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Original article

GRIND-based 3D-QSAR and CoMFA to investigate topics dominated by hydrophobic interactions: The case of hERG K^+ channel blockers

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ABSTRACT

The study compares GRIND-based 3D-QSAR and CoMFA [A. Cavalli, E. Poluzzi, F. De Ponti, M. Recanatini, J. Med. Chem, 45(2002), 3844–53] to investigate a biological topic dominated by hydrophobic interactions, e.g. hERG K^+ channel blocking activity.
As expected, models are found by both methods and there is a fine agreement between statistical and graphical results as well. However, a closer inspection revealed that failures in the prediction of hERG blocking activity for lipophilic compounds were registered for both methods. The study explores the reasons for these failures which are strongly dependent on the chosen method, and gives some suggestions to handle with these topics.

1. Introduction

The human ether-à-go-go related gene (hERG) potassium channel is a key cardiac ion channel that regulates the duration of the plateau phase of the cardiac action potential. Delayed activation of hERG due to chemical blockade, or certain types of inherited dysfunction, results in increased duration of ventricular repolarization, appearing as a prolongation of the time interval between the Q and T waves (LQT) in the electrocardiogram. LQT is considered a major risk factor for torsades de pointes, a life-threatening arrhythmia [2]. Diverse types of organic compounds are believed to disrupt hERG current upon binding within the lumen of the homotetrameric pore domain. The understanding of the chemical requirements for hERG blockade is thus a topic of huge interest in drug design and requires the support of powerful molecular modelling strategies.

3D-QSAR methods are standard tools in medicinal chemistry projects and a lot of software is today available to calculate molecular descriptors and perform chemometric analysis. CoMFA (comparative molecular field analysis) methodology implemented in SYBYL package [3] and GRIND (Grid-INdependent Descriptors)-based 3D-QSAR implemented in ALMOND software [4–6] are the two of the most common and powerful tools in the field, already used to address a number of biological topics [6–10].

Recently, to deeply explore ALMOND methodology, we investigated the influence of ligand flexibility in the generation of the model [11] and the skills of GRIND-based 3D-QSAR approach to reliably predict two different biological activities for the same series of compounds [12]. An additional issue about GRIND-based 3D-QSAR concerns the degree of superposition with CoMFA methodology. To the best of our knowledge, this topic was only addressed by a paper by Menezes et al. [13] which compares the two computational tools to describe the binding mode of a set of estrogen receptor ligands. In this study, the inhibitory activities (expressed as $\log 1/IC_{50}$ and measured in MCF7 cells) calculated with the two methodologies were not in excellent agreement since CoMFA systematically overestimated experimental values, whereas the reverse was true for ALMOND predictions. Graphical results comparison was not specifically addressed by the authors, but visual inspection of reported figures showed similar profiles being key interactions in larger part of polar nature (contribution of steric field and electrostatic field: 40% and 60%, respectively).

These results suggested us to explore in more detail the degree of superposition of ALMOND and CoMFA methodologies applied to a topic for which hydrophobic interactions are largely dominant. This is a highly relevant aspect in the effort of comparing the two computational tools since they differ in at least two characteristics

Abbreviations: CoMFA, comparative molecular field analysis; GRIND, grid independent descriptors; hERG, human ether-à-go-go related gene; LV, latent variable; MIF, molecular interaction fields; PLS, partial least squares; SDEP, standard deviation of errors of prediction; VIP, variable importance in the projection.

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related to the treatment of hydrophobic interactions: (a) the entropy component is taken into consideration by the DRY probe in ALMOND but not by the steric field in CoMFA (in fact to overcome this limit it has been proposed to add a third field, i.e. the molecular lipophilicity potential, MLP [7]) and (b) the alignment of the molecules in CoMFA procedure is very often performed on the most active compound using at least one hydrophobic moiety (generally an aromatic ring). Given these differences, we were interested in understanding which approach works better when the investigated interaction is dominated by hydrophobic interactions.

In the present paper a series of hERG potassium channel blockers were thus submitted to ALMOND software and the resulting model was compared with the corresponding model (= obtained using the same series of compounds) found by a CoMFA procedure and reported in the literature some years ago [1]. Comparison was possible because the study by Cavalli et al. [1] avoids any misunderstanding about both the quality of the biological data (drugs span a potency interval as hERG K^+ channel blockers of more than 5 log units with IC_{50} values expressed exclusively in mammalian cells) and the adopted methodology. In addition, the paper furnishes a clear and complete depiction of the results enriched with a wise discussion.

Results indicate that ALMOND and CoMFA give good and comparable predictions of the hERG potassium channel blocking activity. Both methods show limits in predicting hERG blocking activity of very lipophilic molecules and CoMFA is slightly more accurate when flexible compounds are considered. Finally, a deeper and expert insight into the hydrophobic content of the ALMOND results gave more details about the structural features that are responsible for hydrophobic interactions, but how to link this finding to interpretable features remains doubtful.

