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Short communication

Is it possible docking and scoring new ligands with few experimental data? Preliminary results on estrogen receptor as a case study

P. Cozzini [a,*], T. Dottorini [b]

[a] *Molecular Modelling Laboratory, Department of General and Inorganic Chemistry, University of Parma, Parco Area delle Scienze, 17/A 43100 Parma, Italy*
[b] *Department of Experimental Medicine and Biochemical Sciences, Microbiology Section, University of Perugia, Via del Giochetto, 06122, Perugia, Italy*

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Abstract

Estrogens are steroid hormones playing critical roles in several physiological processes, which bind the estrogen receptors ERα and ERβ. Aim of this work is to analyze, by different docking experiments, the behavior of a set of compounds, mimicking estrogens activity, in order to understand the relationship between ERα and such new ligands. Main goal is to verify, using a widely tested scoring software procedure applied on a set of 10 compounds, the possibility to produce new lead candidate molecules in lack of, or with few experimental data. Our preliminary results reveal the significance of HINT software as a scoring function in docking methodology and specifically, as a mean for assessing the consistency of docking solutions.
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Keywords: Estrogen receptor; Docking; HINT; Scoring function

1. Introduction

The estrogen receptor (ER) is a member of the steroid hormone superfamily of nuclear receptors, which are gene regulatory transcriptional factors. There are two currently known estrogen receptors: ERα and ERβ. ERα displays a characteristic modular domain architecture, which includes two separate transcriptional activation domains, AF1 and AF2. AF1, located at the N-terminus of ERα, is constitutively active and regulated by growth factors [1,2]. Whereas, AF2 located in the C-terminal ligand-binding domain (LBD) of ERα, exhibits a strictly ligand-dependent activity. Hormone binding to LBD of ERα results in a profound reorganization of this domain [3].

Estrogen receptors bind with high affinity to endogenous estrogens, and the dip in hormone production, throughout menopause, enhances bone remodeling [4,5], vasomotor symptoms and heart diseases. Hormone replacement therapies (HRT) have been used for the medical treatment of vasomotor symptoms and the prevention of osteoporosis. However, recent results showed that the HRT increases risks of coronary heart disease and breast cancer [6,7]. These data have stimulated the search for alternative treatments, profiling ER as an important therapeutic target for the treatment and prevention of breast cancer.

This highlighted the need to further develop compounds able to mimick estrogens without their adverse side effect on breast, uterine tissue and cardiovascular system.

Numerous natural and synthetic compounds have been investigated due to their capability of exerting estrogenic effects. Selective estrogen receptor modulators (SERM) are a family of structurally distinct compounds that bind to ER and exert tissue-specific effects.

Selective estrogen receptor modulators such as tamoxifen and raloxifen, have the ability to act both as receptor agonists and antagonists depending on the cellular and promoter context, as well as the ER isoform targeted. Antagonist compounds are designed to have a side chain substituent responsible of AF2 antagonism. The side chain substituent prevents the formation of the coactivator recruitment. Antagonist behavior is generally improved by side chain optimization.

Tamoxifen and raloxifen are currently on the market for the treatment of hormone-dependent breast cancer [8,9] and prevention and treatment of osteoporosis [10], respectively. These two products have distinct pharmacological profiles.

* Corresponding author. Tel.: +39-0521-905669; fax: +39-0521-905556.
E-mail address: pietro.cozzini@unipr.it (P. Cozzini).

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Both compounds demonstrate bone-protective effects in humans, but tamoxifen has been shown to increase the risk of endometrial cancer [8–11], while raloxifen does not appear to be associated with uterine side effects [10]. Nowadays, a wide number of SERMs are currently in clinical trials. Despite a detailed knowledge of the pharmacology of these different classes of ER antagonists, relatively little is known about the molecular mechanisms that define their action.

Analysis of the crystal structures has been critical in understanding the molecular mechanism of the relationship between ER conformation and partial and full antagonism.

As a part of a program aimed at the development of selective estrogen receptor modulators, herein a series of docking experiments is presented, in light of profiling the structural features that greatly influence SERMs' activity. In lack of crystal structures and of exact binding affinity data, it is very difficult to obtain a relationship between structure and ΔG° to predict new ligands. Aim of this work is to find out whether molecular modeling can help understanding the interactions between receptor and ligands to design new lead compounds.

2. Results and discussion

The crystal structures of only three ligand-ERα receptor complexes are currently available: the complex between receptor and (i) estradiol (1ERE), (ii) 4OH-tamoxifen (3ERT) and (iii) raloxifen (1ERR). Several pharmaceutical companies have been providing various additional molecules, having agonist/antagonist activity, however at the present time, none of these has been characterized by X-ray ligand–receptor analysis.

