Ligand based pharmacophore model for Genistein analogs, phytoestrogen with selectivity for the estrogen ß receptor

(Modelo de farmacóforo para análogos de Genisteina, fitoestrógeno con selectividad por el receptor ß estrogénico)

Mary Isabel Cordero de Troconis*, Trina Colman de Saizarbitoria, Milagros Avendaño y Ángel Amesty

Resumen

Se obtuvo un modelo de farmacóforo tridimensional utilizando un grupo de Fitoestrógenos con selectividad por el receptor estrogénico ß y una combinación de técnicas de acoplamiento (docking) en el receptor y diseño de farmacóforo en base a ligandos. Como referencia se utilizó un complejo cristalino de Genisteina (1) con el receptor estrogénico ß reportado en la Base de Datos de Proteínas de Brookhaven (1QKM). Los compuestos estudiados fueron divididos en grupo de ensayo y grupo de validación. Para el estudio de acoplamiento (docking) preliminar se utilizó el programa Scigress 7.0 y los resultados fueron utilizados para escoger los compuestos del grupo de ensayo. Se generaron los modelos de farmacóforo utilizando el modulo GALAHAD del programa Sybyl 8.0. La mejor hipótesis fue identificada por el mayor consenso entre las características del farmacóforo y la parte estérica, y adicionalmente una baja energía; este modelo presenta tres zonas hidrofóbicas y tres zonas aceptoras de protones. Los compuestos más selectivos del grupo de validación, compuestos estructuralmente diversos, presentaron una buena alineación con las características del farmacóforo propuesto. Estos estudios demuestran la aplicabilidad de la combinación de técnicas de acoplamiento con la generación de farmacóforo para identificar compuestos más selectivos. Estos métodos «in silico» también podrían ser de utilidad para el diseño racional de nuevos compuestos con selectividad por este receptor.

Palabras clave: Fitoestrógenos, acoplamiento (docking), farmacóforo, receptor estrogénico ß.

Summary

A three-dimensional pharmacophore model was generated utilizing a set of Phytoestrogens with known selectivity for the estrogenic receptor ß (ER ß) and a combination of docking in the receptor and ligand based pharmacophore modeling techniques. As a reference it was used a crystalline complex between the phytoestrogen Genistein (1) and the ER ß retrieved from the Brookhaven Protein Database (1QKM). The studied compounds were divided into training and validation sets. The docking module of Scigress 7.0 was used for the preliminary docking and these results were used to choose the training set. Pharmacophore models were generated using the flexible align method within the GALAHAD module, implemented in SYBYL 8.0 software. The most significant pharmacophore hypothesis, characterized by the conflicting demands of maximizing pharmacophore consensus, maximizing steric consensus, and minimizing energy, consisted of three hydrophobic zones and three H-Bond acceptor zones. The most selective compounds in the structurally diverse training set showed a good fit with all features of the proposed pharmacophore. These studies demonstrate the applicability of docking combined with pharmacophore modeling to the identification of potentially selective compounds for the ER ß. These «in silico» tools might also be useful in rational design of new compounds with selectivity for ER ß.

Key words: phytoestrogens, docking, pharmacophore, estrogen receptor ß.

Laboratorio de Modelaje Molecular Dr. José Luis Andrade. Facultad de Farmacia. Universidad. Central de Venezuela. Apartado 40109 Caracas, Venezuela

* Email:m_troconis@hotmail.com

Introduction

Phytoestrogens are non steroidal compounds from vegetal origin. Chemically, they can be divided into three main classes: flavonoids such as genistein, naringenin, and kaempferol; coumestans (such as coumestrol) and lignans (such as enterodiol and enterolactone). Phytoestrogens can present similar or opposite effects to the human estrogens (estrogenic or antiestrogenic effects) (Paraskevi, 2007). They show some advantages over synthetic estrogens in hormonal replacement therapy because they present lower risk for cardiovascular accidents (Panay and Rees, 2005). In some cases they can help to prevent some cancers amongst them breast, prostate, uterus and colon (Adlercreutz, 2002). Another phytoestrogens action is against osteoporosis, they augment the osteoblast activity (cells that build bone tissue) and decrease osteoclast activity (cells that destroy bone tissue) (Morabito et al., 2002). There is also increasing evidence that beta estrogen receptor activation is important for mechanisms that underlie estrogen-inducible neuronal morphological plasticity, brain development, and cognition (Zhao and Brinton, 2005, Rissman et al., 2002, Wang et al., 2001 and 2003).

