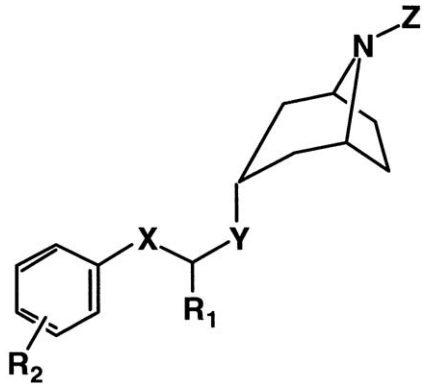
The conformational search was performed using a simulated annealing procedure which was started using the AMBER FF, a distant dependent dielectric constant and a convergence criterion of 0.001 kcal mol^{-1} $Å^{-1}$. The output of the conformational search procedure was submitted to a cluster analysis obtaining subsets of conformers for a specified molecule based on a defined rms (root mean square) value; conformers in each compound were considered within 4 kcal mol^{-1} from the global minimum energy and single conformers for each molecule were then selected combining the FILO procedure [46] with a genetic algorithm (GA) technique. Briefly, conformers in each compound are treated as genes, sets of genes give rise to different chromosomes, i.e. to different combinations of conformers of the dataset molecules. Chromosomes are generally evaluated based on a score function that, in the FILO/GA procedure, is represented by the correlation coefficient R^2 used in the FILO methodology. Here R^2 indicates the correlation coefficient of the function relating Carbo's indexes to the biological activity of the dataset molecules. Carbo's indexes refer to the similarity between the molecular interaction fields (MIFs) of the dataset compounds and the forming MIF of the optimal ligand. In fact, FILO methodology leads to the description of the molecular interaction field (OF, i.e. optimal field) of a ligand with optimal binding affinity that is derived by maximising, via a simplex optimisation procedure, the R^2 value [46]. Combination of FILO and GA techniques will provide a set of chromosomes formed by the combination of conformers (one for each molecule of the dataset) for which the R^2 value is maximized.

PCA and the PLS algorithm were used as implemented in the Almond programme [47]. No scaling or pre-treatment was applied. The optimal dimensionality of the PLS model was determined according to leave-one-out (LOO) and a random groups cross-validation procedure.

GRIND

The compounds in Table 1 were characterized using a new and promising class of descriptors, GRINDs (Grid Independent Descriptors), that allow a detailed understanding of the internal geometrical relationships of receptor regions with which the ligands establish

Table 1. Structure and binding of compounds **1**–**48** (see Reference 43).



N	R_1	R_2	X	Y	Z	K_i nM ± SE		A[a]		S[b]
						σ_1	σ_2	σ_1	σ_2	$\text{Log}[K_i(\sigma_2)/K_i(\sigma_1)]$
R (+) **1**	Me	4-F	O	CO-O	Me	358 ± 27	76 ± 5	−2.55	−1.88	−0.67
S (−) **1**	Me	4-F	O	CO-O	Me		1125 ± 57		−3.05	
1	Me	4-F	O	CO-O	Me	-	557 ± 42		−2.75	
R (+) **2**	Me	4-Cl	O	CO-O	Me		295 ± 12			
S (−) **2**	Me	4-Cl	O	CO-O	Me		1700 ± 130			
2	Me	4-Cl	O	CO-O	Me	> 1000	402 ± 16	−3.00	−2.60	−0.40
3	Me	2-Cl	O	CO-O	Me	65 ± 3	144 ± 8	−1.81	−2.16	0.35
4	Me	3-Cl	O	CO-O	Me	-	590 ± 35			
5	Me	4-Br	O	CO-O	Me	> 1000	821 ± 58	−3.00	−2.91	−0.09
6	Me	4-CF_3	O	CO-O	Me	-	780 ± 68		−2.89	
7	Me	4-CN	O	CO-O	Me	-	4108 ± 352		−3.61	
8	Me	4-tBu	O	CO-O	Me	399 ± 9	275 ± 20	−2.60	−2.44	−0.16
9	Me	3,4-Cl_2	O	CO-O	Me	839 ± 48	320 ± 21	−2.92	−2.51	−0.42
10	Me	4-Ph	O	CO-O	Me	-	781 ± 65		−2.89	
11	Et	H	O	CO-O	Me	-	4187 ± 380		−3.62	
12	Et	4-F	O	CO-O	Me	-	25000.00		−3.40	
R (+) **13**	Et	4-Cl	O	CO-O	Me		703± 68		−2.85	
S (−) **13**	Et	4-Cl	O	CO-O	Me		2169± 69		−3.34	
13	Et	4-Cl	O	CO-O	Me	> 1000	434 ± 30	−3.00	−2.64	−0.36
14	Bn	4-Cl	O	CO-O	Me	-	2116 ± 180		−3.33	
15	Me	H	S	CO-O	Me	1477 ±41	317 ± 30	−3.17	−2.50	−0.67
R (+) **16**	Me	4-Cl	S	CO-O	Me		964 ± 41		−2.98	
S (−) **16**	Me	4-Cl	S	CO-O	Me	374 ± 15	404 ± 20	−2.57	−2.61	0.03
16	Me	4-Cl	S	CO-O	Me	-	553 ± 27		−2.74	
17	Me	4-Br	S	CO-O	Me	1800 ± 39	321 ± 18	−3.26	−2.51	−0.75
18	Me	4-CH_3	S	CO-O	Me	-	1104 ± 80		−3.04	
19	Et	H	S	CO-O	Me	>1000	370 ± 20	−3.00	−2.57	−0.43
R (+) **20**	Et	4-Cl	S	CO-O	Me		267 ± 18		−2.43	
S (−) **20**	Et	4-Cl	S	CO-O	Me		339 ± 31		−2.53	
20	Et	4-Cl	S	CO-O	Me	1173 ± 80	213 ± 16	−3.07	−2.33	−0.74
21	i-Pr	4-Cl	S	CO-O	Me	699 ± 13	332 ± 17	−2.84	−2.52	−0.32
22	Me	H	NH	CO-O	Me	-	>10000		−4.00	
23	Me	4-Cl	NH	CO-O	Me	>5000	>5000	−3.70	−3.70	0.00
24	Me	4-Cl	NMe	CO-O	Me	-	>5000		−3.70	
25	Et	4-Cl	NMe	CO-O	Me	-	2088 ± 160		−3.32	
26	Me	4-Cl	O	CO-O	Bn	86.2 ± 4	518 ± 30	−1.94	−2.71	0.78

Table 1. Continued.

