Study of interactions energies between residues of the active site of Hsp90 and the geldanamycin analogues using Quantum Mechanics/Molecular Mechanics (QM/MM) methods.

Ricardo Vivas-Reyes[1], Alejandro Morales-Bayuelo[2,3], Carlos Gueto[1], Juan C. Drosos[1], Johana Márquez[1], Rosa Baldiris[4], Maicol Ahumedo[1,2], Catalina vivas-Gomez[5], Mauricio E. Orozco-Ugarriza[6].

[1]Grupo de Química Cuántica y Teórica. Programa de química Facultad de Ciencias Exactas y Naturales Universidad de Cartagena.

[2]Grupo de investigación (CIPTEC). Facultad de Ingeniería. Programa de Ingeniería de Procesos. Fundación Universitaria Tecnológico Comfenalco. Cartagena-Colombia.

[3]Ciencias de la Salud, Grupo de Investigaciones Básicas y Clínicas de la Universidad del Sinú (GIBACUS), escuela de medicina, Universidad del Sinú, seccional Cartagena, Colombia.

[4]Grupo de Bioinorganica. Programa de química Facultad de Ciencias Exactas y Naturales Universidad de Cartagena.

[5]Grupo GINUMED. Corporación Universitaria Rafael Núñez. Facultad de Salud Programa de Medicina. Cartagena-Colombia. Enfermera Universidad de Cartagena.

[6]Universidad de San Buenaventura - Cartagena - Facultad de Ciencias de la Salud - Grupo de Investigación Microbiología & Ambiente (GIMA) - Calle Real de Ternera, Diagonal 32, No. 30-966, Cartagena, Código postal: 130010 - Colombia.

Abstract

(Hsp90) 90KDa heat shock proteins are molecular chaperone involved in process of cellular oncogenesis, hence its marked importance as a therapeutic target. An inhibitor of Hsp90 chaperone activity is the geldanamycin, a thready compound that has the power to bind to the binding of ATP in the N-terminal of Hsp90 domain site, however this reported in clinical trials hepatotoxic damage, which I pitting to its disuse. On the other hand, taking advantage that the geldanamycin joins successfully Hsp90, efforts have focused on the search for similar that they have the same or better effect and both exhibited lower effects than its predecessor, as it has been the case with the similar 17-AAG and 17-DMAG. In order to know the chemical factors influencing the growth or decay of the biological activity of such compounds have been evaluated different computational techniques such as docking and 3DQSAR, however it has not been considered in a quantitative way what happens within the active site represented in terms of interaction energy, as it has been evaluated in this work. To evaluate interaction energies found that the Lys58 is essential for the union of the analogs to the active site of Hsp90, and that the activity of these will depend on if these have on the C-11 position of the macrocycle a group of small and at the same time attractor of electrons, as it is reflected with the series C-11 hydroxy and methoxy C-11. Theses outcomes were supported with Quantum Similarity and reactivity indices using the Density Funtional Theory in order to understand the non-covalent stabilization in the active site for theses compounds.

Keywords: Hsp90, geldanamycin analogues, PM6, 3D-QSAR, QM/MM approach, Molecular Quantum Similarity.

1. INTRODUCTION

The molecular chaperones in recent years have been of great interest to the scientific community, since they play an important role in apoptosis and cellular oncogenesis. Maintain the correct folding and the three-dimensional conformation of proteins in the cell and control the balance between synthesis and degradation of many proteins are some of the main functions of the chaperone proteins [1]. Some of them play an important role when, under certain conditions, the protein suffers damage, an example of these is (Hsp90) 90KDa heat shock protein, a dependent ATP [2-6], the Hsp90 molecular chaperone is highly conserved in many species [7-9]. Cell index Hsp90 includes 1-2% of cellular proteins under conditions of non-stress, and can be found in the cytosol, nucleoplasm,

lattice endoplasmatic and mitochondria [10, 11]. Hsp90 is involved in the maturation of oncogenes and plays an important role in the survival, invasion, proliferation, metastasis and angiogenesis of cancer cells [12]. The expression of Hsp90 is associated with many types of tumors including cancer of the breast, pancreatic carcinoma, leukemia, systemic lupus as well as resistance to many drugs [13]. The viability of the Hsp90 as a therapeutic target in cancer is associated to: (1) that Hsp90 is involved in folding and stabilization of a wide range of proteins involved in oncogenesis and malignant progression customers that makes cancer cells particularly dependent on adequate function of Hsp90, (2) the micro environmental conditions found in tumor hypoxia for example low pH and a bad nutritional status may tend to destabilize proteins, making it even more dependent on activity Hsp90. (extraordinary dependence of tumor cells of Hsp90 is consistent with the increase in the concentrations in tumor cells of this protein). [14]

