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Docking studies on PARP-1 inhibitors: insights into the role of a binding pocket water molecule

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Abstract—The binding mode of a series of competitive PARP-1 inhibitors was investigated employing a molecular docking approach by using Autodock 3.0. A particular attention was given to the role played by a water molecule present in some but not all the so far available crystal structures of the catalytic domain of PARP-1. Good correlation between calculated binding energies and experimental inhibitory activities was obtained either by including ($r^2 = 0.87$) or not ($r^2 = 0.84$) the structural water molecule. Closer inspection of our results suggested that this water molecule should be considered part of the hydration shell of polar inhibitors and not as a structural water.

1. Introduction

Poly(ADP-ribosyl)ation is a transient post-translational modification which takes place in eukariotes in response to exposure to DNA-damaging agents, and is involved in fundamental processes related to the preservation of genomic integrity such as DNA repair, chromatin decondensation, and, under certain circumstances, cell necrosis, and apoptosis.[1] Poly(ADP-ribose)polymerase-1 (PARP-1) is the founding member of a still growing family of poly(ADP-ribosyl)ating enzymes, which counts, up to now, at least 18 gene products.[2] PARP-1 uses NAD^+ as a substrate and transfers multiple units of ADP-ribose to acceptor proteins, such as histones, topoisomerases, caspases, and PARP itself. A huge amount of experimental evidences have pointed out PARP-1 as a key player in a variety of pathophysiological processes, and a number of potential therapeutic applications have been proposed and pursued in anticancer therapy,[3] myocardium,[4] and brain ischemia,[5] inflammation,[6] diabetes.[7] While genetic approaches have indicated the viability of mice lacking PARP-1[8] and have thus opened the way to the potential clinical use of PARP-1 inhibitors, there is now a mounting concern about the selectivity of currently used PARP-1 inhibitors, as the interference with other members of the PARP family may lead to severe impairments.[9]

The issue of the selectivity appears to be particularly relevant in light of the very high conservation of the catalytical domain of the so far molecularly characterized

Table 1. Available crystal structures of the catalytic domain (CD) of PARP-1

PDB code[a]	Inhibitor[b]	Water[c]	RMSD[d]	Ref.
2paw	—	—	0.00	11
1pax	PD128763 (**10**)	W65	0.27	12
2pax	4AN (**18**)	—	0.25	11
3pax	3MB (**6**)	W61	0.23	11
4pax	NU1025 (**14**)	—	0.24	11
1uk0	FR257517 (**22**)	W15	0.94	13
1efy	NU1098 (**23**)	W52	0.45	14
1a26	carba-NAD (**24**)	W37	0.33	15
1gs0	—	—	0.96	16

Seven of the nine structures were co-crystallized with competitive inhibitors.

[a] PDB codes of the crystallized CD of PARP-1 and PARP-2 (1gs0).

[b] Seven of the nine structures were co-crystallized with competitive inhibitors. For numbering, see Chart 1.

[c] Water molecules present in the binding pocket and in contact with the inhibitor. The numbering of the water molecule is different as it refers to the original PDB files, but all the listed water molecules occupy the same position in the crystal structures.

[d] RMS deviation of the backbone atoms of the proteins calculated over all the 317 of the CD and relative to 2paw, taken as a reference template.

Keywords: PARP; Docking; Structural water; Desolvation energy.
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PARP enzymes. In the frame of a project aimed at setting up computational approaches for the characterization of novel PARP inhibitors, we have previously generated a predictive QSAR model for a large series of PARP-1 inhibitors.[10] Although efficient in terms of computational speed and reliability of results, this ligand-based approach proved to be ineffective when the issue of selectivity is concerned, given the substantial lack of data on inhibitors acting at PARP enzymes different than PARP-1. Thus, we decided to implement a structure-based screening approach based on automatic docking studies on the available X-ray structure of PARP-1 and PARP-2. In this context, we immediately faced the problem of how to treat the water molecules present in the binding pocket of the above structures. Indeed, survey of the available crystal structures of the catalytic domain of PARP-1 in its apo form or complexed with structurally diverse inhibitors revealed the presence of water molecules in the binding pocket in five of the nine so far reported structures (Table 1).