Table 1
Experimental and calculated pIC_{50} (ALMOND and CoMFA [1] data), differences between experimental and calculated values and log $D^{7.0}$ produced by ADME Boxes software.

	Compound	Exp	Calc (ALMOND)	Exp–calc (ALMOND)	Exp–calc (CoMFA)	Log $D^{7.0}$
	Training set					
1	Astemizole	9.04	8.72	0.32	0.51	4.14
2	Cisapride	8.19	8.69	−0.50	0.23	2.05
3	E4031	8.11	7.94	0.17	0.26	0.34
4	Dofetilide	7.91	7.36	0.55	0.24	0.68
5	Sertindole	7.85	7.92	−0.07	−0.19	2.65
6	Pimozide	7.74	8.49	−0.75	−0.06	5.11
7	Haloperidol	7.55	7.22	0.33	−0.03	1.82
8	Droperidol	7.49	6.99	0.50	−0.33	2.25
9	Thioridazine	7.45	6.71	0.74	0.22	3.83
10	Terfenadine	6.89	7.15	−0.26	−0.33	4.11
11	Verapamil	6.84	7.09	−0.25	−0.21	2.79
12	Domperidone	6.79	6.99	−0.20	−0.09	3.15
13	Loratadine	6.76	6.26	0.50	0.93	4.94
14	Halofantrine	6.70	6.80	−0.10	−0.11	5.68
15	Mizolastine	6.45	6.20	0.25	−0.2	1.96
16	Bepridil	6.26	6.05	0.21	−0.04	4.49
17	Azimilide	6.25	6.64	−0.39	0.1	2.05
18	Mibefradil	5.84	5.31	0.53	0.09	1.96
19	Chlorpromazine	5.83	5.73	0.10	0.15	2.99
20	Imipramine	5.47	6.02	−0.55	−0.51	2.25
21	Granisetron	5.42	5.32	0.10	−0.22	−1.72
22	Dolasetron	5.22	5.34	−0.12	0.23	2.6
23	Perhexiline	5.11	5.48	−0.37	−0.08	2.81
24	Amitriptyline	5.00	5.18	−0.18	−0.66	3.33
25	Diltiazem	4.76	4.41	0.35	−0.26	1.62
26	Sparfloxacin	4.58	4.28	0.30	0.19	−3.4
27	Glibenclamide	4.13	4.50	−0.37	0.06	2.23
28	Grepafloxacin	4.11	4.19	−0.08	−0.24	−1.47
29	Sildenafil	4.00	4.28	−0.28	0.5	2.29
30	Moxifloxacina	3.93	4.18	−0.25	0.11	−2.9
31	Gatifloxacine	3.89	4.10	−0.21	−0.27	−2.96
	Test set					
32	Norastemizole	7.55	6.19	1.36	0.83	0.16
33	Ziprasidone	6.82	7.69	−0.87	−0.1	3.93
34	Risperidone	6.79	6.60	0.19	−0.2	0.71
35	Clozapine	6.72	6.24	0.48	0.54	4.41
36	Cocaine	5.24	4.53	0.71	−0.17	0.41
37	Fexofenadine	4.67	5.90	−1.23	−0.66	1.85

Table 2
Summary of the statistical parameters for the ALMOND model.

	ALMOND	CoMFA [1]
LV	3	3
q^2_{LOO}	0.69	0.77
r^2	0.93	0.95
a[a]	1.00 (±0.05)	N/A
b[a]	0.00 (±0.31)	N/A

N/A, not available.
[a] Coefficients of the relationship between experimental and calculated values calculated from the equation: $pIC_{50}^{exp} = a\ pIC_{50}^{calc} + b$; 95% confidence limits are given in bracket.

2. Methodology

2.1. Data set preparation

The list of compounds to be included in the data set was taken from the paper of Cavalli et al. [1] (chemical structures are available in Supporting information). The original separation into training (31 compounds) and test (6 compounds) sets was also maintained.

The ADME Boxes software (version 2.5, Pharma Algorithm, http://pharma-algorithms.com/) was used to estimate pK_as (Supporting information) and log $D^{7.0}$ (Table 1) and obtain SMILES codes [14], except for **3** whose SMILES was manually built. All compounds bear a positive charge except for **13** and **27** which are in the neutral form and **26**, **28**, **30**, **31** and **37** which are in the zwitterionic form.

The 37 SMILES strings were submitted to Omega (version 2.1.0, OpenEye Scientific software, http://www.eyesopen.com/) as described in detail elsewhere [11]. The lowest energy conformers were selected and checked with MOE (version 2006.08, Chemical Computing Group, Inc., http://www.chemcomp.com/).

