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Molecular dynamics, database screening, density functional and docking studies of novel RAR ligands in cancer chemotherapy

Carlos H.T.P. Silva[a], C.A. Taft[b,*]

[a]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do café, s/n, CEP 14040-903, Ribeirão Preto, SP, Brazil
[b]Centro Brasileiro de Pesquisas Físicas, Rua Dr. Xavier Sigaud, 150, CEP 22290-180, Rio de Janeiro, RJ, Brazil

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Abstract

Due to the major challenge which cancer treatment and cure still imposes after many decades to the international scientific community, there is actually considerable interest in new ligands with increased bioactivity. We have focused on the retinoid acid receptor, which is considered an interesting target for drug design. In this work, we have carried out density functional geometry optimizations and different docking procedures. We have performed screening in a large database (hundreds of thousands of molecules which we optimized at the AM1 level) yielding a set of potential bioactive ligands. Two new ligands were selected and optimized at B3LYP/6-31G* level. A flexible docking program was used to investigate the interactions between the receptor and the new ligands. Molecular dynamics were performed in order to investigate the stability of the two ligands as well as the crystallographic RAR ligand inside the RAR active site. We also investigated the stability of all the main protein–ligand contacts. The parameters of the Rule of Five were investigated. The result of this work is compared with a crystallographic ligand of RAR. One of our theoretically bioactive new ligands indicates stronger and more polar and hydrophobic interactions with the receptor.

Keywords: Cancer; Retinoic acid receptor; Functional density

1. Introduction

Docking, ab initio, density functional and molecular dynamics studies are useful tools for investigating biological receptors and bioactive ligands [1–8]. In the presence of retinoic acids, a large family of nuclear receptor proteins, i.e. the retinoic X receptor (RXR) and the retinoic acid receptor (RAR), activates transcription of vitamine A (retinol) and its biologically active derivatives, affecting homeostasis, vertebrate development and cellular differentiation, as well as the control of gene expression by retinoid signals [9].

The RXR and RAR isotypes (α, β, γ) and their numerous isoforms are related to the thyroid/steroid hormone super family of receptors acting as ligand-dependent transcription factors for different genes [9]. Retinoids, which are involved in the regulation of cell growth, have indicated good results in cancer prevention and treatment. The isotypes of the RAR family have different pharmacological targets.

In this work, we investigate and report a new ligand for RAR isotypes using molecular dynamics, density functional, Natural Bond Orbital (NBO) charges, different docking procedures and a large database of chemicals. Stability of the main ligand–protein contacts, comparison of the orientation and receptor–ligand interactions of the new ligand with the crystallographic bioactive orientation suggest that our proposed new molecule could be a more potent bioactive ligand with more and stronger hydrogen bonds and hydrophobic interactions with the receptor.

2. Methodology

We have optimized at the AM1 level (to obtain partial charges), using the Tsar program [10], and docking (to select ligands with largest scores) ~ 350,000 molecules in

* Corresponding author.
E-mail addresses: catff@terra.com.br, taft@cbpf.br (C.A. Taft).

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the Available Chemical Directory (ACD) database, using Silicon Graphics and IBM RISC Workstations. We have used both the DOCK 5.1 [11] and GOLD 2.1.2 [12] softwares in this work. The final docking calculations were done with GOLD. The merits of different docking techniques or scoring functions have been addressed in reference [13] in which DOCK and GOLD have been rated to be of the same quality. Effectively, GOLD supports the results of DOCK, using different scoring function, suggesting a more potent novel ligand in this work. The first docking calculations of ~ 350,000 molecules were done using the DOCK program. In principle all docking applications include four steps, i.e. identification and preparation of the receptor site, preparation of the ligand(s), docking the ligand(s) and evaluation of the docked orientations. We have used a grid of dimension $20 \times 20 \times 20$ Å^3 centered in the sulfur atom of Met272 of the active site of the RAR (PDB code 1FCX) [9], and a grid spacing of 0.3 Å. We have selected 33 spheres in the bioactive clusters, which were enough to fill the RAR active site. Force field (Amber) charges and Hydrogen atoms were added to the protein using the Insight II program [14]. For the ligands, a flexibility algorithm (anchor-first search) and a contact-clash-overlap of 0.75 were used. Scoring function uses shape, geometry, orientation as well as force fields, whereas terms such as van der Waals and columbic terms are included. We have used this program to perform a screening in the Available Chemical Directory (ACD) database in order to find theoretically more potent RAR ligands (with the largest scoring function). We have optimized ~ 350,000 molecules at the AM1 level in order to obtain the charges for docking calculations. Two promising new ligands with good scores were optimized by Gaussian 03 [15] at the B3LYP/6-31G* level yielding Natural Bond Orbital (NBO) charges. This enables us to have an even better input structure for the next step involving another docking procedure using GOLD. Effectively, the NBO partial atomic charges obtained from the density functional geometry optimization were used for another docking procedure, using GOLD, whereas docking calculations were performed inside a sphere centered in the sulfur atom of Met272 from the RAR (PDB code 1FCX), with radius of 15 Å. Atomic charges for the receptor atoms were obtained using an all atom force field CVFF. Parameters were used, which have been optimized for single docking calculations [12]. As GOLD uses genetic algorithm to perform flexible docking, each docking result is slightly different from the other. Flexible docking was parameterized for 0 ligand bumps, a population size of 100, 5 islands, 100 000 operations, 95 mutations and 95 crossovers, adjusted for 10 dockings. The superpositions of the top 3 solutions (ligand orientations) are within 1.5 Å mean square root deviation (R.M.S.D.).