The inclusion or not of these water molecules may have a substantial impact on the results of automated docking procedures, and we therefore decided to investigate in detail the role of the binding pocket water molecules in the case of PARP-1 inhibitors. Docking experiments were carried out on compound **1**–**21**, reported in Chart 1 and Table 2, for which congruent activity data are available. Compound **22**–**24** have been crystallized in the active site of PARP-1 but no homogeneous biological data are available.

To select the protein structure to be used in the docking simulation, all the structures listed in Table 1 were retrieved from the RCSB Protein Data Bank[26] and superimposed. An average root mean square deviation

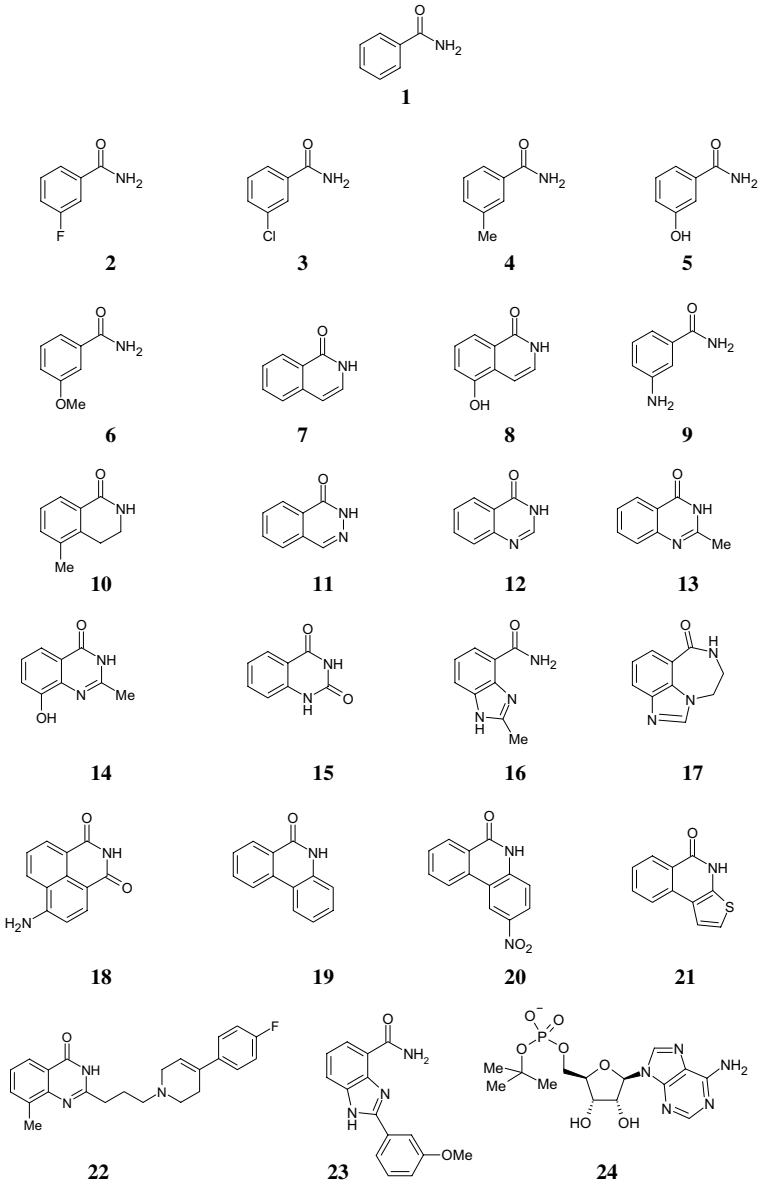

Chart 1. PARP-1 inhibitors.