It is interesting to point out that the binding pockets of 4OH-tamoxifen (4OHT) and raloxifen (RAL) ERα-complexes are significantly different in geometry and shape (r.m.s. 1.58 Å, calculated on the overall structures): differences are addressable to aminoacids involved in the binding activity. A set of 10 ligands was screened by docking the molecules into the binding pocket of the estrogen receptor (Fig. 1). Given the above considerations, the 10 compounds were separated into two sets "tamoxifen-like" and "raloxifen-like" to better compare results coming from different docking methods. The term "tamoxifen-like" compounds groups all the compounds (compound **1–5**, Fig. 1)

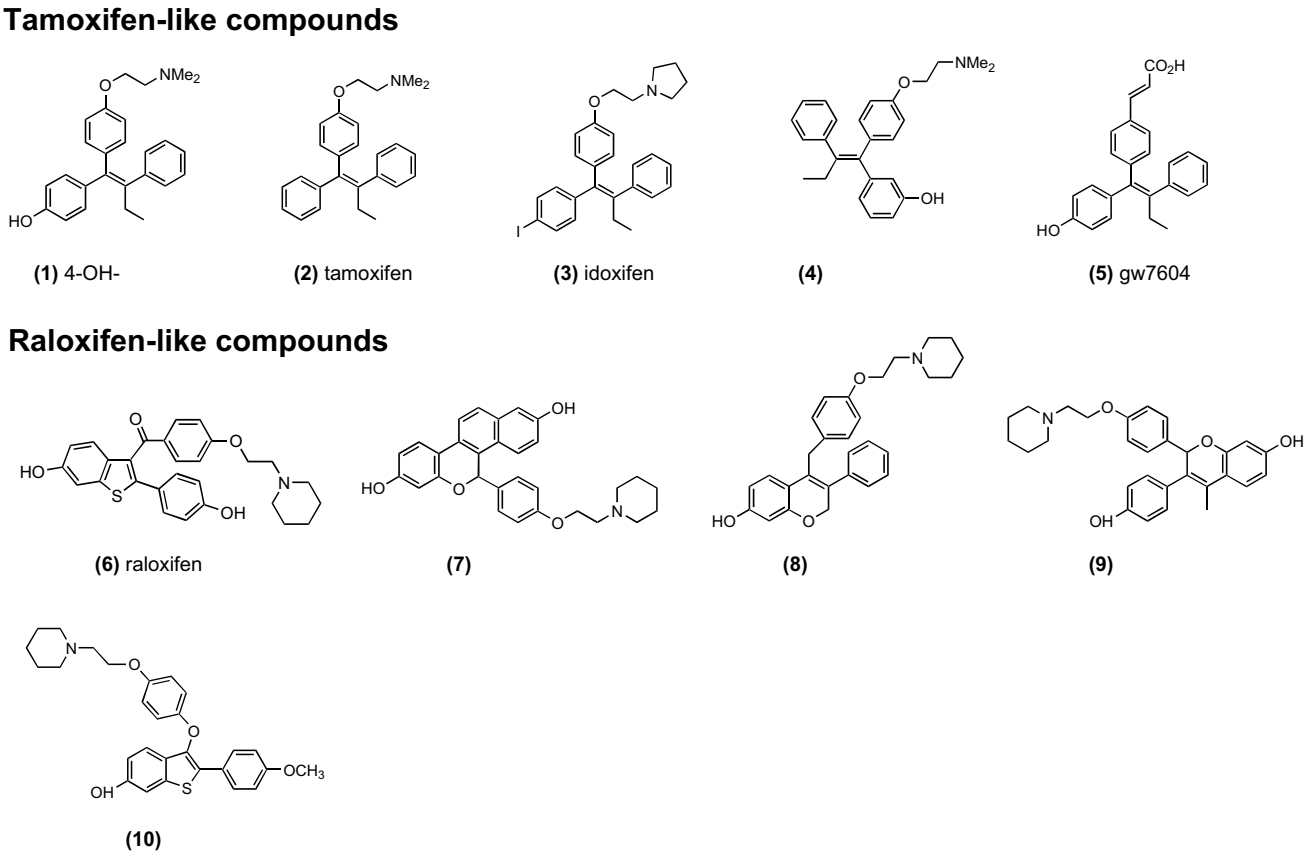

Fig. 1. Ligand compounds. Top panel, "tamoxifen-like" compounds (**1–5**). Bottom panel, "raloxifen-like" compounds (**6–11**). IUPAC names of the compounds: (**1**) 4-{1-[4-(2-Dimethylamino-ethoxy)-phenyl]-2-phenyl-but-1-enyl}-phenol. (**2**) {2-[4-(1,2-Diphenyl-but-1-enyl)-phenoxy]-ethyl}-dimethyl-amine. (**3**) 1-(2-{4-[1-(4-Iodo-phenyl)-2-phenyl-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine. (**4**) 3-{1-[4-(2-Dimethylamino-ethoxy)-phenyl]-2-phenyl-but-1-enyl}-phenol. (**5**) 3-{4-[1-(4-Hydroxy-phenyl)-2-phenyl-but-1-enyl]-phenyl}-acrylic acid. (**6**) [6-Hydroxy-2-(4-hydroxy-phenyl)-benzo[b]thiophen-3-yl]-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methanone. (**7**) 5-[4-(2-Piperidin-1-yl-ethoxy)-phenyl]-5H-6-oxa-chrysene-2,8-diol. (**8**) 3-Phenyl-4-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-2H-chromen-7-ol. (**9**) 3-(4-Hydroxy-phenyl)-4-methyl-2-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-2H-chromen-7-ol. (**10**) 2-(4-Methoxy-phenyl)-3-[4-(2-piperidin-1-il-ethoxy)-benzyl]-benzo[b]thiophen-6-ol.