2.2. ALMOND

Briefly, the ALMOND methodology involves three steps [4–6]: computing a set of molecular interaction fields (MIFs) for

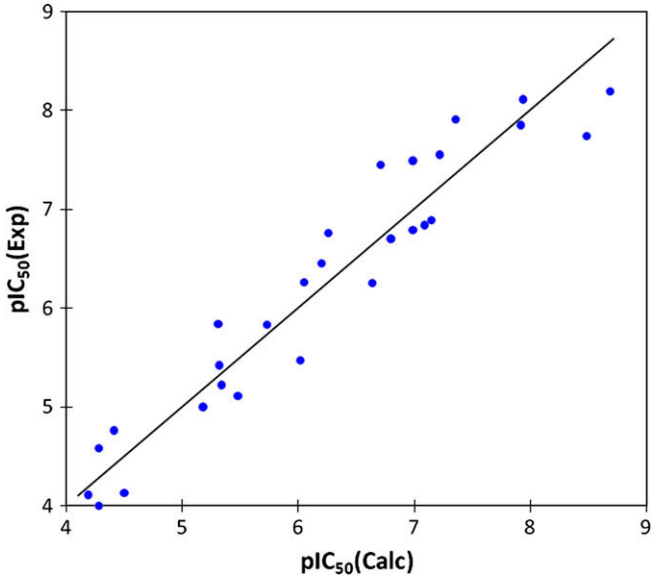

Fig. 1. The linear relationship between the experimental pIC_{50} taken from the paper of Cavalli et al. [1] and the corresponding data calculated by ALMOND.