Table 2. Activity, free energy of solvation, and Autodock energy of the studied inhibitors

Inh.	$-\log IC_{50}$[a]	Sol[b] (kcal/mol)	With water[c]		Without water[d]	
			Cl/Tot	E_b (kcal/mol)	Cl/Tot	E_b (kcal/mol)
1	−1.34[17]	−8.75	1/1	−5.88	1/1	−5.81
2	−1.30[17]	−7.22	1/1	−6.09	1/1	−6.02
3	−1.3[17]	−8.3	1/1	−6.29	1/1	−6.3
4	−1.28[17]	−8.46	1/1	−6.38	1/1	−6.33
5	−0.96[17]	−14.16	1/1	−6.31	1/3	−6.09
6	−1.23[17]	−10.11	1/1	−5.94	1/1	−6.04
7	−0.85[17]	−8.78	1/1	−7.07	1/1	−7.06
8	0.41[17]	−13.42	1/1	−7.5	1/1	−7.24
9	−0.99[17]	−9.17	1/1	−6.77	1/1	−6.38
10	−0.36[18]	−8.5	1/1	−7.9	1/1	−7.84
11	−1.1[17]	−8.8	1/1	−6.96	1/1	−6.94
12	−0.98[17]	−11.75	1/1	−6.99	1/1	−6.97
13	−0.75[17]	−10.5	1/1	−7.5	1/1	−7.47
14	0.40[18]	−16.9	1/1	−7.87	1/1	−7.67
15	0.91[17]	−10.95	1/1	−6.96	1/1	−6.94
16	0.0[19]	−15.56	1/1	−7.78	1/1	−7.31
17	0.64[20]	−15.46	1/1	−8.41	1/1	−8.3
18	0.74[17]	−15.76	0/2	—	1/2	−8.49
19	0.52[17]	−8.12	1/1	−8.4	1/1	−8.41
20	0.46[17]	−8.0	1/1	−8.33	1/1	−8.25
21	0.35[21]	−9.85	1/1	−8.22	1/1	−8.19

Cl/Tot = number of clusters containing the correct solution over the total number of clusters.
[a] For details about experimental assay see Ref. 17–21.
[b] Free energy of solvation, see methods for details.
[c] Autodock cluster analysis and binding energy in presence of W52.
[d] Autodock cluster analysis and binding energy in absence of W52.

(RMSD) of 0.46 Å was obtained over the 347 residues defining the entire catalytic domain (CD) of PARP (Fig. 1), thus indicating that all the available structures have a very similar arrangement of the backbone atoms as well as the side chain atoms in the binding pocket region. We thus decided to use the crystal structure of PARP-1 complexed with a benzimidazole inhibitor (**23**) (pdb code: 1EFY[14]). This structure has a water molecule (water 52, W52), which forms a bridge between Glu988 and the inhibitor.

Two separate sets of docking experiments were performed. In the first case, W52 was included in the simulation while in the second one it was eliminated. The obtained results will be discussed in terms of the impact of structural water in determining the binding affinity of the inhibitors.

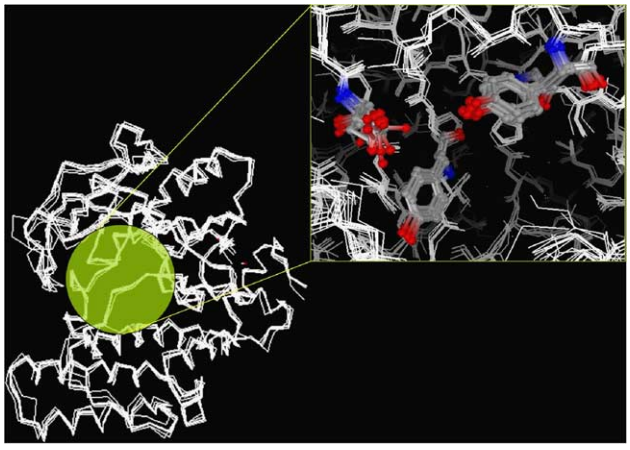

Figure 1. Superposition of the available crystal structures of PARP-CDs: focus on the active site.