N	R_1	R_2	X	Y	Z	K_i nM ± SE		A[a]		S[b]
						σ_1	σ_2	σ_1	σ_2	$\text{Log}[K_i(\sigma_2)/K_i(\sigma_1)]$
27	Me	4-Cl	O	CH_2O	Me	120.9 ± 6	100 ± 8	−2.08	−2.00	−0.08
28	Et	4-Cl	O	CH_2O	Me	220 ± 12	98.5 ± 8	−2.34	−1.99	−0.35
29	Me	4-Cl	S	CH_2O	Me	58 ± 3	62 ± 7	−1.76	−1.79	0.03
30	Et	4-Cl	S	CH_2O	Me	117 ± 11	273 ± 14	−2.07	−2.44	0.37
31	Me	H	O	CONH	Me	-	619 ± 45		−2.79	
32	Me	4-NO_2	-	CO-O	Me	-	1097 ± 80		−3.04	
33	Me	4-NH_2	-	CO-O	Me	-	3995 ± 238		−3.60	
34	Me	4-Cl	-	CO-O	Me	2918 ± 63	329 ±10	−3.47	−2.52	−0.95
35	Me	3,4-Cl_2	-	CO-O	Me	1554 ± 50	310 ± 8	−3.19	−2.49	−0.70
36	Me	4-Br	-	CO-O	Me	1658 ± 45	614 ± 35	−3.22	−2.79	−0.43
37	Me	4-F	-	CO-O	Me	-	701 ± 40		−2.85	
38	Me	4-iBu	-	CO-O	Me	-	419 ± 20		−2.62	
39	Me	4-OMe	-	CO-O	Me	-	>5000		−3.70	
40	Me	4-CF_3	-	CO-O	Me	-	1536 ± 120		−3.19	
41	Me	4-NMe_2	-	CO-O	Me		418 ± 231		−2.62	
42	Et	4-Br	-	CO-O	Me	-	812 ± 48		−2.91	
45	Cl	H	-	CO-O	Me	-	1039 ± 68		−3.02	
46	H	4-Br	-	CO-O	Me	-	3430 ± 207		−3.54	
47	Me	4-Br	-	CO-O	Bn	149.7 ± 9	700 ± 42	−2.18	−2.85	0.67
48	Me	4-Br	-	CH_2-O	Me	316 ± 15	68.7 ± 4	−2.50	−1.84	−0.66
ALOP						2.2 ± 0.1	16 ± 1	−0.34	−1.20	0.86
DGT						69 ± 3	21 ±1	−1.84	−1.32	−0.52

[a]A is the –log of the K_i value multiplied by 10^9; it is related to the pK_i by the following equation: $pK_i = 9 - A$; [b]S is the selectivity ratio, expressed as $S = \log[K_i(\sigma_2)/K_i(\sigma_1)]$.

non-covalent bonds [44]. GRINDs are particularly useful in 3D-QSAR studies, since they provide a suitable description of the studied compounds without requiring their superposition, although they are not invariant to the molecule conformations. GRINDs are generated by means of the Almond programme [47] which is interfaced with the GRID programme [48] for the computation of the MIFs using different probes (by default, hydrophobic (DRY), hydrogen bond (HB), acceptor (O) and HB donor (N1) probes) (Figure 1a). According to a modified Federov selection algorithm [49], the method selects a reduced subset of informative MIFs (Figure 1b) and conveniently transforms them into GRIND variables. These variables represent the product of the field energy of couples of nodes that are separated by certain distances in the 3D space of each compound (Figure 1c). GRIND variables are organized in 'correlograms', each one representing the interactions between couples of nodes of the same field (auto-correlograms) or different fields (cross-correlograms). Thus, six correlograms are obtained using the three default probes DRY (*D*), O (*O*) and N1 (*N*), namely the *DD*, *OO*, *NN* auto-correlograms and the *DO*, *DN* and *ON* cross-correlograms (Figure 1d).

In the present study the following experimental settings were used for obtaining GRINDs: DRY, O and N1 probes, 0.5 Å grid spacing, 150 nodes, 50% of field importance, smoothing window 0.8.

Dataset

The compounds forming the dataset (Table 1) were synthesized according to Refs. 41 and 42; the evaluation of their sigma receptor affinity has been reported in Ref. 43. The compounds, originally synthesised as nootropics [41, 42], were tested as sigma receptor ligands after the publication of Mach's work [50]. Since in the previous project only few compounds were solved into enantiomers, the affinity for the sigma receptors has been evaluated mainly for racemic mixtures. Therefore, in the present study the biological activity of the main part of the dataset refers to the racemates, and the biological values of the R and S forms have been used when available (five com-