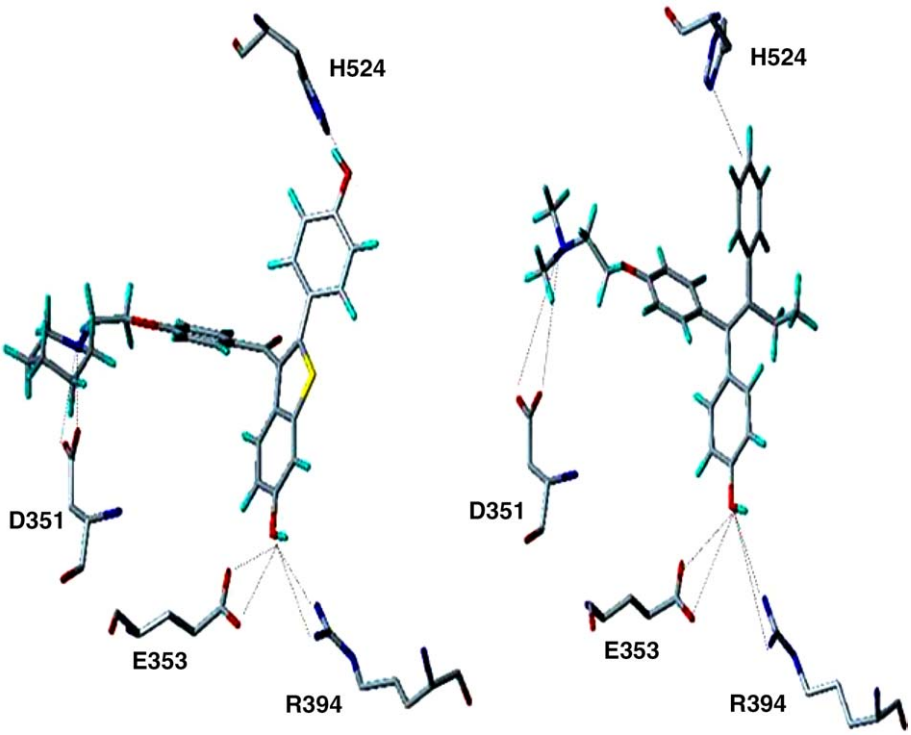

Fig. 2. ERα-ligands salient aminoacidic distances. Left panel, raloxifen site with respect to receptor active aminoacids. Right panel, 4OH-tamoxifen site with respect to receptor aminoacids. Dotted lines are measured ligand aminoacids distances.

with the same "core" of tamoxifen and with quite similar side arms. Likewise, "raloxifen-like" compounds (compound **6–11**, Fig. 1) have basically the same core of raloxifen, while side arms are different.

The interaction mechanism currently suggested [12] is that both estrogenic and antiestrogenic ligands anchor to the binding pocket (aminoacids Glu353 and Arg394) by means of the OH group located at their phenolic site (Fig. 2). The second OH group, when present, interacts with His524, whereas the side chain is projected towards Asp351. Accordingly, we consider these aminoacids as the salient residues describing the binding site (Fig. 2) and changes of their distances from ligand atoms as a measure of variation of the interaction.

Crystal data show that 4OH-tamoxifen is able to form H bonds between its hydroxyl group, Arg394 ND1 nitrogen and carboxylic group of Glu 353, while the terminal nitrogen of the antiestrogenic side chain is able to form H bonds with carboxylic group of Asp351 (Fig. 2).

In the case of the raloxifen complex structure, the "core" shows two terminal OH groups. As mentioned above, the first OH group behaves as in 4OH-tamoxifen, while the second one can make H bond with nitrogen atom of the imidazolic ring of His524. Meanwhile, the antiestrogenic side chain can make acid–base interaction between the carboxylic group of Arg351 and the nitrogen atom of the terminal amide group of the ligand.

Hence, as stated so far, we considered the outstanding measures as a parameter to evaluate the goodness of the binding from a geometric point of view (Fig. 3, Table 1). Studies concerning the influence of water are ongoing, in fact, as previously considered [13], there is a conserved water molecule that is involved in a hydrogen bonding network with the phenolic OH of the ligand and Arg394 and Glu353 of the receptor.

Nevertheless, the role of this water molecule as a pivot in the recognition mechanism is not well defined up to now.

A series of docking analyses was carried out with different docking methods, and then a scoring procedure based on the software HINT [14,15] was applied to evaluate the results.

2.1. Docking methods

The first one is a manual-assisted docking analysis that makes use of a docking module of Sybyl software (version 6.8, www.tripos.com), the second one is the docking procedure based on the FlexiDock module in Sybyl, and the third is based on Autodock/ADT software from A. Olson Research Group at the Scripps Research [16,17]. HINT, a software based on experimental log*P*, able to evaluate a score and to predict molecular associations (protein–ligand, protein–protein and macrocycle–macrocycle) by pondering both enthalpic and entropic contribution to ΔG°, was used as a post-processing scoring function for manual-assisted docking and for FlexiDock, to validate docking results generally more affected by user error; while for Autodock analyses we referred to the Autodock internal scoring function.