Hsp90 is a homodimer, whose domains are important for the development of their chaperone activity, these being: (1) the domain N-terminal highly conserved, whose molecular weight is 25KDa, and which is the site of Union of the ATP, (2) the Middle domain, which is the site of the proteins customers and whose molecular weight is (3) the c-terminal domain that is responsible for the homodimerization of Hsp90 and 40KDa. [15] Because the Hsp90 chaperone activity can be inhibited when it blocks some of the sites specified in any of the three domains, today the Hsp90 inhibitors have been categorized according to the different modes of inhibition in (1) blocking of the binding site for ATP, (2) breakdown of the interactions of the co-chaperone/Hsp90, (3) associations antagonism client/Hsp90 and (4) interference with the modifications Hsp90 pos-transduccionales [14]. That is why inhibition of Hsp90 is a promising drug alternative [13,16]. One of these inhibitors known is geldanamycin, an antitumor antibiotic that has the faculty to join the binding site of ATP in the N-terminal of Hsp90 domain, triggering the loss of chaperone Hsp90 [7,14,17] function with this arrangement. Although the geldanamycin shows a high cytotoxicity testing power, is also reported in clinical trials hepatotoxicity, hence its disuse [8, 14,18]. Currently have synthesized analogs of geldanamycin in which toxic side effects have been reduced and anti-tumour activity has increased, among others the most known are: 17-allylamino-17-demethoxygeldanamicyn (17AAG) and 17-desmethoxy-17-N, N dimethyl amino ethyl amino geldanamicyn (17DMAG) [19,20]. This is why currently seeks to decipher the necessary guidelines to help design the best geldanamycin analogs and which make disappear the toxic effects of its predecessor. This paper presents an analysis based on the combination of different techniques and computational methods (docking, docking-fp6, 3DQSAR semiempirical and residue-ligand interactions) and Quantum similarity, whose purpose is to shed some light onto the role of the changes affecting the activity of the geldanamycin analogs when amending the substituents of c-11 and C-17s of the macrocicle positions.

2 METHODOLOGY

2.1.1 Molecular Mechanic Approach.

2.1.2 Set of study

Experimental Activity date of this study data were taken from the work of Tian *et to the* [21], in this paper was reported the synthesis of analogues 48 of geldanamycin with their biological activities (values of IC_{50}). For analysis only considered analogous 43, due to

others not reported an exact in the value of IC_{50} values. In the Figure 1 the carbon atom C-11 and C-17s of the macrocycle of geldanamycin positions are depicted and in Tables 1 and 2 the substituents of these positions for each analogue are tabulated with their respective biological activities expressed in terms of PIC_{50}.

Please, put here Figure 1, Tables 1 and 2. Here

2.1.2 DESIGN OF STRUCTURES

For the design of the structures of the geldanamycin analogs was taken as template the analog 17-DMAG Hsp90 (code: 1OSF Protein data bank, Figure2a), in order to assist the construction of the other molecules of study as a whole, since such a structure has the bioactive formation (Figure2b). For the design of the structures use the Sybyl 7.3 program. [22].

Please, Put Figure 2. Here

2.1.3 OPTIMIZATION OF STRUCTURES

Once finished the design of the structures of the geldanamycin analogs proceeded to relax all the substituents of the macrocycle different template base using a molecular dynamics. The macrocycle analogues left rigid so that it did not lose the formation bioactiva (Figure3). Upon completion the dynamics performed optimization using the method semiempirical aproach PM6 implemented to MOPAC 2009 [23].

Please, Put Figure 3. Here

2.1.4 DOCKING

With the optimized structures of study as a whole were to perform the docking, which used the Crystal structure of the N-terminal of Hsp90 human domain (code 1OSF PDB). The process of docking was carried out for each of the similar study using the docking AutoDock program version 1.5.4 and its graphic interface ADT 4.2 [24]. The parameters taken into account to perform the docking were: that the ligands were flexible and that the program generates 100 poses for each ligand.

Once obtained the results of the docking, proceeded to optimize all the hydrogens from each of the poses of AutoDock using the semiempirical method PM6 of MOPAC 2009. This in order to retrieve information represented in interactions with other than those considered by AutoDock hydrogen atoms, in addition to obtain energy of interaction to a more sophisticated level of theory.