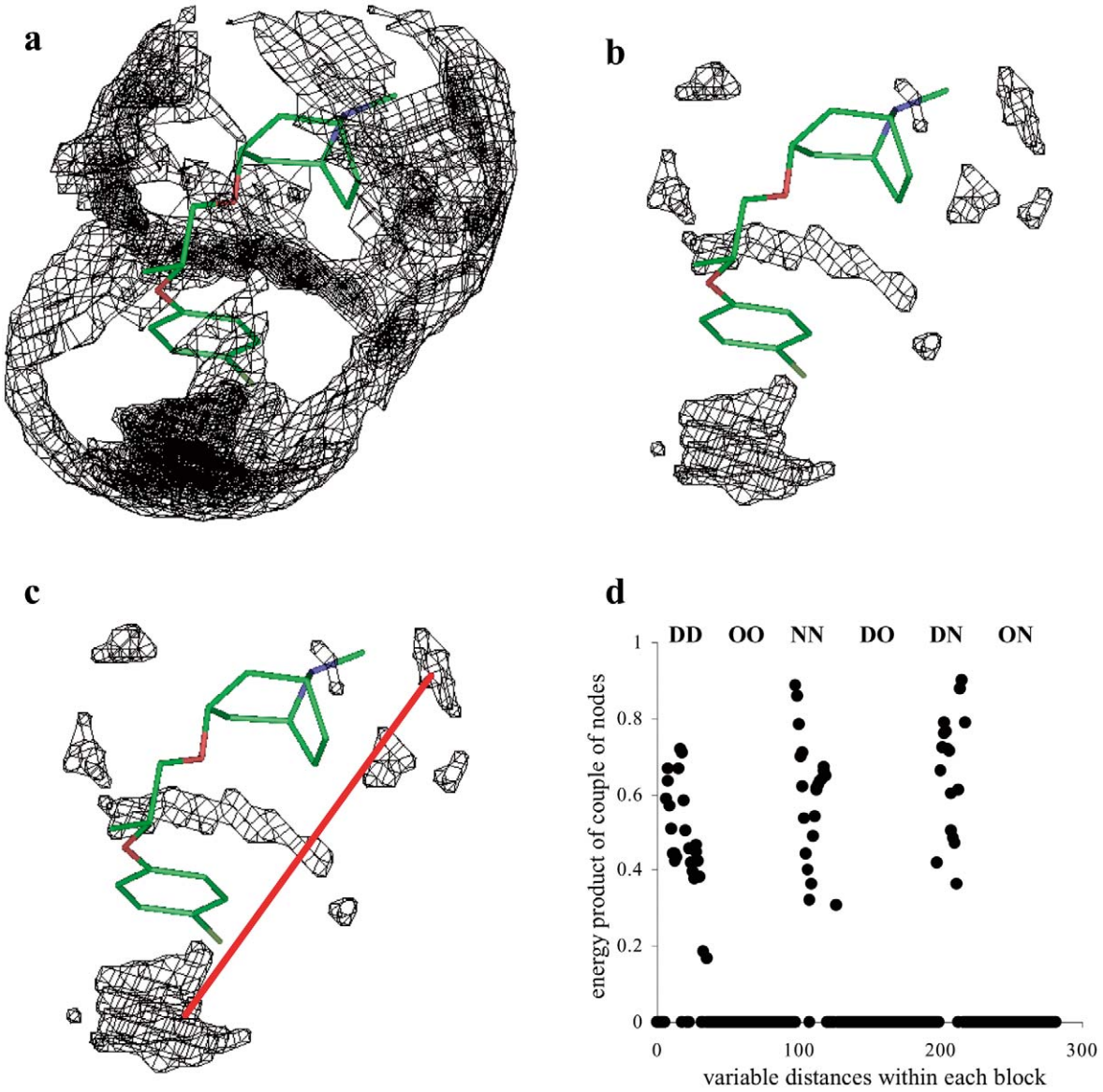


Figure 1. Computation steps of GRid INdependent Descriptors (GRINDs). For clarity only the Molecular Interaction Fields (MIFs) computed with the DRY probe for compound **27** are shown. (a) MIFs computed by GRID program; (b) extraction of a reduced set of informative nodes from the grid field according to a modified Federov's selection algorithm; (c) a couple of nodes (*i* and *j*) separated by the r*ij* distance; (d) graph representation (correlogram) of the energy products of the *i* and *j* field values, found at each different r*ij* distance (see intra).

pounds). It should be noted, however, that the studied α-tropanyl derivatives do not present a clear enantioselectivity in the interaction with sigma receptors: only compound **1** has a relevant eudismic ratio (ER = 15) that decreases in compounds **2**, **13**, **20** and inverts in compound **16**. For this reason (i.e. due to the low enantioselectivity of the studied α-tropanyl derivatives) and due to the generally slightly higher affinity of the R enantiomer, the R form was considered in the QSAR analysis for compounds whose biological activity refers to the racemate.

Results

The computation of the GRINDs was performed for the compounds in Table 1 [44]. Compounds **21**–**23**, **31**, **33**, which in the space of the PCA score (not reported) appeared separated from the rest of the dataset

molecules, probably because of the unique features of their Y or X portion (Y = CONH, X = NH, see general formula in Table 1), were not considered further.

A preliminary inspection of the *OO* autocorrelogram pointed out the marginal contribution of the *OO* interactions in discriminating the sigma affinity. Therefore the *OO* variables, that refer to receptor regions favorable for H-bond acceptor groups, were excluded and the PLS analysis, using the sigma-2 affinity values (Table 1), was performed on the resulting X matrix formed by 235 variables (columns) and 46 structures (rows). FFD variable selection [51] was applied, obtaining a four-component model sufficiently significant to attain a qualitative pharmacophore model for sigma ligands (r^2=0.79, q^2_{LOO}=0.59, q^2_{RG}=0.55). The plot of the experimental vs. calculated sigma-2 affinity in Figure 2A confirms the quality of the obtained model. The predictive ability of the model persists even when compounds **24** and **39**, whose biological activity values are truncated, are removed from the model derivation (r^2=0.77, q^2_{LOO}=0.49, q^2_{RG}=0.47, Figure 2B).

PLS model interpretation is assisted by Almond correlograms, PLS coefficient profile and interactive plots, the latter being the representation in real molecular space of both the nodes involved in GRIND variables and the distances between them.

The plot of PLS coefficients appears as a bar diagram showing the importance of a single GRIND for sigma-2 affinity: positive values of the coefficients indicate a direct correlation to sigma-2 affinity while negative values represent an inverse correlation to the response. Analysis of the PLS coefficients plot derived for the sigma-2 model shows that variables *DD* 31–37, *NN* 15–24 and *DN* 21–24 exhibit a strong inverse correlation to sigma-2 affinity, while variables *DD* 38–41 and *DN* 27–28 are directly linked to sigma-2 affinity (Figure 3A). The numbers associated to variables indicate the distance between two nodes showing favorable interactions with the respective probes and can be converted into Angstrom multiplying by 0.4, according to the two parameter values used in this study (grid spacing and smoothing window, see Materials and methods section).

NN variables refer to receptor regions favorable for H-bond donor groups; in the series of the α-tropanyl derivatives all *NN* interactions are inversely related to sigma-2 affinity and differ in their relative intensity. These interactions are less intensely produced between the tropanic N and the ether O (compounds with Y= -OCH_2) rather than between the tropanic N and the carbonylic O (compounds with Y= -O-CO), which could explain the higher sigma-2 affinity for compounds where Y= O-CH_2 with respect to those where Y= O-CO.

Table 2. Variable influence on sigma selectivity model.