This choice was done because Autodock produces a lot of data in an internal format, so it is not so easy and fast to convert data from Autodock to HINT format. We are devel-

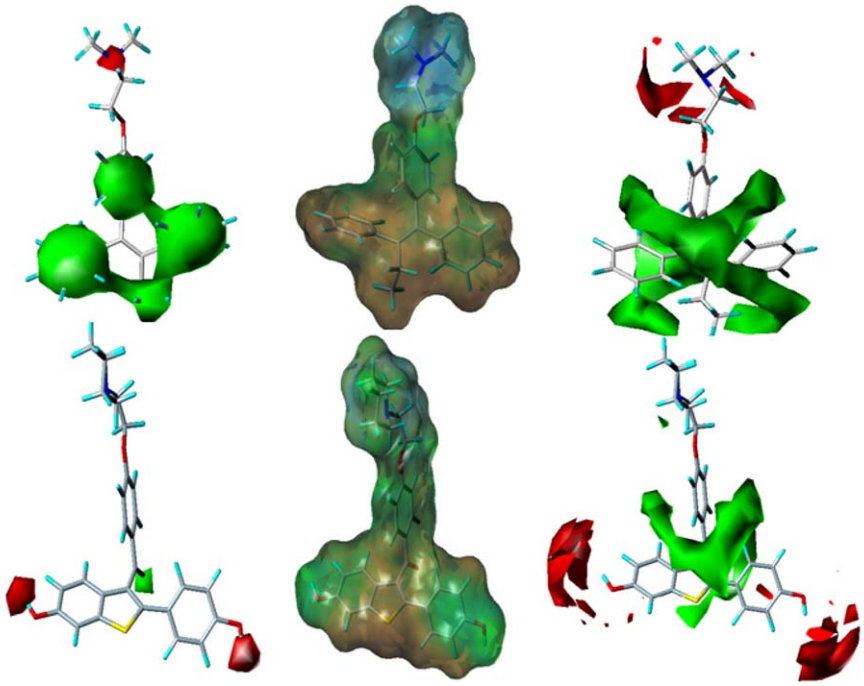

Fig. 3. Hydrophobic-polar, lipophilic and hydrophobic-polar complementary maps (HINT maps). Top panel 4OH-tamoxifen. Bottom panel raloxifen maps. From left to right hydrophobic-polar map of ligands, lipophilic map of ligand and complementary ligand hydrophobic-polar map showing what is the ligand expecting in terms of charge from the binding pocket.

Table 1
List of the two sets of compounds ordered by decreasing HINT score and distances (in Å) between ligands and salient aminoacids within the protein-binding pocket

Name	Glu353	Arg394	Asp351	His524
Tamoxifen-like				
3ERT	3.15	3.98	3.82	4.04
	4.48	5.45	4.81	
Tamoxifen	3.15	3.98	3.82	4.04
	4.48	5.45	4.81	
4OHT	2.42	3.03	3.82	404
	3.26	4.25	4.81	
Droloxifen	3.79	6.08	3.64	3.36
	5.94	7.92	5.28	
Idoxifen	3.31	3.19	3.51	3.01
	3.92	4.28	5.02	
GW7604	2.42	2.42	4.65	4.04
	3.26	3.26	5.94	
Raloxifen-like				
1ERR	2.41	2.97	2,66	2.71
	3.22	4.23	4.13	
RAL	2.41	2.97	4.67	2.71
	3.22	4.23	3.22	
LY353381	2.41	3.23	3.63	5.52
	3.78	4.29	4.86	
LY357489	3.35	3.05	5.31	2.96
	3.85	4.05	5.77	
CHF4056	3.69	3.02	3.77	4.25
	4.17	4.62	5.14	
CP336156	2.52	2.82	3.66	4
	3.43	4.38	5.04	

oping an automatic procedure to export data from Autodock to HINT in collaboration with Autodock group.

To have proper comparison standard values in docking experiments, we extracted the crystallized 4OH-tamoxifen and raloxifen ligands from their relative binding pockets and subsequently, using the above-mentioned docking methods, we re-positioned ligands in their original pocket, to check for the ability of these docking methods to place the inhibitors back into their binding pocket.

As cited above, the r.m.s. between the two structures, 3ERT and 1ERR, is 1.582 Å. This highlights the particular movements of several residues following the entrance of tamoxifen and raloxifen into the binding site. We carried out also a "cross docking procedure", putting raloxifen into the binding pocket of the 4OH-tamoxifen–receptor complex (3ERT) and vice versa putting tamoxifen into the raloxifene–receptor pocket (1ERR). The results of the performed simulations with different procedures yielded worst scores in cross docking, validating the need to dock tamoxifen-like ligands into the binding site of 4OH-tamoxifen and raloxifen-like ligands into the binding site of the raloxifen.