Molecular dynamics simulations (MD) were done with the discover module of Insight II. Previously, the energies of the complexes between RAR and our two novel potential ligands were minimized using 1000 steps of steepest-descent algorithm and the discover/CVFF force field in the Insight II package. No constraints were made during any optimization procedure. We subsequently made a 1.50 ns MD simulation of our RAR potential ligands with an equilibration phase of 80 ps, at 298 K. An implicit solvation condition with a dielectric constant of 80 (water) was employed for both minimization and MD simulation. All the atoms of the ligands as well as the RAR were unconstrained. The input structures of the ligands and their NBO atomic charges were obtained using the density functional method with correlation (B3LYP/6-31G*). Atomic charges for the receptor atoms were obtained using an all atom force field CVFF. The coordinates of the systems were saved every 1 ps during the simulations. From the molecular trajectory of the two systems generated by the molecular dynamics simulations we analyzed the root mean square deviations of each system with respect to all atoms as well as the total energy as function of the time. For calculation of log P and parameters of the Rule of Five [16], we used the Tsar 3.3 software [10].

3. Results and discussion

We give in Fig. 1 the two new ligands proposed in this work: 1-Hydroxy-3-[3-(4-nitro-phenyl)-acryloyl]-pyridinium and 2,5-Bis-(4-fluoro-benzylidene)-cyclopentanone, denoted herewith as HNPAP and FBC, respectively. These two ligands have been selected among the top solutions of DOCK program, after screening the ACD database (containing ~ 350,000 chemicals, and optimized by us at the AM1 level), and yields slightly better energy scores (− 34.0

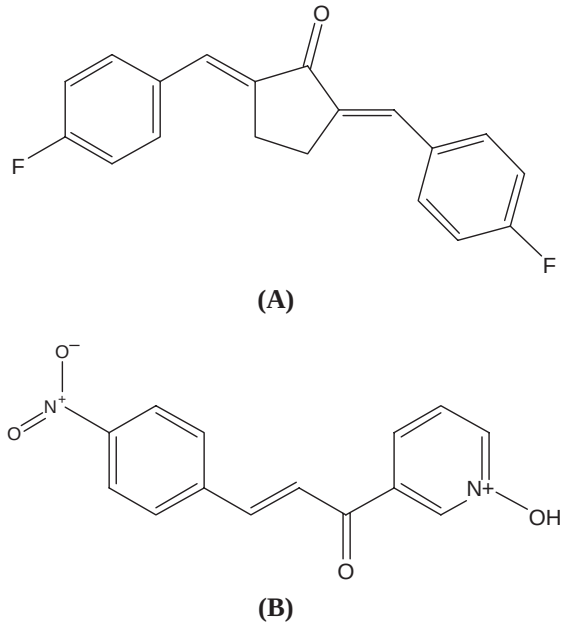

Fig. 1. Structural formulas of the two ligands of highest scores obtained with DOCK by screening in the ACD database. In (A), 2,5-Bis-(4-fluoro-benzylidene)-cyclopentanone, denoted herewith as FBC. In (B), 1-Hydroxy-3-[3-(4-nitro-phenyl)-acryloyl]-pyridinium, denoted herewith as HPAP.

for HNPAP and − 33.2 for FBC) than that obtained for 1FCX ligand (− 32.0). Gaussian was then used for full geometry optimization of the ligands, using B3LYP/6-31G* methods/basis set in the gas phase. The ligand orientations presented here for the two ligands have the best fitness (57.1 for HNPAP and 50.3 for FBC) obtained after single flexible docking using GOLD for the molecules (Figs. 2 and 3). Both molecules indicate good shape complementarity with RAR (Figs. 2 and 3). The orientation of our ligand with best GOLD fitness (HNPAP) has considerable overlap with the X-ray orientation of the bioactive ligand (PDB code 1FCX) (Fig. 4). Both 1FCX (gamma-selective) and HNPAP ligands have a hydroxyl moiety at the same position. Our ligand has a nitro group at the corresponding position of the carboxyl group of 1FCX ligand. This new ligand preserves the pharmacophoric pattern of the 1FCX ligand, as Hydrogen Bond donor and acceptor groups. In addition, HNPAP is a molecular simplification of the hydrocarbonic moiety of the crystallographic ligand.