2. Methods

All compounds were constructed using the fragment dictionary of Cerius2 and geometry optimized using the CVFF 95 force field with the Smart Minimizer protocol of OFF.[22] Atomic charges were computed using the semi-empirical MOPAC/AM1 method. The Lamarckian Genetic Algorithm of the Autodock 3.05 program[23] was used for docking experiments. Details about the methodology used by Autodock are described elsewhere.[23] The region of interest used by Autodock was defined in such a way to comprise the catalytic site of PARP-1. In particular, a grid of 48, 48, and 78 points in x, y, and z direction was built centered on the center of mass of the catalytic site of PARP. A grid spacing of 0.342 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energetic maps. All docked compounds were submitted to 100 runs of Autodock search. The default value of all other parameters were used. Cluster analysis was performed on the docked results using an RMS tolerance of 0.5 Å. Solvation free energies for the training set were computed using the AMSOL program version 6.8, using AM1 Hamiltonian for geometry optimization in the SM5.4 solvation model.[24] All calculations were carried out on a AMD Athlon 1800+ MP based machine running Linux Slackware 9.0 as operating system.

3. Results and discussion

In the first experiment, the 21 chosen inhibitors were docked in the crystal structure of PARP-1 by keeping the structural water molecule present in the binding pocket (W52, number is according to '1efy' pdb structure[14]). Table 2 shows the results of the docking experiments and it is noteworthy that only one cluster could be found for all but one inhibitors. Inspection of this cluster revealed that the full interaction pattern seen in the crystal structures of PARP-1 co-crystallized with inhibitors was reproduced (Fig. 2). This interaction pattern includes: (i) a hydrogen bond between the amido moiety of the inhibitor and Gly866 and (ii) and stacking interaction between the aromatic ring of the inhibitor and Tyr907.

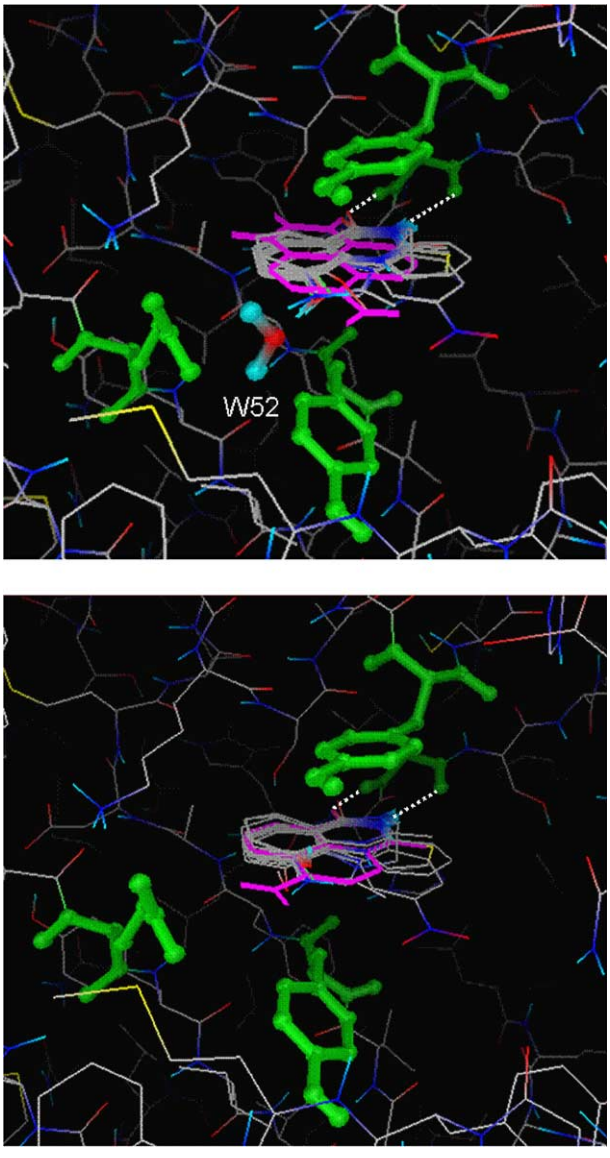

Figure 2. Superposition of docked solutions of studied inhibitors in presence (top) and in absence (bottom) of W52. In magenta is shown 4AN (**18**).