As previously demonstrated [18–20] we tried to find a relationship between experimental data ($\Delta G°/K_i$) and HINT score values of the best conformer docked into the pocket and scored with HINT. However, in this case the variability of the experimental data, including the type of measured parameters, the values of K_i and/or IC50 and the different protocols used, was too high. Even though it is impossible to have a unique experimental affinity scale to classify the performance of our compound activity [21]:

4OH – Tamoxifen > Raloxifen > Extradiol > 17Bextradiol

Table 2
Autodock results. All compounds analyzed with the Autodock software are listed considering: binding energy, r.m.s. from the crystallized related set compound, docking energy and distances (in Å) between ligands and salient aminoacids within protein-binding pocket

Ligand	Conformer number	Binding energy	r.m.s.	Docking energy	Asp351	Glu353	Arg394	His524
CP336156	5	–13.5	1.57	–14.4	3.25	2.7	2.5	3.99
					5.2	3.6	4.5	
Raloxifen(RAL)	5	–15.8	1.78	–18.1	3.39	3.03	2.65	2.71
					5.1	3.07	3.07	
CHF4056	8	–11.5	1.49	–13.3	3.24	3.28	3.37	3.62
					5.2	3.54	3.75	
Ly353381	7	–10.6	1.62	–12.5	3.93	3	2.5	2.78
					5.21	3.25	3.42	
Ly357489	8	–11.8	1.41	–12.8	3.34	3.6	3.99	3
					3.6	5.02	4.03	
4OH-Tamoxifen (4OHT)	2	–11.3	1.7	–14	2.63	2.95	2.55	4.01
					4.76	3.48	3.81	
GW7604	1	–9.14	0.77	–11	3.7	2.63	2.75	4.15
					5.14	3.39	3.98	
Tamoxifen	8	–10.8	2.03	–13.4	2.88	3.23	3.57	4.12
					5.07	4.63	5.34	
Idoxifen	10	–10.4	1.25	–12.6	3.09	3.18	3.61	2.95
					4.96	3.89	4.38	
Droloxifen	9	–10.9	2.33	–13.7	2.64	3.01	3.45	5.2
					4.82	3.74	4.43	

Moreover, another possible activity classification based on Jordan work [12], a qualitative scale, separated for tamoxifen-like and raloxifen-like subset, can be defined as follows:

T-scale (tamoxifen-like)

GW7604 > 4OH – Tamoxifen > Tamoxifen > Droloxifen

R-scale (raloxifen-like)

CP336156 > CHF4056 > Raloxifen > LY357489

We refer for the discussion of our results to these binding affinity scales. First of all, we focused on proper locating ligands active groups with respect to the salient aminoacid (Asp351, Glu353, Arg394 and His524). Afterwards, we focused on ligand proper core positioning, first considering the ligand core nature (lipophilicity, hydrophobicity and polarity) and the complementary ER pocket charges requested for the binding afterwards (Fig. 3).

As shown in Fig. 3 (top panel) tamoxifen has a hydrophobic–lipophilic core with a polar charge positioned in the nitrogen atom; as a result, a complementary charged binding pocket is required for tamoxifen to proper bind the ER.

Instead, raloxifen has polar charges positioned in the two active groups (Fig. 3, bottom panel), with a small hydrophobic core, thus requiring a differently charged environment to bind the receptor.

We obtained for the tamoxifen-like subset the scale reported in Table 3 that is in agreement with the affinity–activity scale mentioned above (T scale).

All ligands of this subset are extremely similar in the core and show small differences in the side arm (Figs. 4 and 5). As a result, it turns out that all tamoxifen-like compounds exhibit the same position and interact with the same salient aminoacids.

In particular GW7604 shows small differences in side arm substituents, the absence of nitrogen and ether oxygen and the presence of vinyl carboxyl group, thus this peculiarity region of the side arm can better interact with Asp351. In fact, this positive–negative charge interaction as stated by Jordan [12], could be confirmed by HINT highest fitting score.

Given what stated above, the docked solutions best fitting the affinity scale were those obtained from manual docking, due to the possibility for the user to tune the docking result, by introducing specific constraints, and due to the high degree of reciprocal core similarity of the tamoxifen-like compounds. The results from FlexiDock presented slight variations with respect to manual docking, being FlexiDock more sensitive not only to user specifications but also to the resolution algorithm adopted (genetic algorithms). In summary,

Table 3
Selected conformers scored using HINT method

Subset	Compound	HINT score
4OH-Tamoxifen-like	GW7604	3038
	4OH-Tamoxifen(4OHT)	2898
	Tamoxifen	2386
	Droloxifen	1738
Raloxifen-like	CP336156	3179
	Raloxifen (RAL)	2477
	CHF4056	2327
	LY353381	2070
	CHF3316	1763
	LY357489	1032