The predominant contributions of the 1FCX ligand to the RAR γ-selectivity can be attributed to the interaction of the Met272 residue from the receptor with the close hydroxyl group (Fig. 4). Comparison of our proposed HNPAP with 1FCX ligand indicates 2.80 Å and 3.29 Å distances between Met272 sulfur atom and the nearest non-hydrogenic atom of the ligands (hydroxyl oxygen for both HNPAP and 1FCX ligands), respectively [9]. We thus note that our new ligand may also be RAR γ-selective due to the slightly shorter ligand–Met272 distance as well as to the presence in our ligand of the hydroxyl group.

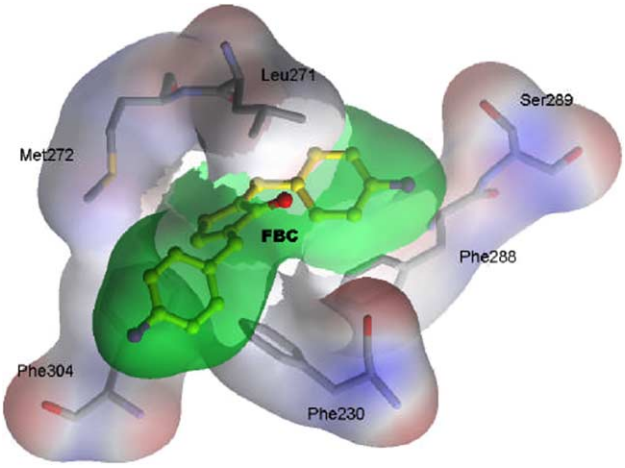

Fig. 3. Orientation of the top solution obtained with GOLD for the FBC ligand selected after screening the ACD database using the DOCK program. The new ligand (carbon atoms in yellow and density surface in green) has only hydrophobic interactions with selected atoms of the RAR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We note (Fig. 2) that in our new ligand we find hydrogen bonds between its nitro group and the side chain hydroxyl group (2.5 Å) as well as the main chain nitrogen (at 3.1 Å) of Ser289 of the receptor. The corresponding distances of the carboxyl group of the 1FCX ligand with the same atoms of the receptor are 2.5 and 3.7 Å.

We observe hydrophobic interactions between the new ligand and certain residues of the receptor, such as Phe230,

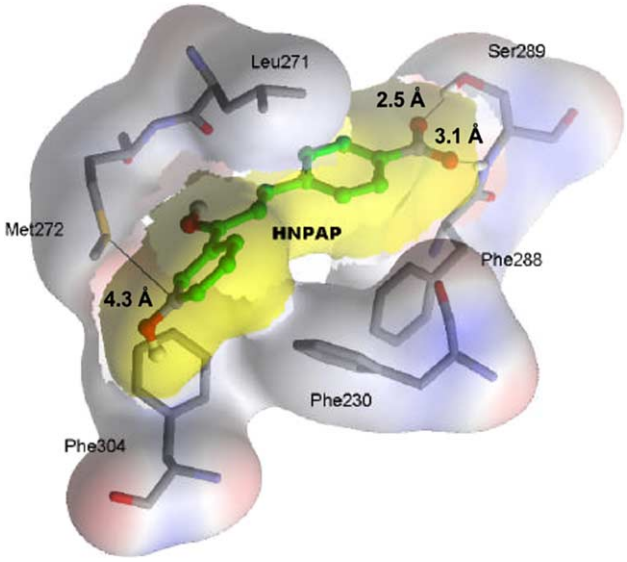

Fig. 2. Orientation of the top solution obtained with GOLD for the HNPAP ligand selected after screening the ACD database using the DOCK program. The new ligand (carbon atoms in green and density surface in yellow) has hydrophobic, ion–dipole and hydrogen bond interactions (represented by lines) with selected atoms of the RAR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