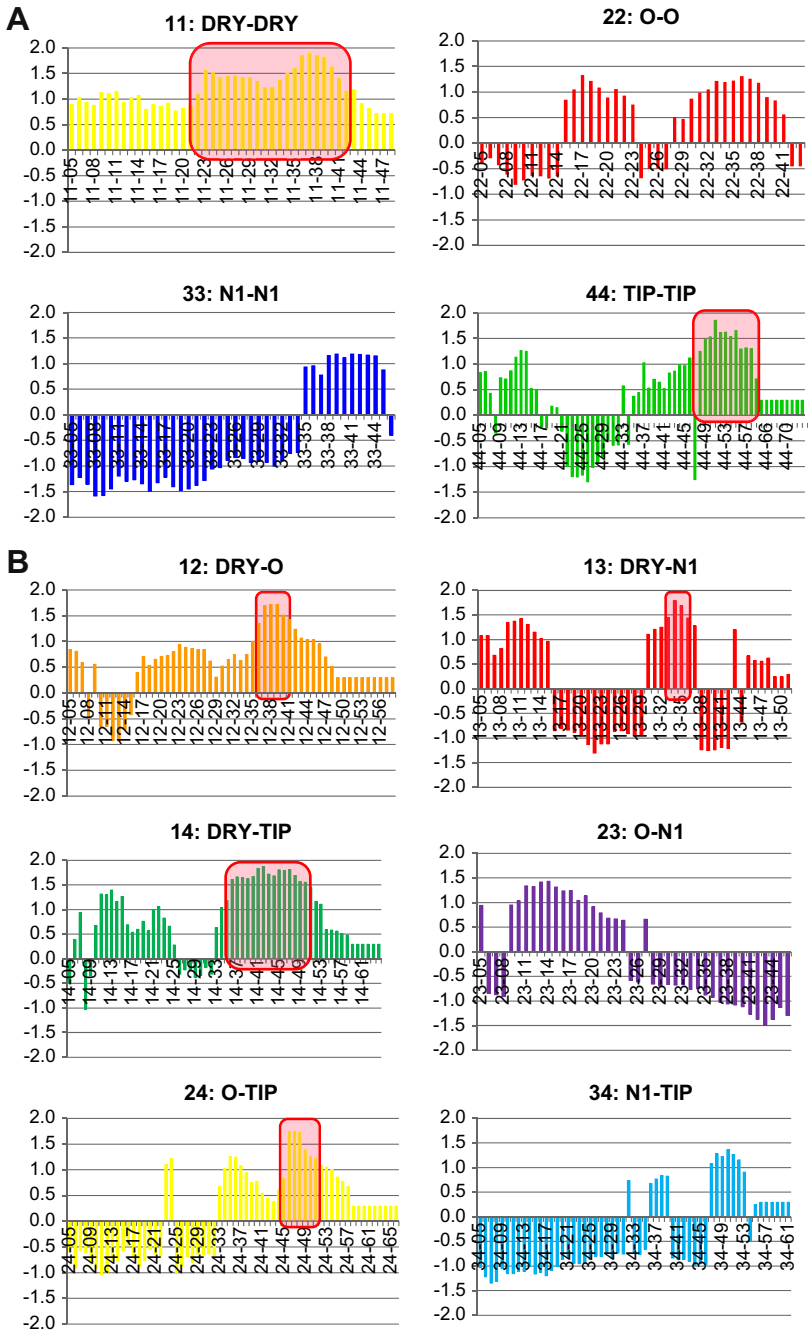

Fig. 2. Plots for ALMOND PLS model (VIPs are represented on the positive or on the negative axis on the basis of the corresponding PLS coefficient sign. See text for details). (A) VIPs corresponding to the four ALMOND auto-correlograms: 11 (DRY–DRY), 22 (O–O), 33 (N1–N1) and 44 (TIP–TIP). (B) VIPs corresponding to the six ALMOND cross-correlograms: 12 (DRY–O), 13 (DRY–N1), 14 (DRY–TIP), 23 (O–N1), 24 (O–TIP) and 34 (N1–TIP).

molecules in the data set, filtering the MIFs to extract the most relevant regions, and encoding the filtered MIFs (also called final nodes where a node represents a favourable probe–target molecule interaction region) into GRIND variables. This procedure works on the final nodes and computes the product of the interaction energy for each pair of nodes. The products are then ranked according to the distance between the nodes. Distances are grouped in a discrete number of categories and in each category, only the product with the highest value is stored and represents one GRIND variable. GRIND variables are then grouped into blocks (= correlogram) representing distances between the pairs of nodes. Finally, the GRIND variables constitute a matrix of descriptors that can be analysed using multivariate techniques (see below).

The training set of 31 compounds was used as an input for ALMOND (version 3.2, Molecular Discovery Ltd., http://www.moldiscovery.com). Default probes (DRY, O, N1 and TIP) were used: the DRY probe represents hydrophobic interaction, O is an sp^2 carbonyl oxygen and has hydrogen-bond acceptor (HBA) properties, N1 is a neutral flat NH and has hydrogen-bond donor (HBD) properties, and the TIP probe is a molecular shape descriptor. Default values were used for all ALMOND parameters.

2.3. Chemometric analysis

Chemometric analysis was carried out using the statistical tools included in ALMOND together with the SIMCA-P statistical package (SIMCA-P, version 10.0.2.0, Umetrics, http://www.umetrics.com/).

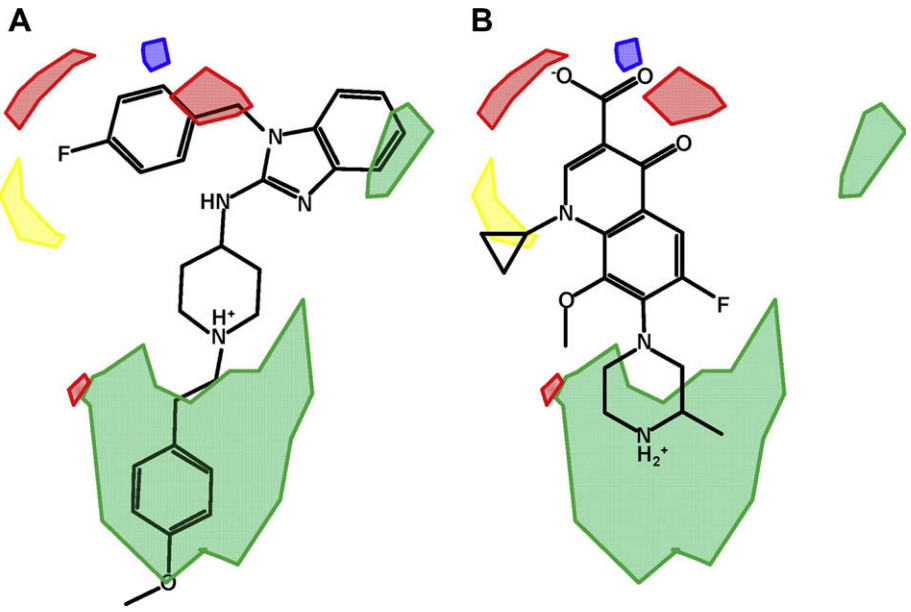

Fig. 3. Schematic representation of CoMFA results extracted from the paper of Cavalli et al. [1]: favourable steric regions are green, the yellow contour indicates that increasing bulk is detrimental for the activity, while the red and blue contours indicate a prevalent effect of positively and negatively charged groups, respectively. (A) The most potent compound, astemizole (**1**). (B) The least potent compound, gatifloxacin (**31**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The GRIND descriptors calculated by ALMOND were then related to hERG K^+ channel blocking activity of compounds (Table 1) [1] by means of partial least squares (PLS) analysis. The GRIND descriptors (used without scaling) were the *X* variables, whereas the corresponding inhibitor potencies were the *Y* variables.