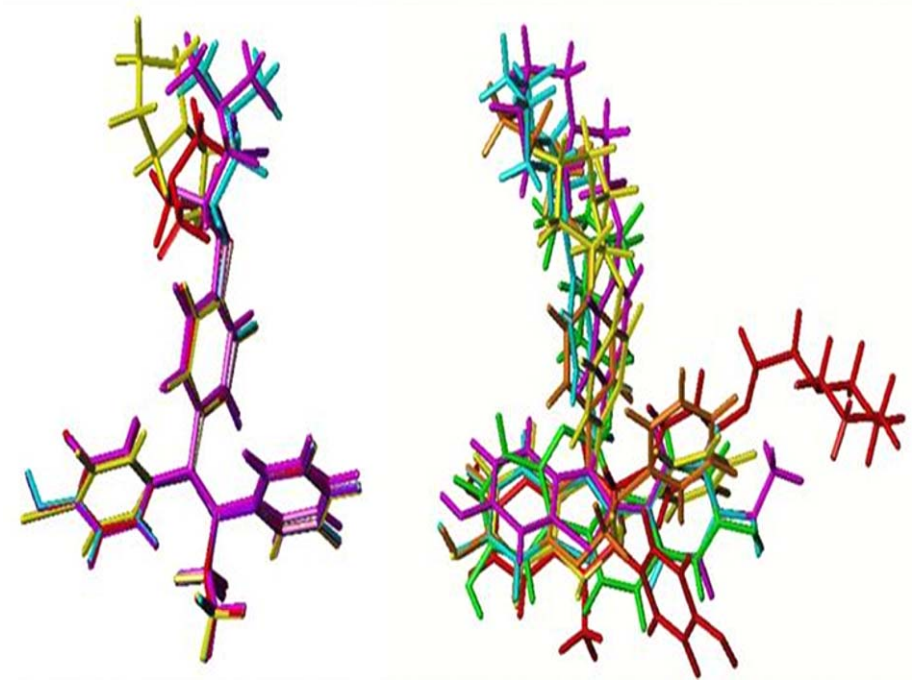

Fig. 4. Ligands overlapping. Left panel, "tamoxifen-like" compounds; Right panel, "raloxifen-like" compounds. Overlapping highlights core similarities and side arm differences.

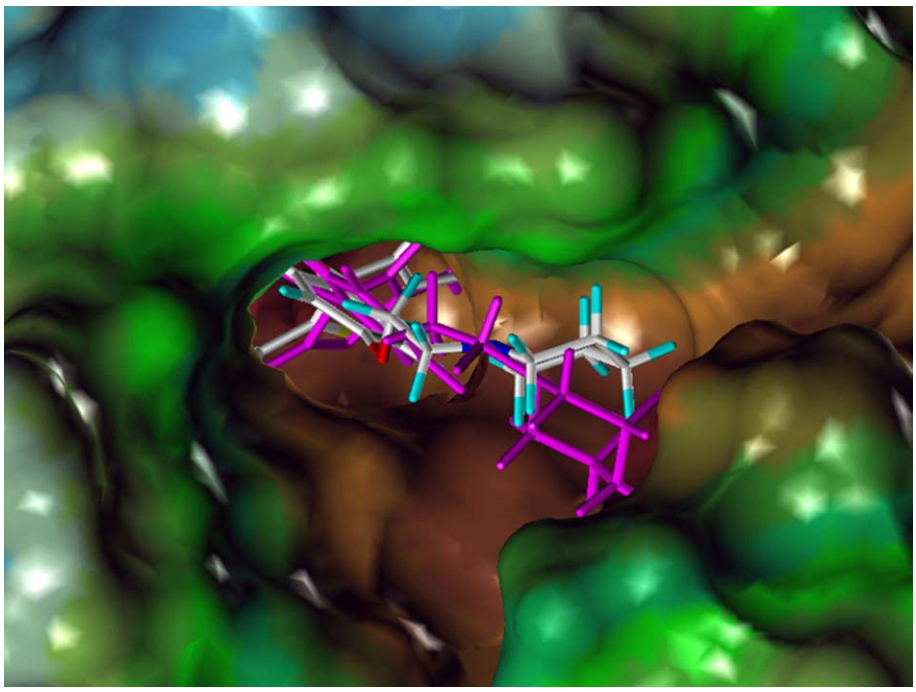

Fig. 5. Example of docking results for ligands with no experimental data: connolly surface of compound LY353381 (capped-sticks, colored by atom type) depth into raloxifen (capped-sticks, magenta color) binding pocket.

having in hand 3D templates, ligand core similarities and same active groups, the most suitable and more appropriate method for the described situation was manual docking.

As well, some Autodock result analyses were not in accordance with the T scale: we found that GW7604 and 4OH-tamoxifen, raloxifene and idoxifen were, respectively, inverted. Considering the R scale, the same situation was encountered for some ligands. It should be stressed out that energy values of docked compounds were within the 3 kcal/mol, so it was difficult to discriminate between the set of conformers obtained (data unpublished).

The r.m.s. values for the out of scale compounds were higher (r.m.s. > 2.0 Å) than those belonging to conformers which follow the correct progression, this behaviour seems to be peculiar when the compounds considered are not in agreement with the proposed scales.

In the case of the compound with unpublished affinity constant values, LY353381, a series of docking tests was performed as well. The main guideline followed in this specific case was, first of all, to place the active group of the compound in the same position of that of the root element, defining the subset to which the ligand would belong then, as a second step, to position the ligand core and, as a third step, to set the side arm properly. Geometrical positioning into the binding pocket of these ligands, and the HINT score related values of the best docked simulation, gave us the possibility to address these molecules in our scale, providing a suggestion about their position in the computational affinity scale (Table 2 and Fig. 5).

3. Conclusions

Docking ligand molecules into a specific receptor binding protein is strictly related to crystallographic templates and to well defined biochemical affinity analysis data, which are seldom available, and also difficult to sort out: differences in K_i, IC50, system and protocols in collecting values, and in same cases complete lackness of experimental values, result in ambiguity. Is this a limiting step in docking analysis? We are confident these difficulties can be overcome and that it is possible to perform docking analysis based on few accurate crystallographic data.