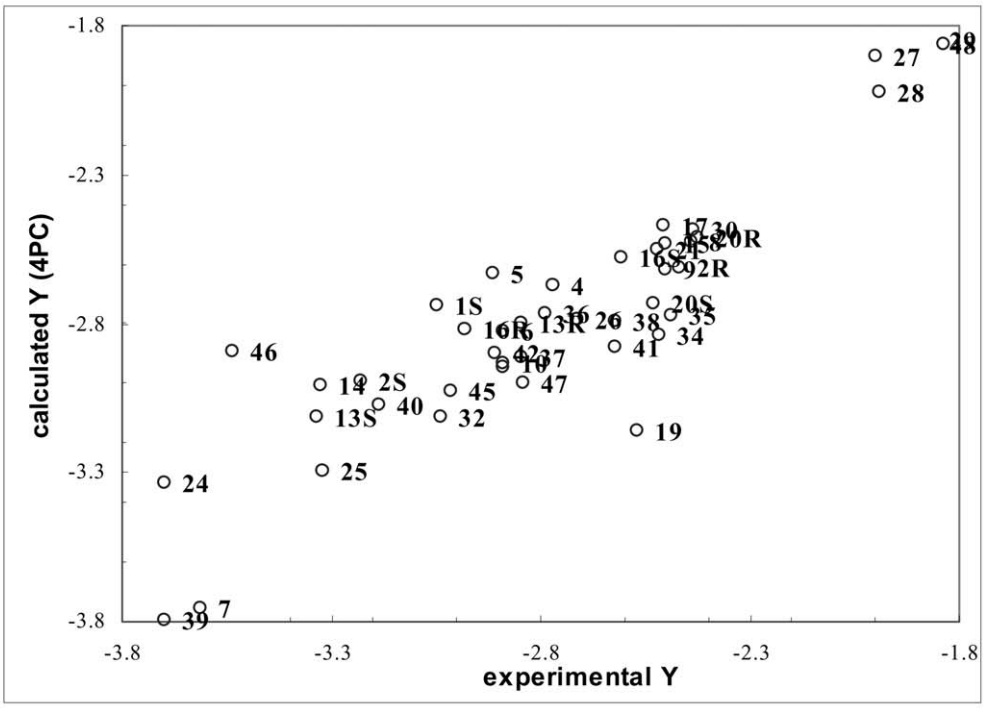
Direct impact	Inverse impact
DD 27 – 33	*DN* 29 – 34
DD 35 – 40	

DN variables refer to receptor regions favorable to H-bond donor and hydrophobic groups. As stated above, *DN* 27–28 variable codes correspond to distance interactions of about 10.8–11.2 Å between the hydrophobic region 1 interacting with the Ar moiety and the H-bond donating region interacting with the tropanic N (Figure 3B).

DD variables refer to receptor regions favorable for hydrophobic groups. These regions mainly arise from the interactions of the Ar moiety (hydrophobic-*1* region) and the tropanic ring system (hydrophobic-*2* region) with the DRY probe. Distances of 15.2–16.4 Å (variables *DD* 38–41) between the two receptor hydrophobic regions are associated to compounds with the highest sigma-2 binding affinity while distances between 13.6 and 14.8 Å (variables *DD* 34–37) are characteristic of compounds with lower affinity.

A selectivity study was undertaken considering all those compounds (23) for which both sigma-1 and sigma-2 affinity were measured, in an attempt to understand the requirements that lead to sigma-1 or sigma-2 affinity. The PLS analysis, performed on the 23 α-tropanyl derivatives, relates the selectivity values S ($\log(K_i\sigma_2/K_i\sigma_1)$ of Table 1) to 68 *DD* and *DN* variables selected via a fractional factorial design and results in a two-component model (r^2=0.72, q^2_{LOO}=0.46). Long-distance *DD* variables are directly related to the response, i.e. to compounds with higher sigma-1 selectivity (Table 2). The *DD* 37–41 (14.8–16.4 Å) variables are more intensely produced in compounds like **26** and **47** (the N-benzyl derivatives) and refer to distances between the hydrophobic-*1* and hydrophobic–*2* receptor regions. It can be seen from Table 3 and Figure 4 how greater *DD* distances (*DD* 35–40, 14–16 Å) are associated to compounds showing sigma-1 selectivity (i.e. **47**). As the distance between the hydrophobic-*1* and -*2* regions decreases,

(A)



(B)

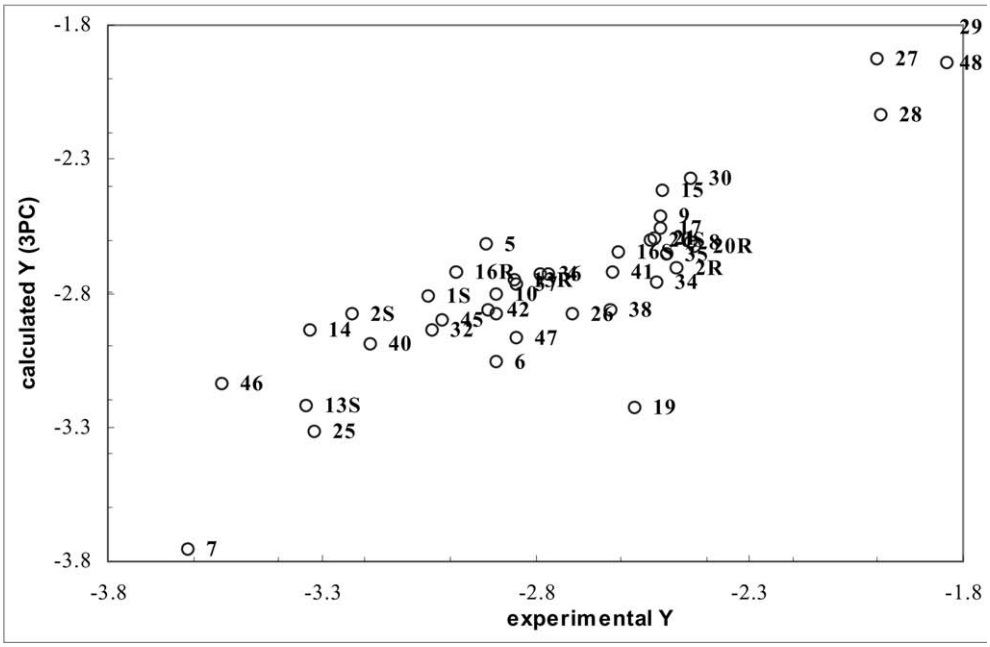


Figure 2. (A) PLS experimental vs. calculated (4LV) sigma-2 affinity values of the α-tropanyl derivatives. (B) PLS experimental vs. calculated (3LV) sigma-2 affinity values for the model derived without compounds **24** and **39**.