The optimum number of PLS components (latent variables, LVs) was chosen by monitoring the changes in the model's predictivity index (q^2_{LOO}, leave one out) evaluated by applying the cross-validation procedure available in ALMOND.

To interpret the statistical model correctly, the variable importance in the projection (VIP) plot was combined with the PLS coefficient table [15].

All calculations were performed on a Linux-based server and on standard PCs operating with Microsoft Windows Vista.

3. Results and discussion

3.1. The ALMOND model

Since in a previous paper [12] we demonstrated that the best ALMOND model is obtained by the use of a data set of the lowest energy conformers generated by a conformational analysis, in this study, GRIND descriptors [4] were calculated for the 31 compounds belonging to the training set generated by Omega (see Section 2) and related to hERG K^+ channel blocking activity of compounds, expressed as pIC_{50}, by means of PLS analysis. A three-LV model was obtained (Table 2): the model shows an r^2 of 0.93 and a q^2_{LOO} of 0.69, with SDEP = 0.37. Table 2 also shows the coefficients (i.e. the slope, *a*, and the *Y*-intercept, *b*) of the linear relationship between the

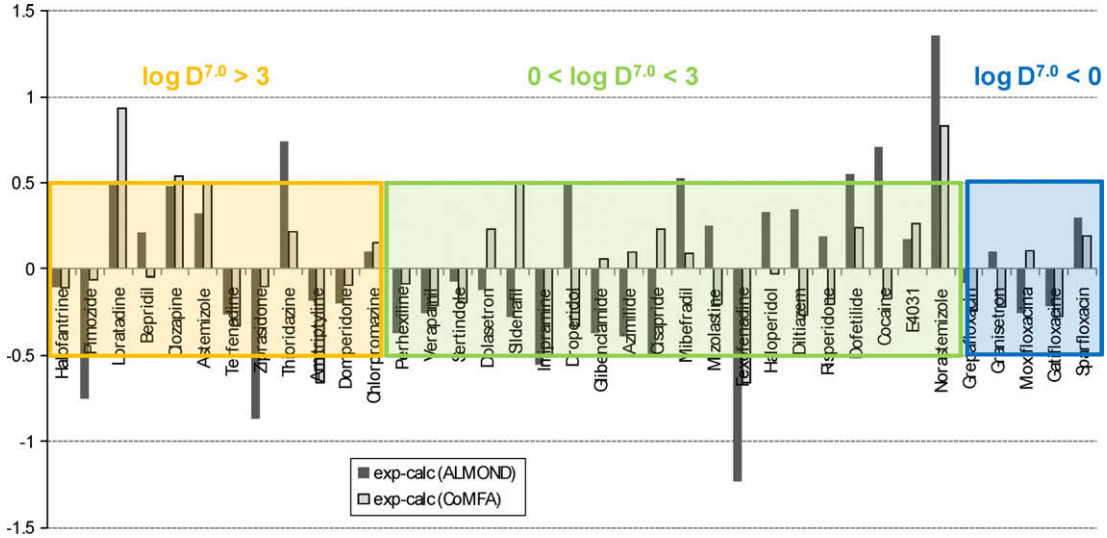

Fig. 4. Differences between experimental (exp) and calculated (calc) values: ALMOND data are in dark grey and CoMFA data are in light grey. Compounds are ordered by their log $D^{7.0}$ value: the most hydrophilic (sparfloxacin) is on the right side, whereas the most lipophilic (halofantrine) is on the left side. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

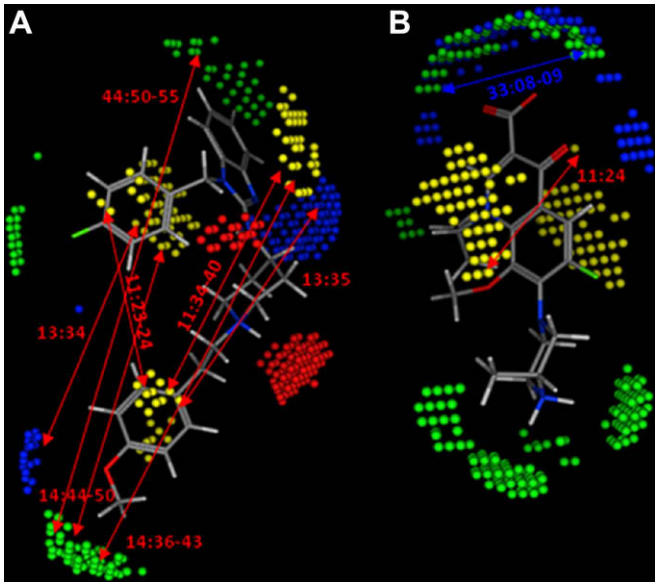

Fig. 5. Filtered MIFs obtained with DRY (yellow), O (red), N1 (blue) and TIP (green) probes and the distance ranges corresponding to GRIND with VIPs value greater than 1.5. (A) The most potent compound, astemizole (**1**), GRIND 24:46-48, 12:37-40 and 33:08-09 are absent in the molecule. (B) The least potent compound, gatifloxacin (**31**), GRIND 11:25-40, 12:37-40, 13:34-35, 14:36-50, 24:46-48 and 44:50-55 are absent in the molecule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