For one inhibitor, compound **18**, two clusters were found, no one containing the experimentally determined position of the inhibitor. Indeed, visual inspection of the binding pocket indicated that W52 occupies the binding site of the amino group of this inhibitor as it is observed in its crystal structure (pdb code: 2pax).[11] Thus, in the best docking solution the hydrogen bond network between the phthalazide moiety and Gly866 was disrupted whereas the amino group was flipped away from Glu988, preferentially interacting with the hydroxyl group of Tyr896. A regression analysis between binding energies and activity for the other 20 inhibitors, expressed as $\log(1/IC_{50})$, yielded a correlation coefficient (r^2) of 0.87 (Fig. 3).

The second docking experiment was carried out on the same set of 21 inhibitors after having removed W52 from the binding site of PARP-1. In this case, an appropriate docking solution could be found for all the 21 inhibitors. Only one cluster containing the experimentally determined position of the inhibitor was found for 19 inhibitors. Two and three clusters were found for compound **5** and **18**, respectively. In these cases, the experimentally determined position was found in the first cluster and chosen for further analysis. The regression analysis of the binding energies and the activity of the selected 21 inhibitors gave a correlation coefficient (r^2) of 0.84 (Fig. 4).

The comparison of the correlation coefficients obtained in presence ($r^2 = 0.87$) or absence ($r^2 = 0.84$) of W52 pinpoints a marginal role of this water molecule for the correct prediction of the binding potency of the chosen set of inhibitors. Moreover, in the case of compound **18**, the presence of W52 into the binding site of PARP-1 hampers the correct binding position of this inhibitor as observed in its crystal structure. Since this water molecule is nevertheless present in many crystal complexes, we wondered whether W52 was indeed a structural water or, rather, structured by the presence of particular types of inhibitors.

Thus, the structure of 1EFY[14] was used for a Dowser calculation after the removal of inhibitor **23** from the enzyme. Briefly, the Dowser program explores a protein's structure to locate internal cavities and assesses the hydrophilicity of these cavities in terms of the energy of interaction of a water molecule with the surrounding atoms.[25] Surprisingly, no structural water molecules were placed on the position of water 52 as it is observed in the binding site of PARP-1 complexed with the benzimidazole inhibitor (**23**, pdb code 1EFY[14]). The easiest explanation for this apparent conflicting result is that W52 could belong to the solvation shell of the inhibitor, which undergoes only a partial desolvation before entering the binding site of the enzyme. To test this hypothesis, we calculated solvation energies for the whole set of 21 compounds (Table 2). A regression analysis between the solvation energies and the activities of the inhibitors gave no correlation ($r^2 < 0.1$, results not shown). Closer examination of the data indicated that compounds **5**, **8**, **14**, **16**, **17**, and **18** have much higher values of solvation energy compared to the remaining

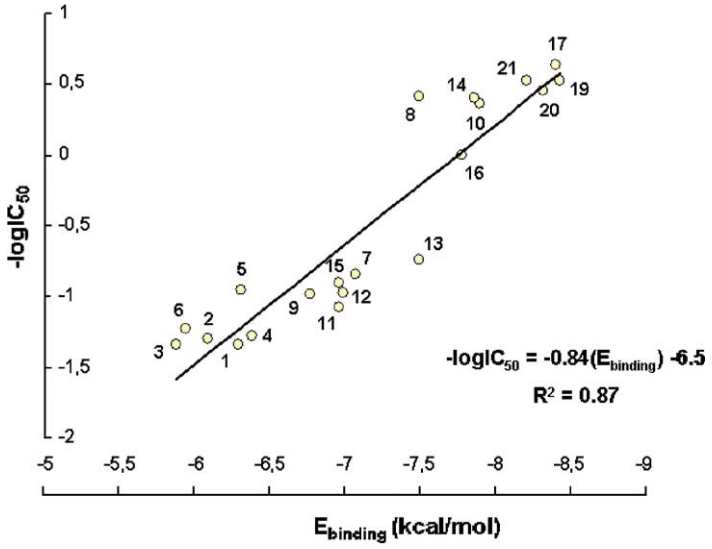

Figure 3. Regression analysis of activity ($-\log IC_{50}$) versus binding energy in the presence of the W52 water molecule.