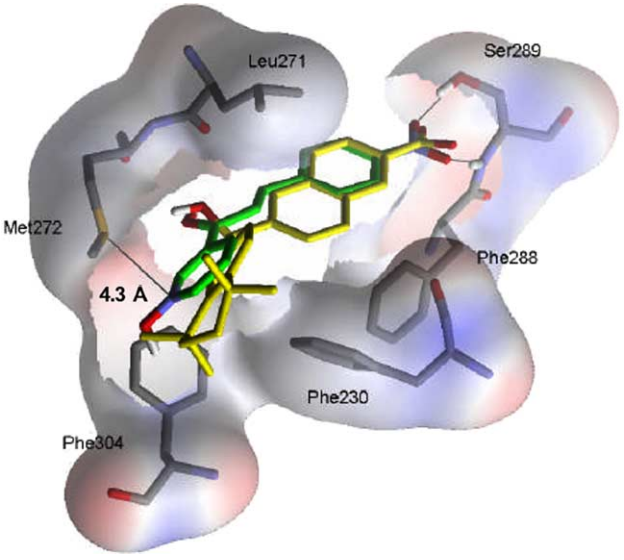

Fig. 4. Superposition of the crystallographic orientation of the 1FCX ligand (carbon atoms in yellow) with our best ligand proposed in this work (HNPAP, carbon atoms in green), selected by docking search procedure. The main interactions (hydrogen bonds and iondipole) between selected residues of the RAR active site and the new ligand are indicated by lines. Our ligand, in contrast to the crystallographic ligand, would have one additional ion–dipole interaction between the pyridinium nitrogen and Met272 of RAR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

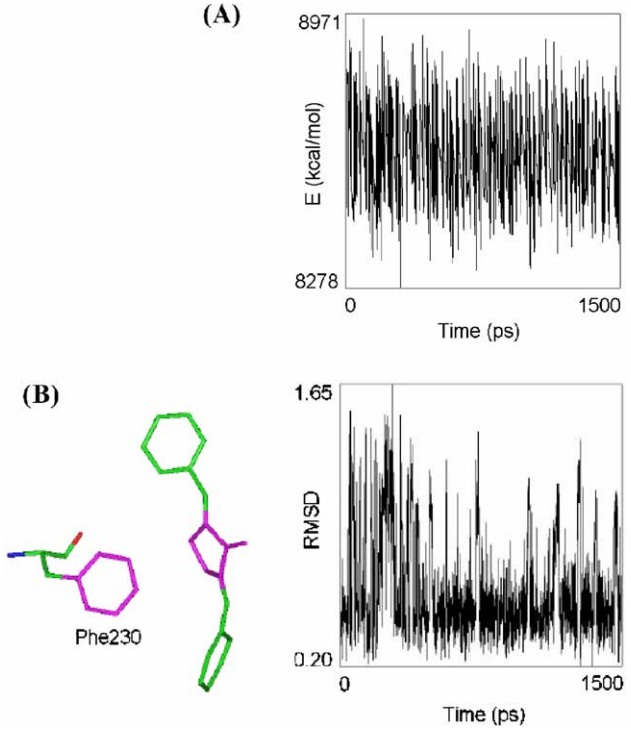

Fig. 5. Plots of (A) energy vs. time for the FBC ligand and (B) root mean square deviation (RMSD) with respect to main ligand–protein contact (selected atoms in magenta). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

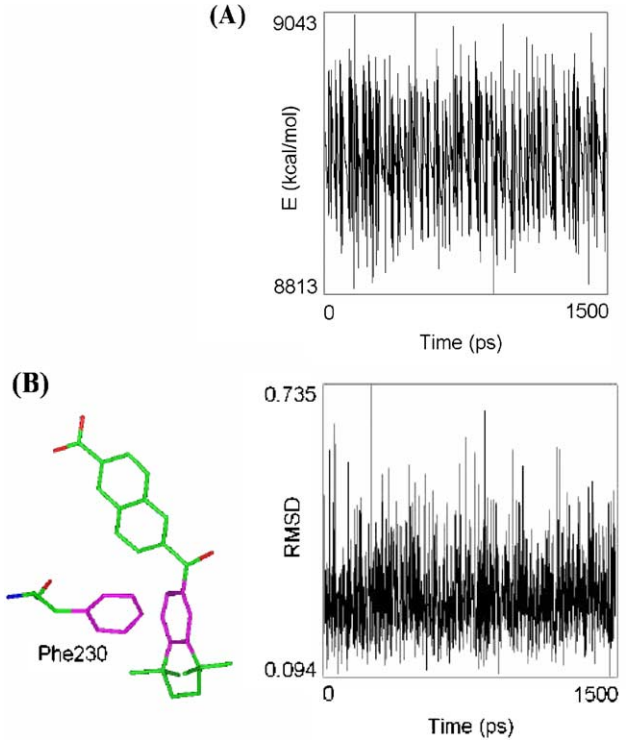

Fig. 6. Plots of (A) energy vs. time for the RAR crystallographic ligand and (B) root mean square deviation (RMSD) with respect to main ligand–protein contact (selected atoms in magenta). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