experimental and the calculated values shown in Fig. 1. Values close to 1 for the slope and close to 0 for the *Y*-intercept also support the good quality of all predictions. The test set prediction (Table 1) was also acceptable (SDEP = 0.90). A poor prediction of hERG K^+ blocker activity (= difference between experimental value and calculated value significantly larger than 0.5 (absolute value)) was found for **6**, **9**, **32**, **33**, **36** and **37**. Conformational reasons are expected to be responsible for the behavior of **6**, **9** and **33**. By changing the stereochemistry of the protonated nitrogen, cocaine prediction (4.95) showed a better agreement with the experimental value (4.53). To understand the reasons of the failure in the prediction of **37**, a standard molecular dynamics simulation in solvent was performed (data not shown) and interestingly the conformation with lowest energy and forming an intramolecular HB (see Fig. 6A) showed a better agreement (4.86) with the experimental data (4.67). Finally, we were unable to give an explanation as to norastemizole anomalous behavior.

In general, an ALMOND model gives graphical as well as statistical results. By having in mind that for each GRIND descriptor (a) the VIP value reflects the importance of terms in the model with respect to both *Y* and to *X* and (b) the PLS coefficient represents the contribution to the model only with respect to *Y*, we prepared a series of histograms (Fig. 2A and B) in which each GRIND descriptor was represented with a vertical bar whose height is the VIP value and whose sign corresponds to the sign of the PLS coefficient (with positive values that increase the hERG K^+ blocker activity of the compound and vice versa). Vertical bars with high absolute value (>1.5, cutoff arbitrary chosen after various assays) correspond to GRIND that largely govern the model.

Fig. 2A shows the four auto-correlograms. Among them DRY–DRY and TIP–TIP are the most relevant since both show many vertical bars > 1.5, all of them with a positive sign. Moreover, these bars are located on the right side of the plot that for ALMOND means large distances between GRIND nodes. Fig. 2B shows the six cross-correlograms. As discussed above, the most important for the chemical interpretation of the ALMOND model are DRY–TIP, O–TIP, DRY–O and DRY–N1. The DRY–TIP correlogram confirms the analysis of DRY–DRY and TIP–TIP auto-correlograms. A careful inspection of cross-correlograms due to the O and N1 probes (O–TIP and DRY–O and DRY–N1) evidences that only a limited number of distances between hydrophobic moieties and polar groups are relevant for the model.

Taken together ALMOND graphical results suggest that (a) large molecules with peripheral aryl moieties are more preferably bound by the hERG K^+ channel than small polar structures, (b) the presence of an HBD group (not related to the positive basic nitrogen but rather to the second nitrogen present in the molecule) far from one aromatic moiety is important for activity, (c) the presence of an HBA group far from aryl moieties increases biological potency and (d) approximately, the distances between the HBD and the HBA groups with the distal aromatic moiety should be similar.

Finally, it must be mentioned that in 2005 Cianchetta et al. [16] published predictive models for hERG potassium channel blockers using GRIND descriptors. Since the study was performed on a larger data set (hundreds of compounds, some of them with unknown chemical structure), results are difficult to compare with those reported here.

3.2. The CoMFA model by Cavalli et al. [1]

After a careful alignment of the investigated structures using a hypothetical pharmacophore built with a constructionist approach, the CoMFA strategy produced a number of excellent statistical results most of which are reported in Table 2. Poor predictions of pIC_{50} were obtained for **13**, **24**, **32** and **37**. Moreover, calculations revealed that the steric field contribution was more than two times larger than the electrostatic field contribution. This was clearly outlined by the analysis of the contour maps (= graphical results schematically reported in Fig. 3) which showed (a) the presence of a large sterically favourable region around an aromatic moiety of the pharmacophore, (b) a small favourable steric contour around a second aromatic moiety of the pharmacophore and (c) a region of polar interactions and forbidden bulk hindrance around the third aromatic moiety of the pharmacophore.

3.3. Comparing ALMOND and CoMFA results

Statistical results obtained with the two methods are of comparable quality as shown in Table 2. Interestingly, both models fail in predicting biological potency for norastemizole (underestimated) and fexofenadine (overestimated). The occurrence of more complex factors (e.g. binding to hERG K^+ channels in different states, and/or in different domains of the protein) is expected to occur for norastemizole, whereas conformational reasons explain the failure in the prediction of **37** (see above).