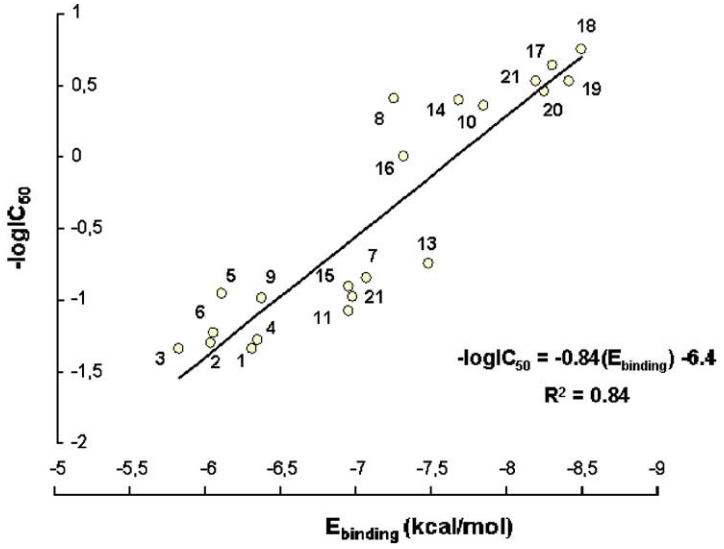

Figure 4. Regression analysis of activity ($-\log IC_{50}$) versus binding energy in the absence of the W52 water molecule.

inhibitors (Table 2). These six compounds are characterized by the presence of polar groups on the aromatic moiety (see Chart 1). We thought that these inhibitors would bring a water molecule as part of their solvation shell into the binding site of PARP-1 thus saving a high cost in terms of desolvation energy. Support to this hypothesis came from the inspection of affinity maps for the oxygen atom calculated with Autogrid[23] in the binding site of PARP-1. These affinity maps reported the conditionally existence of minima points in the region of W52 only if 'polar' inhibitors were considered in the calculation (Fig. 5). Finally, as a further test of the above hypothesis, we used our two regression models to predict the activity of compound **23** (NU1098). Indeed, if W52 is structured by **23** upon binding, the first model, which considers explicitly the water molecule, should give a better prediction of the activity which, in turn, reflects a correct evaluation of the binding energy of **23**. The first model (Fig. 3) gives a prediction of $-\log IC_{50} = 1.245$ ($IC_{50} = 0.057\,\mu M$). The second model (Fig. 4) assigns a $-\log IC_{50} = 0.85$ ($IC_{50} = 0.14\,\mu M$) for compound **23**. Accordingly, our models suggest an activity ratio of 17.5 between **23** and its closest structural analog **16** (first model, W52 present) and 7.14 (second model, no W52). The former data is in agreement with the experimentally determined ratio of 16.5 between the K_i for **16** and **23**.[14] Thus, inclusion of the water molecule led to a correct evaluation of the binding energy of **23**.

These results confirm the hypothesis of a partial desolvation of polar inhibitors (compounds **5**, **8**, **14**, **16**, **17**, **18**, **23**) before entering the binding site of PARP-1 and turn out that W52, being stabilized upon binding of polar inhibitors, can be considered as structured water.

This hypothesis is also in agreement with the topology of the binding site of PARP-1, which is characterized