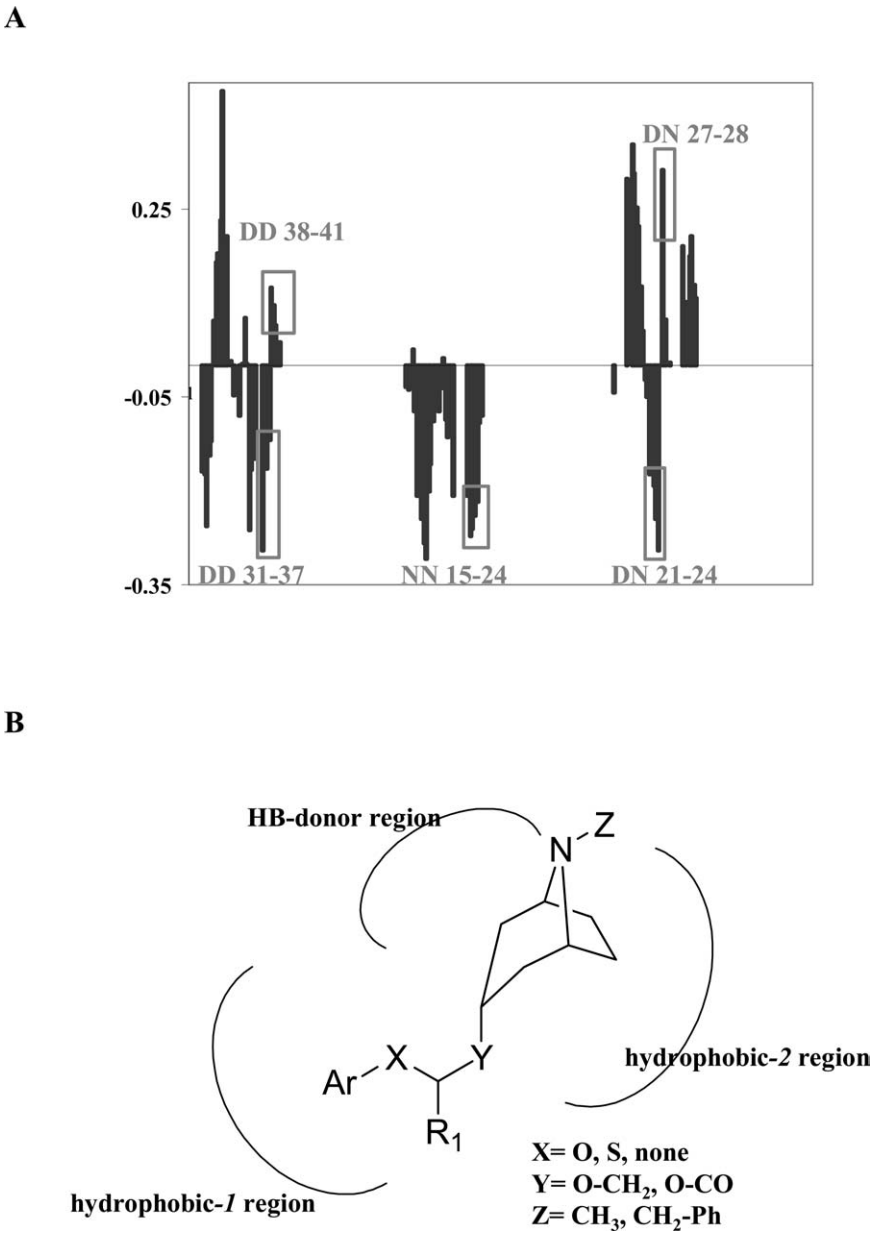


Figure 3. (A) PLS coefficient plot for the sigma-2 model. (B) Schematic sigma receptor-α-tropanyl derivatives complex.

sigma-2 affinity increases: in fact, *DD* 27–33 (10.8–13.2 Å) variables are mainly activated in compounds such as **29**, **34** and **48** which show high sigma-2 affinity but no selectivity (**29**) and/or sigma-2 selectivity (**34**, **48**).

Discussion

Over the last few years, several investigations have dealt with the definition of the structural requirements for sigma receptor binding. Studies have been addressed both qualitatively (SAR) [25, 27–30] and quantitatively (QSAR) [26, 31, 32], both on homologous series and on heterogeneous sigma ligands generally showing higher sigma-1 than sigma-2 affinity. Through structural modification of a series of

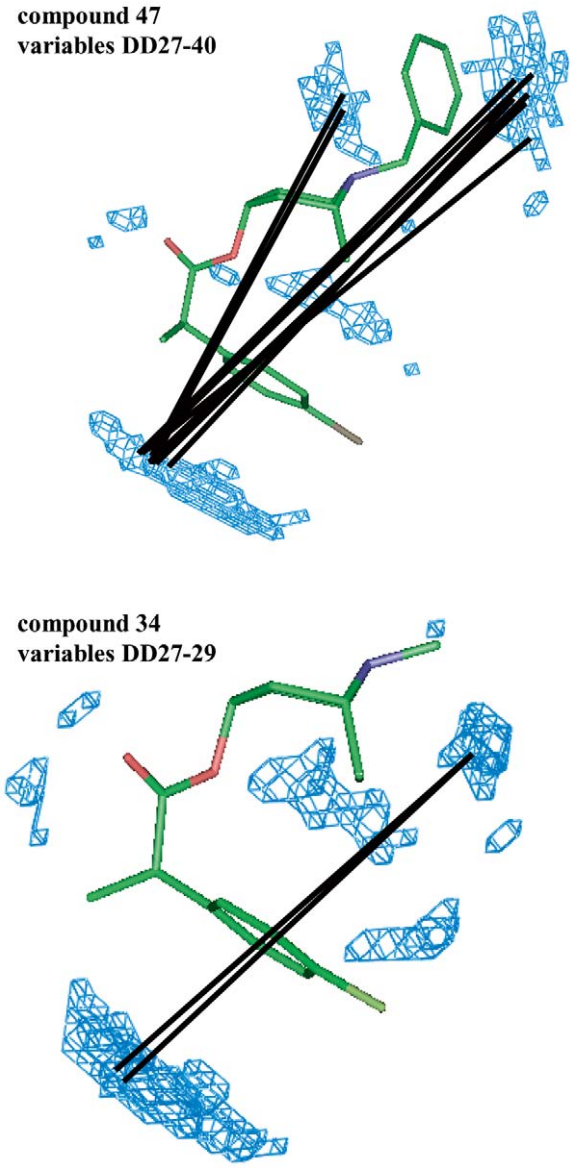


Figure 4. Representation of *DD* GRIND variables for compounds **47** and **34** in the selectivity model. The contour plots of GRID fields obtained with the DRY probe are shown in blue. The fields are filtered according to the GRINDs derivation procedure.