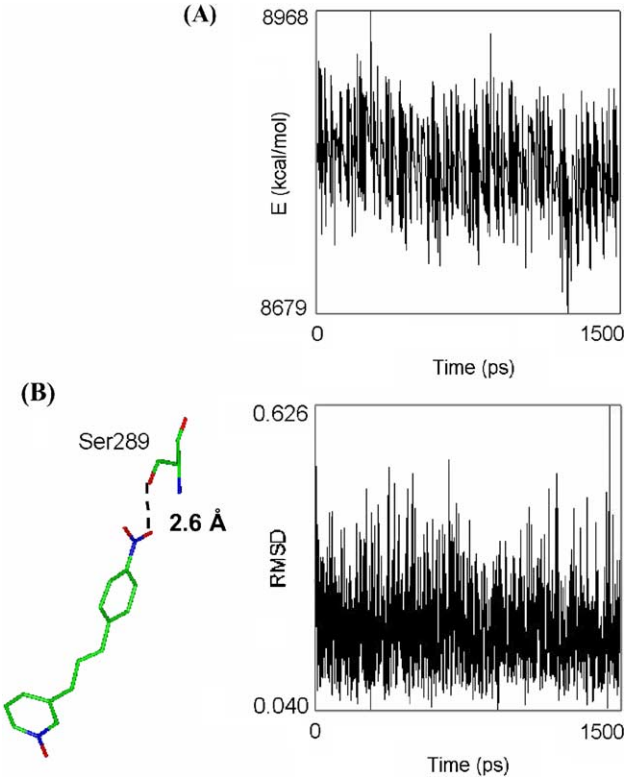

Fig. 7. Plots of (A) energy vs. time for the HNPAP ligand and (B) root mean square deviation (RMSD) with respect to main ligand–protein contact (hydrogen bond between the hydroxyl group of Ser289 and the nitro group of HNPAP).

Leu271, Phe288, Phe304 and Met272. We find however an apparently stronger network of hydrophobic interactions between the phenyl moiety of the new ligand and the Phe230 (inter-carbons distance of 4.34 Å), Met272 (carbon–sulfur distance of 3.50 Å), Phe288 (intercarbon distances of 3.43 Å), and Phe304 (inter-carbon distances of 3.33 Å) residues (Fig. 2). In addition to the hydrophobic interactions between 1FCX and RAR, HNPAP also has an ion–dipole interaction (4.3 Å distance) between its pyridinium nitrogen and the Met272 sulfur atom of the receptor.

We performed molecular dynamics simulations of 1.50 ns with FBC, the crystallographic ligand as well as HNPAP. The results are shown in Figs. 5–7, respectively. Our results suggest that both HNPAP and FBC are stable inside the RAR active site, such as the RAR crystallographic ligand.

Table 1
Absortion, Distribution, Metabolism and Excretion (ADME) parameters of the *Rule of Five* for our two new ligands and the crystallographic ligand (PDB code 1FCX) for comparison

ADME properties	Ligands		
	Crystallographic ligand	FBC	HPAP
Molecular weight	388.50	296.33	271.27
H bond acceptors	3	1	4
H bond donors	2	0	1
Log *P*	6.81	4.41	2.94
Rule violations	1	0	0

We also calculated the stability of the main ligand–protein contacts. For FBC and the RAR crystallographic ligands, the main contact is an hydrophobic interaction between Phe230 and the closest rings of the ligands (Figs. 5 and 6). For HNPAP the main protein–ligand contact is an hydrogen bond between the hydroxyl group of Ser289 and the nitro group of our proposed new ligand. The RMDS of the main protein–ligand contact for this new proposed ligand attains the largest value of 0.626, which is the smallest for all the ligands investigated. We calculated the parameters that define the Rule of Five. None of our ligands violated this rule (Table 1).

4. Conclusions

Summarizing, we suggest that our proposed ligands could be γ-selective such as the 1FCX ligand. We also observe an increased number of stable polar interactions as well as a large network of hydrophobic interactions between our ligand and certain residues of the receptor. Consequently, if this ligand penetrates a living organism, it could theoretically interact strongly with the receptor yielding a potent bioactive RAR ligand. Ab initio, density functional, molecular dynamics and docking studies can be very useful to investigate orientations of ligands in their respective RAR active sites as well as the receptor–ligand interaction energies.

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