In previous Molecular Modeling work it was determined that some structural characteristics and electronic properties are important for the interaction of Phytoestrogens with the ß estrogenic receptor (Colman et al., 2005). These facts and the therapeutic potential of these natural products justify the research for a pharmacophore that will help in the finding of new active compounds.

A pharmacophore according to the IUPAC (Wermuth et al.,1998) *«is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response»*. In rational drug design a pharmacophore is «the highest common denominator of a group of molecules exhibiting a similar pharmacological profile and which are recognized by the same site of the target protein» (Wermuth, 2006 and van Drie, 2007). This definition differs from the initial tendency in medicinal chemistry to call pharmacophores some specific functional groups, especially if they appear to be often associated with biological activity. Actually the design of a pharmacophore is widely used in the rational drug design process in academy and in pharmaceutical industry. In such way, pharmacophore approaches have become one of the major tools in drug discovery (Kubinyi, 2006).

In the present work we study several phytoestrogens, especially Genistein (1) (Zhao et al., 2005), phytoestrogen with reported selectivity for the ß estrogenic receptor. Selective compounds are interesting because they could have potential use against degenerative diseases aging related, such as Alzheimer disease, without the synthetic estrogens proliferative effects over the uterus and breast. These secondary effects are mediated by the estrogenic receptor. Other potential therapeutic advantages associated with the beta receptor are regulation of estrogen vascular protection actions (Makela et al., 1999) and development of new sites for pharmacological intervention in diseases such as depression, colon cancer, prostate cancer, obesity and leukemia (Gustafsson, 2003).

For our research we used an estrogen receptor ß (ER ß) structure complexed with Genistein (Pike et al., 1999). This complex was obtained experimentally and retrieved from the Brookhaven Protein Data Bank (Berman et al, 2000) (Figure 1). It was used to run a preliminary docking of compounds 1-17 using Scigress Explorer® 7.0 from Fujitsu Limited (Scigress Explorer, 2008). The results were useful to study key interactions with the receptor and to choose compounds with similar docking to Genistein (model compound). The docking process takes into account the conformation and orientation the ligand must adopt in order to bind the receptor active site. The chosen compounds were used to generate a model pharmacophore using GALAHAD, a Tripos software (Richmond et al., 2006, Shepphird and Clark 2006, and Clark and Abrahamian, 2009).

A pharmacophore generation is a very important task because it helps to study key interactions in the drug receptor complex. The combined use of these two methodologies, docking and pharmacophore generation, has been used successfully by other

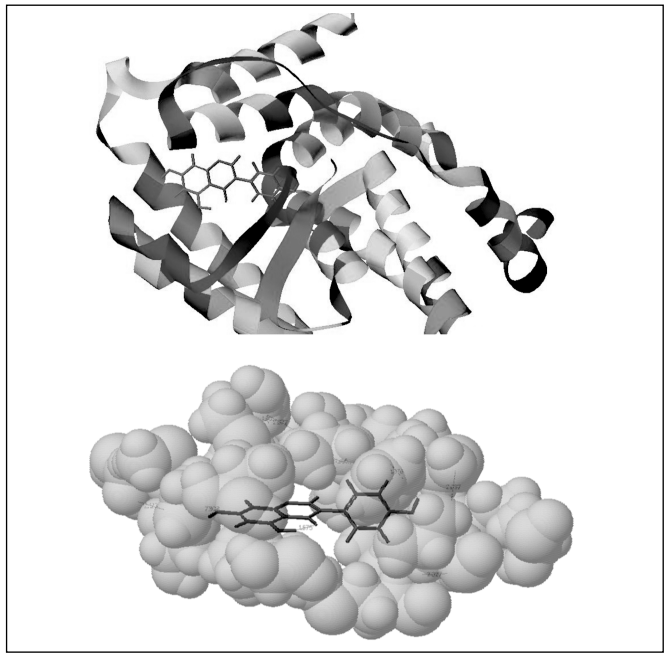

Figure 1. Genisteín in the active site of the estrogenic receptor ß (1QKM) (Pike et al., 1999).