phenylalkylamines, focused on the spacer between the pharmacophoric groups, Glennon and coworkers [25–28] have come up with a model of interaction with the sigma-1 receptor which suggests a H-bond acceptor group and two aromatic rings, placed at certain optimal distances to each other, as important features for binding. Substitution at the aromatic rings has been studied by Huang [31, 32] through the synthesis of a series of (benzylpiperidinyl)arylacetamides, which

Table 3. Activation of *DD* 27–40 variables in some representative compounds whose sigma-2 affinity and selectivity values are also reported.

	47	3	29	48	34
DD 27–29	x	x	x	x	x
DD 33	x	x	x		
DD 35	x	x			
DD 36	x				
DD 37–40	x				
sigma-2 affinity (Ki, nM)	700	144	62	68.7	329
Selectivity	0.67	0.35	0.03	−0.66	−0.95

allowed discovery of some halogenated derivatives, potentially useful for PET (positron emission tomography) or SPECT (single photon emission computed tomography) studies of sigma-1 receptors. In most cases, the common opinion has been that the presence of a basic nitrogen atom in the ligands represents an essential pharmacophoric feature. In the case of CoMFA investigation, attention was focused on which elements were to be considered for alignment purposes, since the superposition of molecules is a critical step for compounds undergoing 3D-QSAR studies.

The scarcity of high-affinity sigma-2 selective compounds has prevented the formulation of analogous models for sigma-2 receptors, however, also in some selective sigma-2 ligands, such as siramesine [38] or CB64D [52], the pharmacophoric groups important for interacting with sigma receptors (i.e. a H-bond acceptor and two lipophilic groups) are also present.

The availability of a pharmacophoric model helps to design compounds with improved potency and selectivity, especially if they are derived using descriptors arising from 3D-level procedures. Since such models have been developed only for the sigma-1 receptor, we have now applied a 3D-QSAR analysis [44] to a series of α-tropanyl derivatives showing some selectivity for the sigma-2 receptor (Table 1). These compounds were originally tested as analgesics and nootropics, acting through the central cholinergic system [41, 42]; after the publication of Mach's work, [50], they were evaluated for sigma binding. Although not possessing high affinitiy or selectivity, they show, like few other azabicyclo derivatives [36], a preferential trend of affinity toward sigma-2 receptor. Their 3D-analysis can give useful information to find some of the requirements for interaction with the sigma-2

receptor and to design new molecules with improved potency and selectivity.

As already stated in the Materials and methods section, only few of the studied compounds have been tested as enantiomers, and therefore the choice to use for the racemates only the R enantiomer to derive the model may appear inaccurate. In this respect, two considerations should be done. At first, enantioselectivity is not always a peculiarity of sigma-2 receptor: in fact, in the literature only few chiral sigma-2 ligands are reported to bind with high to moderate enantioselectivity (for instance, pentazocine [53] and CB64D [52]) while many other chiral substances do not bind [52, 54–57].

Moreover, it should be noticed that the relevant interactions in the region of the chiral center are mainly hydrophobic and may be established by both aryl and alkyl groups on the chiral carbon. As a consequence, the distance between groups does not substantially vary when R or S enantiomers are considered. Such a consideration points out an important feature of GRINDs descriptors, i.e., they are not dependent on the chirality centers. The inspection of correlograms, i.e the ensemble of variables associated to each compound, of enantiomer pairs in Figure 5 highlights this statement: variables of R or S enantiomers almost match one to another except for the variability due to the computational parameter 'smoothing window'.

SAR studies, carried out mainly on sigma-1 ligands, highlight the presence of a H-bond acceptor group as an essential pharmacophoric element. In most cases, such an element is identified in a basic nitrogen atom [27]. However, substances are known (e.g. steroids) which bind to sigma receptors but lack a basic nitrogen atom [13]. All the molecules in Table 1 contain a basic nitrogen but some of them also show another electron-rich center (Y=O-CO) able to behave as a strong H-bond acceptor. This group, when present, may represent an alternative counterpart for the receptor H-bond donor region (*primary anchor point*).

Sigma-2 model. From the analysis of the PLS coefficients of *DN* 21–28 variables (Figure 3A), it can be hypothesized that the α-tropanyl esters (Y=O-CO) may establish interactions with the receptor H-bond donor region using either the basic tropanic N atom or the carbonylic O atom. The 10.8–11.2 Å distance, corresponding to the *DN* 27–28 variables directly linked to the response, is optimal only for molecules showing Y= O-CH_2, where the HB-acceptor group of the ligand interacting with the *primary anchor point* of the receptor is the tropanic N. In those compounds where Y= O-CO, the Ar moiety could not extend optimally to interact with the hydrophobic-*1* region, thus determining a decrease in sigma-2 affinity which is particularly evident in the series of 2-phenylalkanoic acid esters (X = none, compounds **32**–**47**); it is also likely for some of these compounds to establish interactions between the R1 chain and the Ar moiety responsible for less extended molecule conformations which prevent an optimal binding mode with the hydrophobic-*1* region.

The *DD* variables can be conveniently used to explore the features of the receptor regions on opposite sides with respect to the receptor *primary anchor point*. Distances of 15.2–16.4 Å (variables *DD* 38–41) between the two receptor hydrophobic regions are associated to compounds with the highest sigma-2 binding affinity while distances between 13.6 and 14.8 Å (variables *DD* 34–37) are characteristic of compounds with lower affinity and mainly refer to the α-tropanyl esters (Y=O-CO). It is likely that the distance between the two hydrophobic regions flanking the receptor *primary anchor point* is critical for sigma-2 affinity. In cases where the molecule binding mode involves the tropanic nitrogen as the H-bond acceptor group, few conclusions can be drawn; in fact, in our dataset the occupancy of the hydrophobic-*2* region is not sufficiently explored, since only two compounds (**47**, **26**) present an additional lipophilic group (Z=Bn). However, if the molecule binding mode involves the carbonylic oxygen as the H-bond acceptor group, the compounds possess a distance between the two hydrophobic regions not adequate for sigma-2 binding affinity. As the distance between the hydrophobic groups present in the ligands increases (15.2–16.4 Å), so does sigma-2 affinity. However, it should be considered that those compounds for which variables *DD* 38–41 (15.2–16.4 Å) are activated (Z=Bn, **47** and **26**) present a more pronounced sigma-1 than sigma-2 affinity, thus suggesting that this feature is crucial for a sigma-1 binding requirement rather than for a sigma-2 one. In fact, considerations concerning the basic structural features of the studied ligands for binding to sigma-2 receptor have to take into account the appreciable ability of most of the compounds in Table 1 to bind even to sigma-1 receptor, in most cases with comparable values of affinity.