In summary, we found that the best fitting ligands were those following this guideline: (i) delineate ligand-template core positioning (this implies core similarities), (ii) locate functional groups, checking atom and bond types correctly to define the molecule properly, (iii) have complementary ligand and pocket from hydropathic point of view and (iv) position the side arms defining additive functionality (i.e. antagonism).

It must be noted that it is very difficult to obtain a quantitative range or scoring scale for these ligands even though the results are able to drive further QSAR analysis and even synthesis. In this work we neglect the behavior of the crystallographic water molecules because as stated [12], they are not involved in the binding process as a bridge but they participate to position the ligand core into the pocket.

New development of our work will be to consider explicitly the water contribution and the protonation state [19] using a new version of the software HINT recently developed.

4. Experimental protocols

4.1. Model building and computational methods

All calculations were performed on a Silicon Graphics (Mountain View, CA) Octane workstation (300 MHz MIPS R12000 processor) and a Pentium Linux Red Hat 9.0. All ligands structures were constructed using the Sketch module of Sybyl (version 6.8, www.tripos.com) or using the Spartan Builder (Spartan version 5.0, Wavefunction, Inc www.wavefunction.com) and then optimized.

Reference protein coordinates used for docking were taken from X-ray structures deposited in the Protein Data Bank (www.rcsb.org): ERα in complex with 17-β-estradiol (1ERE, monomer A) [22], ERα in complex with 4OH-tamoxifen (3ERT) [13], ERα in complex with raloxifen (1ERR) [22]. We found that choosing the crystal coordinates of ERα in complex with different ligands was a reasonable choice since the active site is differently opened to accommodate the diverse ligands. The X-ray structures were imported as PDB files into Sybyl, and then checked for atom and/or bond type correctness on the complex, with particular attention to the ligand and the residues involved in binding pocket. Ligand molecules as well as all water molecules were removed from the PDB structure. Hydrogen atoms were added when necessary using standard Sybyl geometries, and minimized with the Powell algorithm with a gradient of 1 kcal/(mol Å) and 1000 cycles to remove potential bad contacts. For each protein target, the active site was defined as the collection of aminoacids enclosed within a 6.5 Å radius sphere centered on the bound ligand.

4.2. Manual docking

We used a manual docking procedure based on templates (4OH-tamoxifen and raloxifene, respectively) and driven by the Dock utility in the Compute module of Sybyl 6.8. We also performed manual adjustments to determine the most energetically favorable binding location and orientation for all ligands. Each ligand was first located in the position of the crystallized antagonist (4OH-tamoxifen and raloxifen), and then manually adjusted. Finally, the complexes structures were energy-minimized using Powell minimization, with a gradient of 1 kcal/(mol Å) and 1000 cycles to remove potential bad contacts.

4.3. FlexiDock

This automated docking procedure [23], with manual adjustments to determine the most energetically favorable binding location and orientation for several ligands, was used as a Sybyl software module. An energetically favorable docking conformer was selected for each ligand between several possible models, which were subjected to a test of consistency with HINT (see below). During flexible docking, only the ligand was defined flexible with rotatable bonds. After the hydrogen atoms were added to the receptor, atomic charges were recalculated using Kollman all-atom treatment for the protein and Gasteiger–Hückel for the ligand. Ligands were variously pre-positioned in the putative binding cavity using the original position of the crystallographic inhibitor as a template and as a starting point for the FlexiDock search. Default FlexiDock parameters were set at 3000-generation or 20000-generation for the genetic algorithm. Finally, the com-

plexes structures were energy-minimized by using Powell minimization with a gradient of 1 kcal/(mol Å) and 1000 cycles to remove potential bad clashes.

4.4. AutoDock

The protein targets and the ligands were prepared for docking using Autodock 3.05 [16] and AutoDockTools 1.1 [17]. All "heteroatoms", including water molecules and ions, were removed from the original files. The positions of polar hydrogen and charges were assigned using the Kollman algorithm [24]. In the preliminary global docking experiment, ligand-centered grid maps were generated with 0.375 Å spacing by the AutoGrid program for the whole protein target. Lennard–Jones potential 6–12 (supplied with the program package) was used for modeling H bonds and Var der Waals interactions. The distance-dependent dielectric permittivity of Mehler and Solmajer [25] was used for the calculations of the electrostatic grid maps; finally, the Lamarckian Genetic algorithm (LGA) method was used with default parameters suggested by the Autodock authors. Random starting positions on the entire protein surface, random orientations and torsions were used for all structures. For all simulations the populations in the genetic algorithm was settled to 50 conformers. Each simulation comprised 2.5×10^6 energy evaluations, with a 0.02 rate of gene mutation and with a 0.8 rate of crossover. Each docking experiment consisted of a series of 100 simulations.

The search was carried out performing a cluster analysis with r.m.s. cluster tolerance of 0.5 Å i.e., the lowest-energy solution was used as a reference, and all other conformations were binned using r.m.s. deviation threshold of 0.5 Å. Other parameters of the docking simulations were identical to that of the global search.

Docked conformations of the ligand were sorted in order of increasing binding energy. For the whole-molecule docking procedure, clustering was carried out without taking any ligand conformation as a reference.