In general terms, ALMOND predictions are less accurate than CoMFA. In Fig. 4 we compared the differences between experimental and calculated values of CoMFA results (in light grey) and the corresponding values registered with ALMOND method (in dark grey). In Fig. 4 compounds are ordered by their log $D^{7.0}$ value (Table 1): the most hydrophilic (sparfloxacin) is on the right side, whereas the most lipophilic (halofantrine) is on the left side. Apart from norastemizole and fexofenadine, Fig. 4 shows that both methods give better predictions for hydrophilic rather than lipophilic compounds.

Graphical results obtained with the two methods are also in agreement. Fig. 5 illustrates ALMOND graphical output for compounds **1** (Fig. 5A) and **31** (Fig. 5B) endowed with the highest and the lowest hERG K^+ channel blocking activities, respectively. The distance range corresponding to GRIND (see Section 2) with VIP values above 1.5 (= the most relevant distances, as discussed in detail elsewhere [12]) are shown in red when the corresponding PLS coefficient is positive and in blue when it is negative

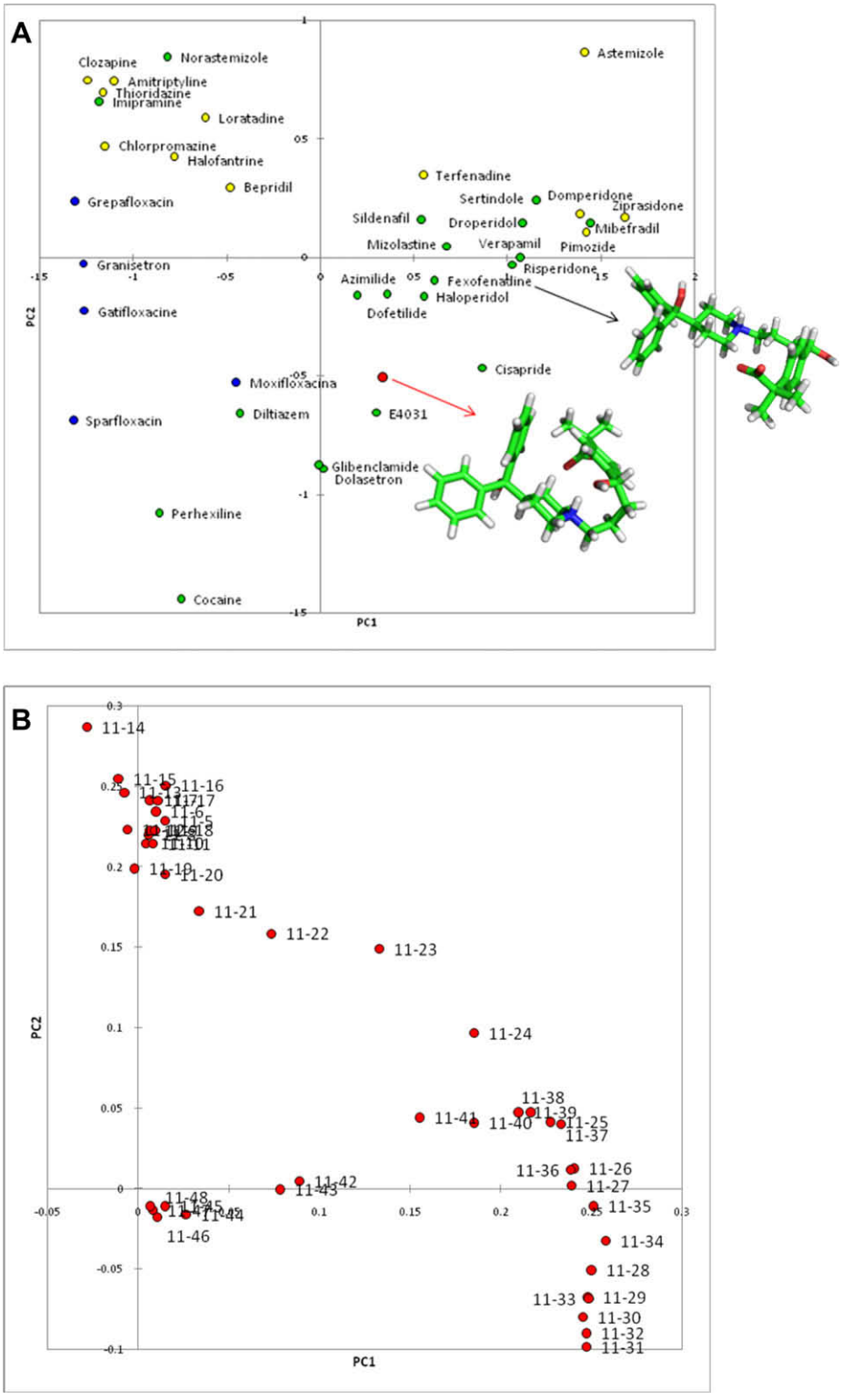

Fig. 6. PCA plots (GRIND DRY–DRY, see text for details). (A) Scores plot. The dots colour code is as follows: yellow = $\log D^{7.0} > 3$; green = $0 < \log D^{7.0} < 3$; blue = $\log D^{7.0} < 0$. Two conformations of fexofenadine are shown: the original built with Omega (black arrow) and the one resulting from a molecular dynamics simulation in aqueous solvent (red arrow). (B) Loadings plot. The labels significance is the same as in Fig. 2A: 11 refers to DRY–DRY whereas the second number is related to the distance between nodes, the highest the most distant.

(colour codes: DRY (yellow), O (red), N1 (blue) and TIP (green)). Fig. 5 was reported to enable comparison with Fig. 3 in which contour maps of the two same compounds are shown. Fig. 5 indicates for astemizole (the most active compound) a high number of favourable ALMOND distances, most of which are rather long and related to aromatic moieties. This is in line with CoMFA contour maps that evidence that aromatic portions of astemizole fit favourable steric regions. On the other hand, gatifloxacin (the least potent compound) shows only one favourable (in red) ALMOND distance and one defavourable (in blue) which is around the

carboxylate region. Interestingly, in the CoMFA graph gatifloxacin does not make any contact with favourable steric regions and its carboxylate function falls close to a region where a positive charge is required.

Since one of the ALMOND's most powerful feature is the treatment of the hydrophobic interactions (see Section 1), a PCA run of the 37 compounds using only DRY–DRY GRIND was performed. The first two components explain 52% and 22% of the total variance, respectively. Fig. 6A and B shows the PCA scores and loading plots, respectively. Fig. 6A indicates that PC1 highly discriminates the activity of molecules since compounds with the highest hERG blocking activities are located on the right of the scores' plots, whereas the reverse is true for weaker hERG blockers. According to the loadings plot, this location indicates that the most active compounds share the presence of two or more hydrophobic regions separated by a large distance.