researchers in rational drug design (Perola et al., 2006; Gopalakrishnan et al., 2005; Claussen et al., 2004; Good et al., 2003)

Materials and methods

In the present study it was carried out a preliminary docking of several Phytoestrogens and other related compounds with affinity for the beta estrogenic receptor (Shi et al., 2001) using Scigress Explorer® 7.0 from Fujitsu Limited (Scigress Explorer, 2008). As reference it was used a receptor complex with Genistein retrieved from the Brookhaven Protein Data Bank (Berman et al., 2000) identified as 1QKM (Pike et al., 1999) (Figure1). The complex was converted from .pdb to .csf format using standard functionality from the Workspace Scigress Explorer module.

The estrogenic receptor complex is formed for four chains. Since the four active sites in the different chains are equivalent, the receptor complex structure was simplified to a monomer (A chain). This monomer is a complete subunit that contains all the residues and the cofactor necessaries for the drug receptor interaction. The water molecules outside the active site were deleted. Then the residues in the active site were determined using the ligand Genistein (1) in the complex and selecting the residues in a range of 3Å around the ligand. This search showed that the active site was formed by the following residues: methionine 30 (MET 30), leucine 33 (LEU 33), threonine 34 (THR 34), leucine 36 (LEU 36), alanine 37 (ALA 37), glutamic acid 40 (GLU 40), leucine 74 (LEU 74), methionine 75 (MET 75), leucine 78 (LEU 78), arginine 81 (ARG 81), phenylalanine 91 (PHE 91), isoleucine 108 (ILE 108), isoleucine 111 (ILE 111), histidine 205 (HIS 205), leucine 206 (LEU 206) and methionine 209 (MET 209). These residues form a pocket with important hydrophobic characteristics to accommodate the different ligands.

For the evaluation it was selected a 0.3 Å/grid resolution. The tridimensional coordinates for the ligand visualization were assigned by the Scigress software. Before the ligand docking study, a validation test was made in order to find the best parameters for the docking process with the program. The docking performance was evaluated using the Root Mean Square Deviation (RMSD) for each one of the different docking positions of the ligand Genistein with respect to the crystalline structure. The ligand Genistein was docked again in the active site and the best pose showed an RMSD value of 1.3Å and a ΔG of -55,891 Kcal/mol

Ligand 3D structures of different Phytoestrogens and related compounds reported by Shi et al. (Shi et al., 2001) were built using the Scigress Explorer v7.0 software editor. Then they were minimized using Molecular Mechanichs (MM) using the Allinger MM3 Augmented force field (Allinger et al., 1989), using Block Diagonal Newton Raphson and 0.001 Kcal/mol convergence value. The conformer with lower energy for each compound was obtained using Conflex. These conformers were used for the docking simulation with the ER β. Molecular structures of the studied compounds and the target receptor were prepared and docked through Scigress Explorer v7.0 (Fujitsu) allowing flexibility to ligand and active site residues. The analysis was made by triplicate, using genetic algorithm and a box size of 15, 15, 15 Angstroms for x,y,z for the simulation. The results for the training set are reported as ligand receptor interaction ΔG mean values and are shown in Table I.