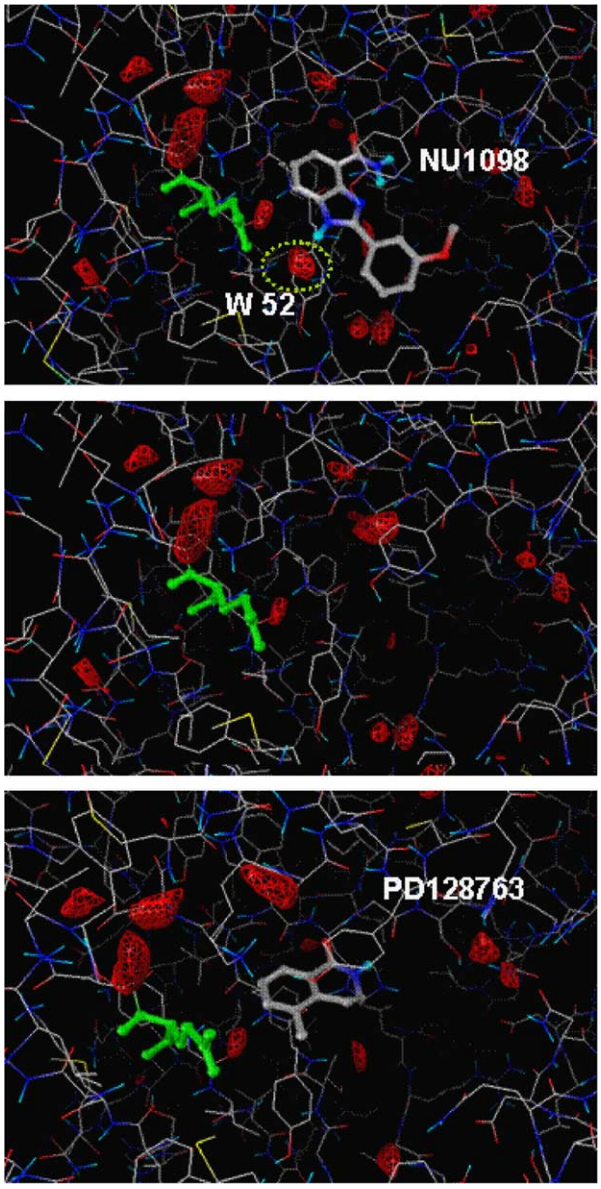

Figure 5. Autogrid affinity maps for the oxygen atom in the presence of inhibitors with a polar (top), nonpolar (bottom) group on the aromatic moiety and without any inhibitor (middle).

by a large cleft exposed to the solvent. This scenario would justify an apparent observation that good correlations can be obtained either by including or by removing W52 from the structure to be used for the docking studies. Nevertheless, the presence of W52 is needed for a correct evaluation of the binding energy of polar inhibitors, which becomes evident when a polar inhibitor external to the training set is considered. Furthermore, the presence of W52 is needed to avoid the high cost of desolvation that inhibitors with polar substituents on the aromatic ring should otherwise pay for. Compound **18** constitutes an apparent exception. Indeed, **18** must undergo a full desolvation to properly interact with the PARP-1 binding pocket. In comparison to the other 'polar' inhibitors, **18** should pay an additional cost of desolvation of about 7 kcal/mol (Amsol energy, see Table 2). Since **18** is very potent, this additional cost must be compensated by an equivalent increase in the binding energy, which is not recognized by the Autodock derived binding energies. Technical reasons might be at the basis of this apparent contradiction. First of all, the Autodock binding energies and the Amsol solvation energies are not congruent (the former must be seen more as a scoring function than 'real' energies). Therefore, they can be compared only qualitatively and cannot be mixed when quantitative correlations are sought. Second, specific interactions not explicitly perceived by Autodock may play a role for compensating the desolvation energy in **18**, for example, an increased π–π interaction between its electron poor aromatic system and Tyr907.

In summary, the following points can be concluded from our data: (i) Autodock 3.05 provides very good results in predicting the geometry and also the relative energy of binding for a set of competitive PARP-1 inhibitors. This capability makes Autodock a possible method of choice for the virtual screening of libraries in search for selective PARP inhibitors, given the availability of the crystal structure of the CD of PARP-1 and PARP-2 (ii) our results are controversial regarding the inclusion or not of water molecules eventually present in the binding pocket of the chosen X-ray structure for docking studies. Indeed, our correlation analysis clearly indicated no real advantages in the inclusion of water molecule. Rather, the presence of this molecule may hamper the identification of the correct binding orientation for individual inhibitors. Nevertheless, a good estimation of binding energies is achieved only when the water molecule is considered to complete the binding environment of inhibitors (iii) a further consequence of the above results is that the water molecule observed in the binding pocket of five crystal structures of the CD of PARP-1 must be considered a structured water, brought inside the binding pocket as a part of the hydration shell of polar inhibitors.

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