This structural feature is not caught by the CoMFA procedure (Fig. 3) and evidently cannot be extracted by the analysis of a bulk descriptor as $\log D^{7.0}$ (many compounds with high $\log D^{7.0}$ and lower hERG blocking activity are located on the left side of the plot).

4. Conclusions

ALMOND and CoMFA are the two widely used 3D-QSAR methods. Some studies in the literature (many by people related to the companies releasing the software) describe their application to a number of biological topics and list their advantages and drawbacks. Since ALMOND and CoMFA strongly differ in the treatment of hydrophobicity we were interested in performing a close comparison of the two computational tools when the investigated biological interaction is dominated by hydrophobic forces. We thus undertook this study which compares ALMOND and CoMFA in the prediction and interpretation of hERG K^+ channel blocking activity. To do that we performed ALMOND calculations and compared the results with those obtained by Cavalli et al. using the CoMFA procedure [1] on the same data set.

Results indicate that both the methods give good and comparable activity predictions and reasonable identification of the main molecular moieties responsible for the interactions. Nevertheless both of them show limits in predicting hERG blocking activity for lipophilic compounds as unfortunately most drug candidates are. The study highlights how these limits could be ascribed to different reasons. The second hydrophobic region caught by ALMOND as crucial for the interaction with the hERG receptor (see Section 3) but not by CoMFA, is probably due to the poor treatment of hydrophobic interactions of the latter method which accounts for only enthalpic contribution. Conversely, since highly lipophilic compounds often share high flexibility, the absence of the alignment renders ALMOND procedure more dependent on the choice of the starting conformations and explains the larger differences between experimental and predicted values and thus the higher number of outliers.

Taken together, these findings therefore suggest that the best usage of 3D-QSAR tools results from the combination of the two methods but unfortunately the accessibility of this solution is often strongly limited by the commercial nature of many packages and the amount of time required. We thus suggest before performing a 3D-QSAR study to run a preliminary alert procedure based on the identification of (a) the main features of the interaction (polar or hydrophobic) and (b) the lipophilicity and flexibility of compounds (e.g. by using simple descriptors such as $\log D$ and the number of rotatable bonds). Moreover, if ALMOND is the chosen procedure and the alert procedure suggests that hydrophobicity is highly relevant in the interaction, a close inspection of the information due to DRY probe must be performed in deep details. On the other side, if CoMFA method is adopted, caution should be used in designing new compounds based on CoMFA contour maps since some crucial region of interaction could not be present on the maps.

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Appendix. Supporting information

Supplementary data associated with this article can be found in the online version, at 10.1016/j.ejmech.2008.11.009.

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