Table I
G values obtained in the docking study for the training set

Compound	ΔG, (Kcal/mol)
Genistein,1	-56,239
17β Estradiol,2	-36,014
Chalcone,3	-40,775
Coumestrol,3	-53,729
Daidzein,5	-52,580
Formononetin,6	-8,389
Myricetin,7	-47,587

The training set was determined using the previous docking results and trying to include phytoestrogens with different structures and previously reported affinity for the beta receptor (compounds in the training set are shown in Figure 2). For each compound it was selected the conformation that best fit the receptor using the best docking score in order to be used for the pharmacophore generation. This training set was used to build a database using Sybyl 8.1. After that it was used GALAHAD®, pharmacophore generation module of SYBYL, to generate the pharmacophore models. GALAHAD aligns a set of molecules that share a common mode of biological activity, and develops a pharmacophore hypothesis for them. GALAHAD uses a genetic algorithm, pharmacophore triplet/quartet finger prints and a multi-objective scoring function in order to evaluate the different pharmacophore models.

The pharmacophore models are Hypermolecules each of which contains data from all the molecules in the training set. This data is implicit in the ligand alignment and overlapping pharmacophoric features. The returned models are ranked by their Paretto score, and can then be examined in order to choose the model that better fits the following terms:

- HBond is the scoring function related to the overlap of pharmacophoric features. Despite

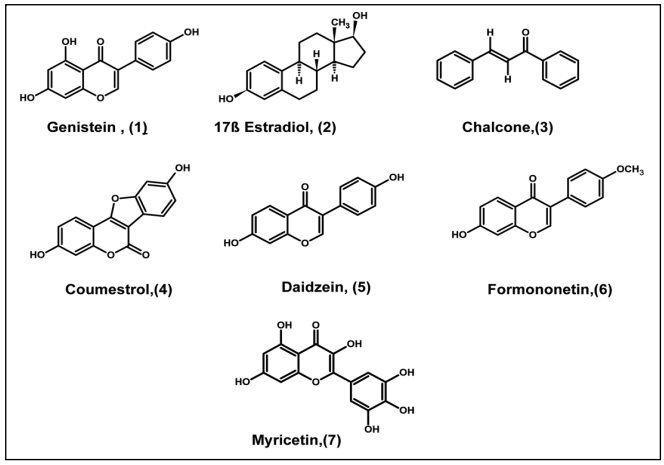

Figure 2. Compounds included in the training set used in the pharmacophore model generation with GALAHAD.

the name, it considers all features, not just hydrogen bonding.

- Sterics is the scoring function that assesses shape similarity.
- Energy term indicates the total energy of all the molecules in the training set for the conformations encoded in the torsional chromosome.
- Specificity is a logarithmic indicator of the expected discrimination of each query, based on the number of features it contains, their allotment across any partial match constraints, and the degree to which the features are separated in space.
- N_HITS is the number of molecules in the dataset that hit the query when a Unity search is performed

Additionally it was made a visual review of the different models. As it was known that all the studied molecules interact with the same receptor, is reasonable that they will adopt similar positions in their union with it. In that way models that do not present a good overlap are not candidates to be the best pharmacophoric model. The experimental values for the different models are shown in (Table II). Using these values and the visual review, model 3 was selected as the proposed pharmacophore.

In order to validate the chosen pharmacophore model, a new database for the validation set was built and is shown in Figure 3 (compounds 8-17). The compounds in the validation set are aligned individually with the proposed pharmacophore, and a validation table is generated (Table III). Finally the alignments are compared in order to determine if the reported selective compounds present a good fit with the proposed pharmacophore.

Results and Discussion

It has been reported that Estrogens exert their physiological effects through at least two estrogen