4.5. Hydropathic analysis

The hydropathic analysis consists in (i) partitioning the protein and the ligand molecular models calculating the log $P_{o/w}$ (partition coefficient for water/1-octanol) and (ii) computing the score for each complex with the software HINT (version 2.35S; www.edusoft-lc.com/hint/) using the following equation [14]:

$$B = \sum\sum b_{ij} = \sum\sum a_i S_i a_j S_j R_{ij} T_{ij} + r_{ij}$$

where a is the hydrophobic atom constant, S the solvent accessible surface area, R_{ij} an exponential of the distance (e^{-r}), r_{ij} an implementation of the Lennard–Jones potential and T_{ij} a logical function for polar–polar interactions. For the protein, HINT calculates the log $P_{o/w}$ using a table of parameters based on residue type and solvent condition [14], while the ligands log$P$ is determined using an adaptation of the Hansch and Leo's CLOG-P method [15,26,27]. Neutral solvent condition was chosen, relating to fact that the ionization states of the residues were not changed (i.e., considering Lys, Arg, Glu and Asp charged). In the log$P$ calculation the hydrogen treatment was carried out as follows: only the polar hydrogen atoms were treated explicitly in the case of the protein, whereas, for the ligands all the hydrogen were treated explicitly. Biomolecular association scores for each atom–atom interaction (non-covalent atom–atom interactions) were then considered as further evaluation for docking results by subsequent manually modifying docking interactions.

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References

[1] L. Tora, J. White, C. Brou, D. Tasset, N. Webster, E. Scheer, P. Chambon, Cell 59 (1989) 477–487.
[2] B. Cenni, D. Picard, Trends Endocrinol. Metab. 10 (1999) 41–46.
[3] D. Moras, H. Gronemeyer, Curr. Opin. Cell. Biol. 10 (3) (1998) 384–391.
[4] M. Sato, T.A. Grese, J.A. Dodge, H.U. Bryant, C.H. Turner, J. Med. Chem 42 (1999) 1–24.
[5] J.A. Kanis, Bone 19 (1996) 185S–190S.
[6] D.J. Torgerson, Exp. Opin. Pharmacother. 1 (2000) 1163–1169.
[7] S. Palacios, Int. J. Fertil. Menopausal Stud 39 (S2) (1994) 93–98.
[8] M. Dutertre, C.L. Smith, J. Pharmacol. Exp. Ther 295 (2000) 431–437.
[9] D.P. McDonnell, Trends Endocrinol. Metab 10 (1999) 301–311.
[10] M. Maricic, O. Gluck, Expert Opin. Pharmacother 3 (2002) 767–775.
[11] I.N.H. White, Carcinogenesis 20 (1999) 1153–1160.
[12] V.C. Jordan, J. Med. Chem 46 (2003) 1081–1111.
[13] K.A. Shiau, D. Barstad, P.M. Loria, L. Cheng, P.J. Kushner, D.A. Agard, G.L. Greene, Cell 95 (1998) 927–937.
[14] G.E. Kellogg, G.S. Joshi, D.J. Abraham, Med. Chem. Res 1 (1992) 444–453.
[15] D.J. Abraham, A. Leo, J. Protein-Struct. Funct. Genet. 2 (1987) 130–152.
[16] D.S. Goodsell, G.M. Morris, A.J. Olson, J. Mol. Recognit 9 (1996) 1–5.
[17] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, J. Comput. Chem. 10 (4) (1996) 293–304.
[18] P. Cozzini, M. Fornabaio, A. Marabotti, D.J. Abraham, G.E. Kellogg, A. Mozzarelli, J. Med. Chem 45 (2002) 2469–2483.
[19] M. Fornabaio, P. Cozzini, A. Mozzarelli, D.J. Abraham, G.E. Kellogg, J Med Chem 46 (2003) 4487–4500.
[20] A. Marabotti, L. Balestreri, P. Cozzini, A. Mozzarelli, G.E. Kellogg, D.J. Abraham, Bioorg. Med Chem Lett 10 (18) (2000) 2129–2132.
[21] S. Aliau, H. Mattras, E. Richard, J.C. Bonnafous, J.L. Borgna, Biochemistry 41 (25) (2002) 7979–7988.

[22] A.M. Brzozowski, A.C. Pike, Z. Dauter, R.E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G.L. Greene, J.A. Gustafsson, M. Carlquist, Nature 389 (1997) 753–758.

[23] R. Judson, Genetic algorithms and their use in chemistry, in: K.B. Lipkowitz, D.B. Boyd (Eds.), Computational Chemistry, 10, VCH Publishers, New York, 1997, pp. 1–73.

[24] L. Chiche, L.M. Gregoret, F.E. Cohen, P.A. Kollman, Proc. Natl. Acad. Sci. USA 87 (1990) 3240–3243.

[25] E.L. Mehler, T. Solmajer, Protein Eng 4 (1991) 903–910.

[26] D.J. Abraham, G.E. Kellogg, J.M. Holt, G.K. Ackers, J. Mol. Biol 272 (1997) 613–632.

[27] C. Hansch, A.J. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, J. John Wiley and Sons Inc., NY, 1979.