Selectivity model. It must be noted that the selectivity of the molecules in the dataset is low; nevertheless, to try to understand the structural requirements that address selectivity, a study was carried out on the 23 α-tropanyl derivatives for which both $\sigma 1$ and $\sigma 2$

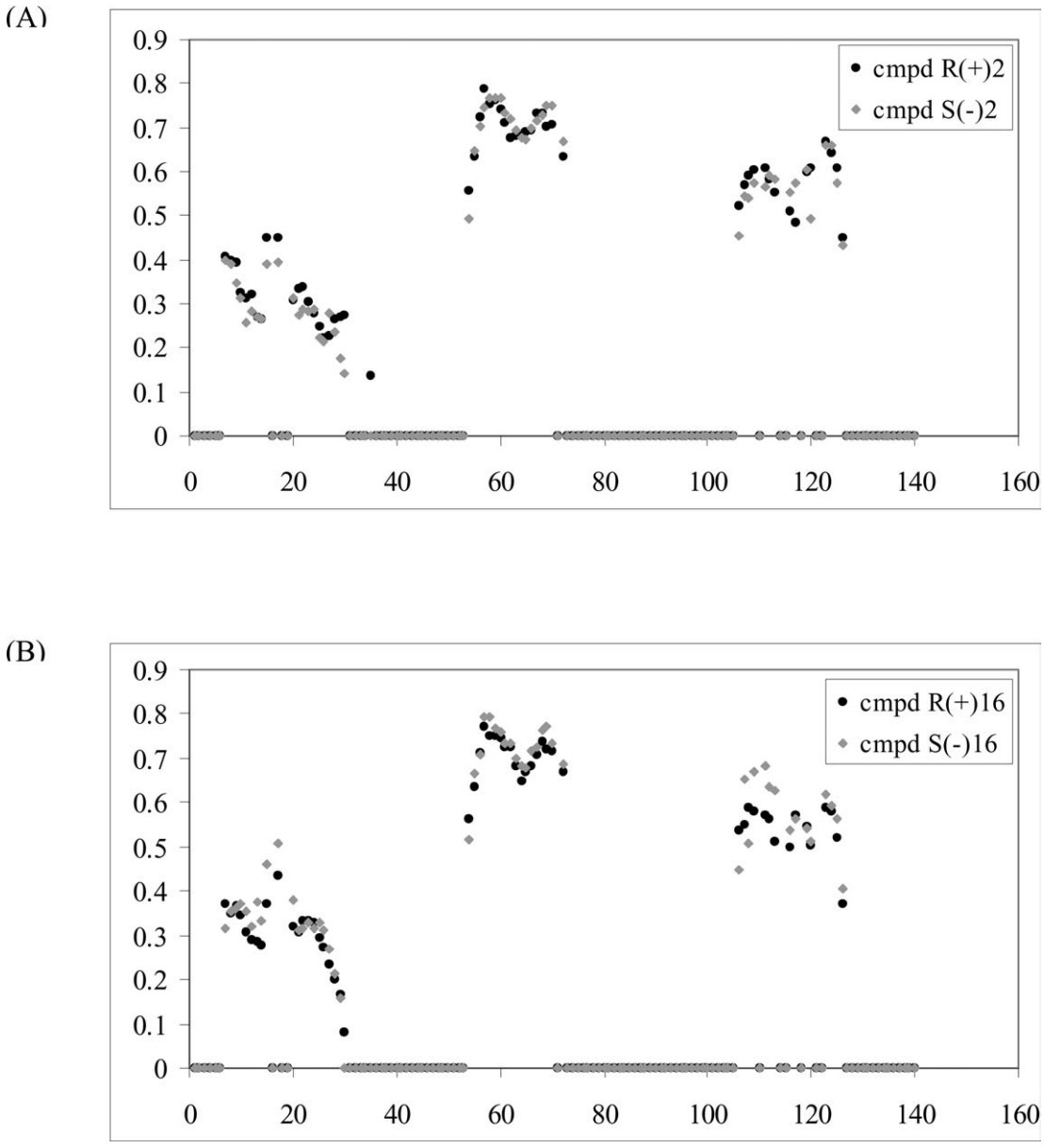


Figure 5. Correlograms of enantiomer pairs: (A) compounds R(+)**2** and S(−)**2**; (B) compounds R(+)**16** and S(−)**16**.

activity values were measured, using the selectivity ratios as dependent variable. As shown in Figure 4, distances of 14–16 Å (*DD* 35–40) are associated to compounds showing sigma-1 selectivity (i.e. compound **47**); while shorter distances (10.8–13.2 Å, *DD* 27–33) between the hydrophobic-*1* and -*2* regions are present in compounds such as **29**, **34** and **48**, which show high sigma-2 affinity but no selectivity (**29**) or sigma-2 selectivity (**34**, **48**). Sigma-2 selectivity seems to be more linked to *DN* 29–34 (11.6–13.6 Å) variables (Table 2) arising from the hydrophobic-*1* receptor region and H-bond donor receptor region interacting with the tropanic N. Thus, the property of the sigma-2 model is confirmed: an optimal interaction between the hydrophobic portion of ligands and the hydrophobic-*1* cleft of the receptor is obtained when Y= -O-CH_2- and the tropanic N acts as the H-bond acceptor group. However, some of these variables are also activated in sigma-2 selective compounds like (*R*)-**1** and **34** where the Y= -O-CO moiety is present.