Table II

Experimental values for the different generated pharmacophore models

Model number	Specificity	N Hits	Feats	Energy	Sterics	Hbond
1	2.479	7	8	7.28	198.6	144.1
2	3.685	6	8	186.68	216.8	133.7
3	3.425	7	8	12.66	211.7	135.3
4	2.773	7	6	4891.76	215	138.8
5	3.667	7	8	11.52	192.6	146.3
6	3.664	6	8	11.95	200.2	145.6
7	2.575	5	9	19.32	201.9	140.8
8	3.753	7	7	6.61	212.8	121.3
9	2.871	6	7	14.33	215.2	133.2
10	3.628	6	7	7.92	207.98	129.5
11	3.702	7	8	51449.36	216.4	170.2
12	3.95	6	8	903.46	221	129.1
13	3.42	6	8	17.3	215.8	130.6
14	4.054	7	6	12.93	210.9	136.8
15	3.402	5	9	50.17	210.8	138.7
16	2.619	6	9	7.34	202.5	123.7
17	3.668	7	8	22.96	215.7	123.8
18	3.592	5	7	7.49	203.7	131.1
19	3.201	5	10	9.02	209	133.2
20	3.751	6	7	38.41	207.4	137.3

receptor (ER) subtypes, ER α and ER ß (Green et al., 1986; Mosselman et al., 1996). They are members of the family of nuclear hormonal receptors and the act as transcription factor when activated by the ligand (Nuclear Receptors Nomenclature Committee, 1999). In the present study the interest is to generate a 3-D pharmacophore model for compounds with selectivity for the ER ß. As a model it was used a complex between ER ß and Genistein, phytoestrogen with known selectivity (Morito et al., 2001; Zhao and Brinton, 2005), available in the Brookhaven Protein Data Bank identified as (1QKM) (Pike et al., 1999).

Given the availability of the estrogen receptor affinity data for several Phytoestrogens and related compounds (Harris et al., 2005; Good et al., 2003; Shi et al., 2001) it was decided to exploit this information to develop a molecule-derived pharmacophore model that would capture the primary chemical features common to these compounds. This is a powerful method for finding novel ligands and has been used extensively in drug discovery research in academia and pharmaceutical industry (Gopalakrishnan et al., 2005; Claussen et al., 2004).

The training set used to generate the pharmacophore includes compounds with known selectivity for the ER ß receptor (Shi et al., 2001; Harris et al., 2005) the natural ligand 17ß Estradiol (2) and Chalcone (3). These compounds presented a docking similar to Genistein in the previous docking evaluation (Results shown in Table I).

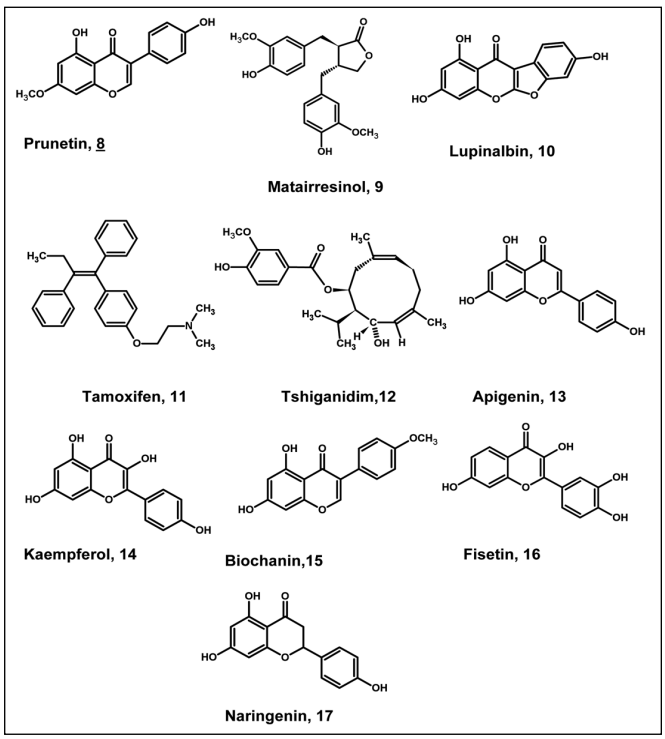

Figure 3. Compounds in the validation set.

The training set was used for the generation of an extended pharmacophore model by applying GALAHAD, pharmacophore generation module of SYBYL modeling software from Tripos. The generated models were analyzed based in their visual superimposition, their paretto graphics, and the obtained values for hits number, specificity, energy, steric and HBond. Model 3 was chosen as the best model using the program ranking and the visual analysis. This model presents three hydrophobic features colored in blue and four HBond acceptor features (colored in green) Figures 4 and 5.