To perform the optimization with the semiempirical approach was considered waste within a radius of 5Å with centre in the ligand.

Once completed the previous procedures, make the selection of the best poses of each methodology (AutoDock and methodology hybrid AutoDock PM6), taking into account the following parameters: a) For the selection of the best pose of each analogue studio used as

criterion the pose that you reported AutoDock of lowers energy within the histogram of greater population and b) For the selection of the best pose given by methodology hybrid (AutoDock-PM6) used selection criteria was based on the energy of the complex, i.e. was selected as the best pose that the complex was more sTableenergy. Highlighting the fact that the energy calculation of quantum-mechanical type.

Subsequently be proceeded to generate three models 3DQSAR, obtained from to: a) simple alignment based on the superposition of structures optimized analogues of geldanamycin **(model A)**, b) an alignment based on the superposition of structures obtained from docking (**model B**) and c) an alignment based on the superposition of structures obtained from the methodology hybrid AutoDock PM6 (**model C**). The alignments were made using the Sybyl 7.3 program, and were generated using CoMFA method, using the PLS (Partial Least Squares) to a maximum of 10 components.

In contrast, the energy of interaction (E.I) was calculated in order to observe what is happening within the site asset of Hsp90 once bind analogues of geldanamycin used in this study, and with the information obtained is pretend to explain the influence of certain interactions in pro or against biological activity in quantitative terms, and to a certain extent identify according to the observed trend which are "critical" possible residues which are linked to this fact.

The idea of the calculation of the energy of interaction, was performed in order to find due to information obtained from the 3DQSAR model do not understand of a whole, why to vary the substituents at positions C-11 and C-17s of the macrocicle of the geldanamycin changed activities, accompanied by the fact that certain analogues have high activities with respect to others, so apparently no corresponding because between them there is a very marked structural difference.

In order to perform this study, they were obtained complexes and subsequently proceeded to defragment waste around each analogue, in order to calculate the energy of interaction each of them. The energy of interaction was calculated using the Equation 1.

Interaction energy ligand - residue (E.I) = E_{LM} - E_L – and E_M , where E_{LM}, E_L, and E_M denote the energy ligand-residue, energy of the ligand and evaluated residual waste respectively.

2.2.1 Molecular Quantum Similarity Measure. Generalities.

The DFT context is used to analyze the non-covalent stability of the compounds on the active site. In this order of ideas the Quantum Similarity and reactivity indices frameworks were used. A Molecular Quantum Similarity Measure (MQSM), between two systems A and B, denoted Z_{AB}, is a comparison between two molecules that can be constructed using their respective Density Functions (DFs). Both DFs can be multiplied and integrated over all the respective electronic coordinates, in turn weighed by a defined positive operator $\Omega(r_1,r_2)$ **[25-27]**:

$$Z_{AB} = \langle \rho_A | \Omega | \rho_B \rangle = \int\int \rho_A(r_1)\Omega(r_1, r_2)\rho_B(r_2)dr_1dr_2 \qquad \textbf{(1)}$$

The nature of the operator used in the equation **1** will provide the information that will be compared between the two systems and at the same time will name our measure of similarity; when the operator chosen is the Dirac delta function (a function that founds a very useful approach for functions with high peaks, such as electronic density, and constitutes the same type of mathematical abstraction as a charge or point mass.), this is $\Omega(r_1,r_2)= \delta(r_1- r_2)$, we obtain an overlapping MQSM, one of the first similarity measures used; another widely handled possibility is the use of the Coulomb operator, this is, $\Omega(r_1,r_2)= |r_1- r_2|^{-1}$, obtaining a coulombic MQSM. A measure of similarity can be applied between two molecular systems any, including the case in which the two molecules are equal, in this case the measurement is called measure of self-similarity (Z_{AA} for the case of molecule A) **[26]**.

Given a group of N molecules, we can obtain, for each of them, a measure of similarity with respect to each of the group's molecules, including itself. From all these obtained measurements we can construct a symmetric matrix. The i-th column of the matrix can be considered as the collection of all measures of similarity between the i-th molecule and each element of the group, including itself. This is why each vector (each column of the matrix) can be considered as a discrete N-dimensional representation of the i-th structure. These collections of vectors can be considered as a group of molecular descriptors. However, this collection of columns of the similarity matrix does not constitute simply another group of molecular descriptors like those that are generally used to theoretically describe a given molecule; each descriptor has the following particular characteristics **[26,27]**:

- **i.** Universality, in the sense that it can be obtained from any group of molecules and for any molecule within the group.
- **ii.** Impartiality, since in the construction process, there are no other options than those provided by the knowledge of the density functions and the similarity measures involved.