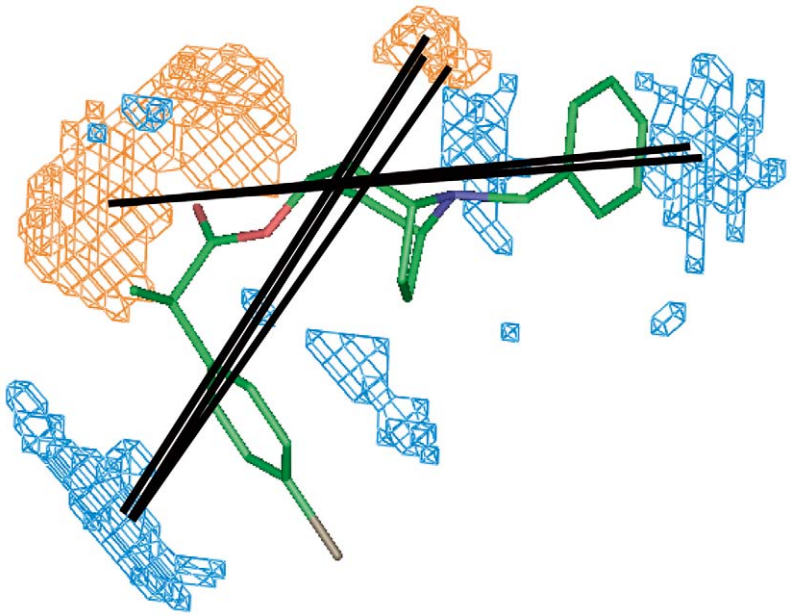


Figure 6. Representation of *DN* GRIND variables for compound **47** in the selectivity model. The contour plots of GRID fields obtained with the DRY (blue) and N1(orange) probes are shown. The fields are filtered according to the GRINDs derivation procedure.

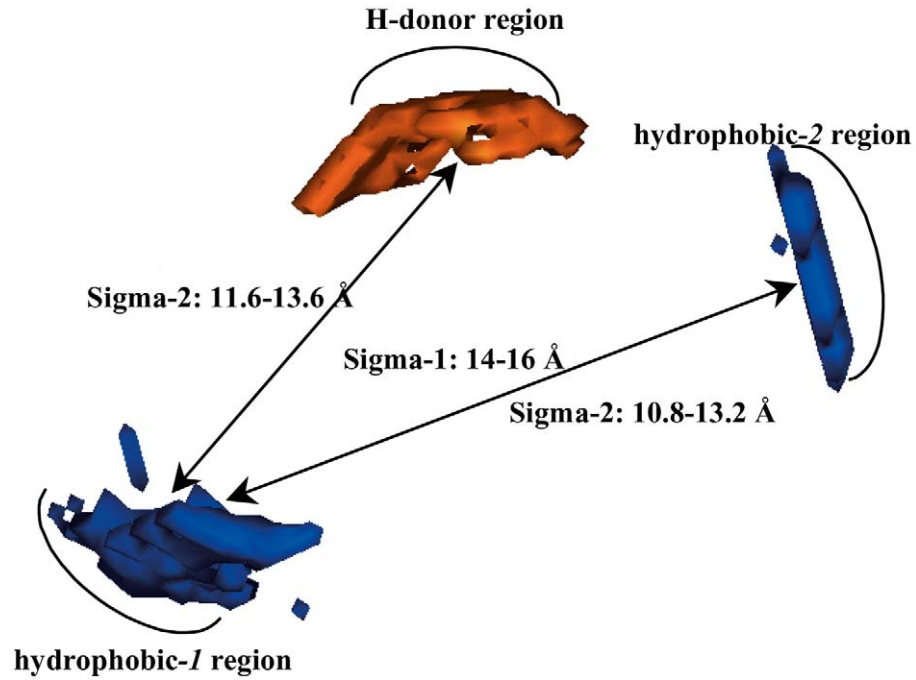


Figure 7. Proposed geometrical relationships and maps of the main interaction areas for the sigma receptor.

It is likely, owing to a closed geometry assumed by these compounds, that they perform the interaction with the receptor H-bond donor group by means of the basic nitrogen of the tropanic ring. An interesting situation arises from the inspection of the *DN* variables activated for compound **47**: the optimal *DN* distance between the receptor hydrophobic-*1* and the HB-donor regions can be reached also considering an alternative, flipped mode of binding for which the Ar moiety and the tropanic ring exchange the receptor hydrophobic areas of interaction (Figure 6). The nearly symmetrical nature of these compounds with respect to the H-bonding acceptor carbonyl group allows the Ar moiety and the tropanic ring to bind with the hydrophobic-*1* and the hydrophobic-*2* regions, respectively, and vice versa.

Combining information from *DD*, *DN* and *NN* variables, a map of the main interaction areas has been built which depicts the internal geometrical relationships of the sigma receptor binding site with which the ligands establish non-covalent bonds. The interaction site appears to be formed by two hydrophobic regions (hydrophobic-*1* and -*2*), spaced 14–16 Å from each other in the sigma-1 receptor but closer to each other (10.8–13.2 Å) in the sigma-2 one, and a H-bond donor area at about 11.6–13.6 Å from the hydrophobic-*1* region, which represents an essential anchor point for ligands which bind to the sigma-2 receptor (Figure 7). These results are consistent with the Ablordeppey et al. [26–28] model where the phenyl-A and -B regions correspond to the hydrophobic-*2* and -*1* areas, respectively.

Conclusions

The series of α-tropanyl compounds used to derive the pharmacophore model for the sigma receptor, although showing low potency or selectivity, displays a preferential trend of affinity toward the sigma-2 receptor, a peculiarity shared by only a few other sets of compounds reported in the literature. Despite the limited number of the enantiopure compounds present in the dataset (only a few racemates have been solved), the nature of the descriptors used allows us to consider one enantiomer or the other without affecting at all the computation of GRINDs, their interpretation, and therefore the model.

The analysis performed using the GRIND descriptors has confirmed the presence of two hydrophobic areas and a H-bond donor moiety in the binding site of the sigma receptor, interacting with the lipophilic groups and the electron-rich center of the molecules. The obtained PLS model nicely calculates the sigma-2 activity of the α-tropanyl derivatives involved in the study, while results from the selectivity analysis highlight the distance within the two hydrophobic areas as the major sensitive element for sigma selectivity.

Acknowledgements

The Italian funding agency of MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca) is thanked for financial support. The authors are grateful to Molecular Discovery (http://www.moldiscovery.com) for supplying the GRID and Almond software programs, free of charge, to the academic institution.

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