The selected pharmacophore model was validated by making individual alignments of the compounds in the validation set with the model. The validation set includes compounds with reported selectivity for the ER ß similar to Genistein (Shi et al., 2001; Harris et al., 2005), Prunetin (8) a Genistein prodrug (Joseph et al., 2007), Matairesinol (9) a lignane with flexible structure (Niemeyer et al., 2003 and Ivon et al., 2005) and Lupinalbin (10), a rigid Phytoestrogen with selectivity for the ER ß (Miller et al. 2003). Structures shown in Figure 3. Of special interest was the aligment with Lupinalbin (10) because of its rigid structure and selectivity for the receptor. The obtained values for the alignments are shown in Table III.

The compounds that presented the worst alignment were Tamoxifen (11), compound with low selectivity for ER ß and Tshiganidim (12) compound with a ten member ring with flexibility. They had lower values for Hbond and presented a partial alignment with the receptor. Alignment examples are

Table III

Individual alignment values for the validation set

Compound	Energy	Sterics	HBond
Prunetin,8	4.99	272.4	406.9
Matairresinol,9	4.27	84.6	1191.7
Lupinalbin,10	2.09	436.5	666.6
Tamoxifen,11	7.67	199.7	0
Tshiganidim,12	90.2	122.5	57
Apigenin,13	2.9	719.5	462.6
Kaempferol,14	4.07	2067.3	3052.1
Biochanin,15	4	661.7	359.1
Fisetin,16	4.32	526.7	2950
Narigenin,17	2.46	49.1	128.3

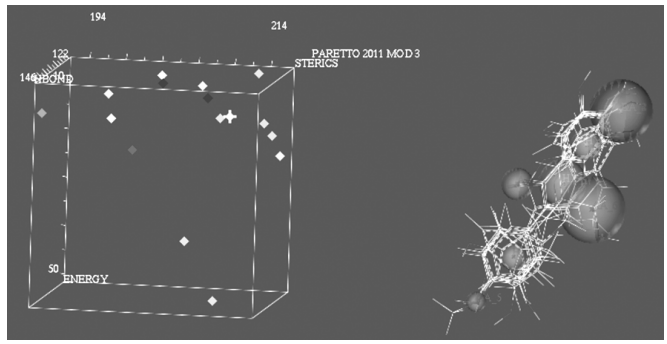

Figure 4. Paretto graphic of Model 3.

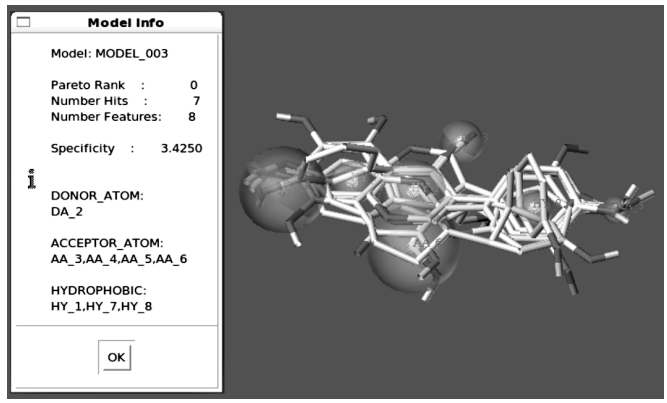

Figure 5. Pharmacophore GALAHAD Model 3 obtained from seven compounds in the training data set includes three hydrophobes(blue), one HBond donor atom(not shown), and four HBond acceptor atoms (green). The sphere sizes indicate query tolerances.

shown in Figure 6. From the obtained values and the alignments visual analysis, it can be concluded that the compounds with similar affinity to Genistein present a good alignment with the model, low energy and relatively high values for sterics and Hbond (higher values indicate best alignment with the pharmacophore). Lupinalbin (10), the rigid phytoestrogen with selectivity for ER ß also shows a very good superimposition with the model and a similar docking with the receptor (Figure 7).

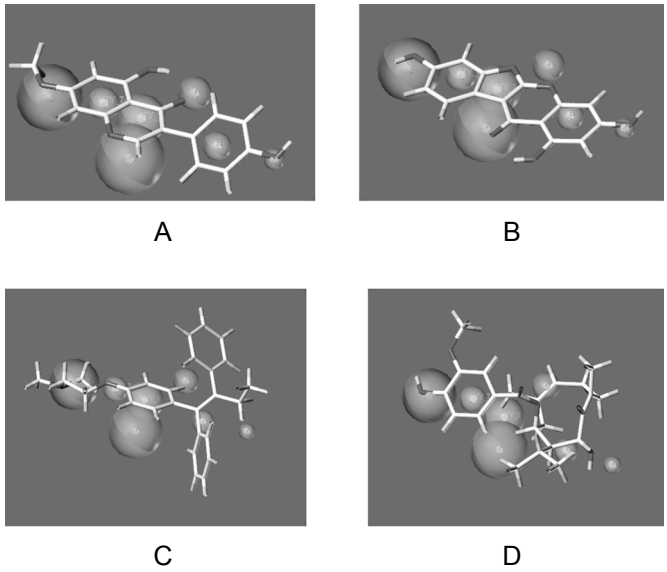

Figure 6. Examples of Alignment with Model 3. A: model 3+ Prunetin; B: model 3 + Lupinalbin; C: model 3 + Tamoxifen and D: model 3 + Tshiganidim.

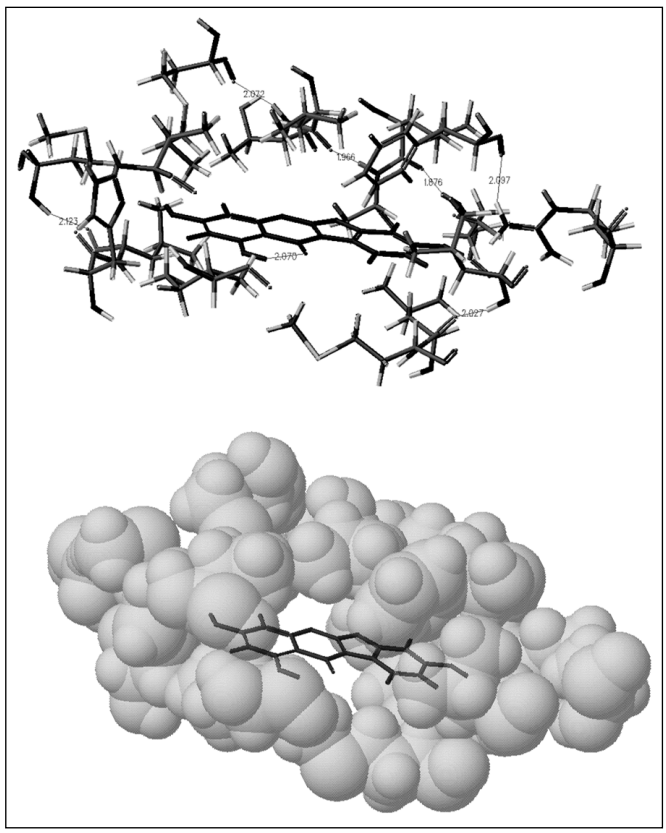

Figura 7. Lupinalbin docked in ER ß active site of, G= -62,506 kcal/mol.

Conclusions

From our results we can conclude that the obtained pharmacophore model has the requirements to produce a higher selectivity for the ER ß and can be used to identify new compounds with selective affinity. This pharmacophore model provides a hypothetical picture of the main chemical features in Phytoestrogens responsible for the selectivity for the beta receptor and broadened the vision for the generation of more selective compounds. It would be useful for the future development of more potent analogues based on rational design. It also would be used to optimize and enhance the selectivity of the lead known compounds and to evaluate how well any newly designed compound maps in the pharmacophore developed in this study.

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