2.2.2 Manipulation of MQSM and visualization techniques. Similarity indexes.

Once we have chosen a group of study objects and the operator related to the MQSM in equation **1**, the measure of similarity obtained for the group is unique; however, it is common practice to transform or combine these measures to obtain a new class of auxiliary terms that can be called Quantum Similarity Indices (QSI). There is a vast amount of possible manipulations of MQSM that lead to a variety of QSI definitions. The most used are the following and by this reason are used in this work **[28-30]**:

2.2.3 Carbó's similarity index between two molecules I and J is constructed like this:

$$C_{IJ}(\Omega) = z_{IJ}(\Omega)\left[z_{II}(\Omega)z_{JJ}(\Omega)\right]^{-1/2} \quad \textbf{(2)}$$

That corresponds to the cosine of the angle subtended by the density functions involved, taken as vectors, for which this index is also called the cosine-like similarity index. This Carbo QSI, for any pair of molecules compared, it has a value between 0 and 1, which depends on the similarity associated with the two molecules (when I = J the index will approach 1) **[28-30]**.

2.2.3 The quantum Similarity using the euclidean distance:

Considering the similarity equation **3**.

$$D_{IJ}(k,x,\Omega) = \left[k\left(z_{II}(\Omega) + z_{JJ}(\Omega)\right)/2 - xz_{IJ}(\Omega)\right]^{1/2},\ x[0,\ k] \qquad \textbf{(3)}$$

For which, if k= x= 2, it is reduced to the so-called euclidean distance index. We can also define the index **3** of the form:

$$D_{IJ}(\infty,\Omega) = max\left(z_{II}(\Omega),\ z_{JJ}(\Omega)\right) \qquad \textbf{(4)}$$

This equation **4** constitute the distance index of infinite order **[31]**.

2.2.4 MQSM definition used in this work.

The measures of quantum similarity are based on psychological perception and they are based on the obvious principle of similarity: "*the most similar between two molecules, the most similar between their properties*". This statement requires producing a comparison between two molecules, to obtain a quantitative measure of quantum similarity of the range of similarity between two molecules, they are based on the comparison of their densities. For the range of similarity between two compared systems, previously we obtain the technique that will be used for the static description. Generally, the MQSM can be defined with the meaning of the integral computational measure between two tentative density functions involving molecular systems. The density functions are multiplied and integrated for the electronic coordinates in the convenient domain, the MSQM can be single defined at the scale of the first order molecular density functions associated with the compared molecules, and with positively defined operators **[26-29]**.

$$Z_{IJ}(\Omega) = \langle \rho_I \,|\, \Omega \,|\, \rho_J \rangle = \int\int \rho_I(r)\Omega(r_1,r_2)\rho_J(r_2)dr_1dr_2 \in R^+ . \qquad \textbf{(5)}$$

Where A and B are the two quantum objects studied, $\{r_1\ ,\ r_2\}$ they are the set of electronic coordinates associated with the corresponding wave function, $\{\rho_A\ ,\ \rho_B\}$ of the first order and $\Omega\ (r_1,r_2)$ positively defined supported in the operator, dependent on the coordinates of the electrons **[24-29]**.

2.2.5 Types of measures in molecular quantum similarity.

It depends essentially on the information required, seriously on the selection of the supported operators, producing different types of MSQM **[8-10]**.

2.2.6 MQSM de overlap considering the equation 2:

It is the simplest and most intuitive usual choice of a positively defined operator, it is the distribution Dirac's delta, $\Omega\ (r_1,\ r_2) = \delta\ (r_1,\ r_2)$. This selection transforms the general definition of MQSM specifically to calculate the overlap MQSM, which obtains measurements of the volume enclosed in the superposition of both electronic density functions **[26-29]**.

$$Z_{IJ}(\Omega) = \int\int \rho_I(r_1)\delta(r_1 - r_2)\rho_J(r_2)dr_1dr_2 = \int \rho_I(r)\rho_J(r)dr \qquad \textbf{(6)}$$

The Dirac delta function comes intuitively from physical definition, and is computationally compliant. The MQSM comes from information on the concentration of electrons in the molecule, and indicates the degree of overlap between the molecular comparison **[25-30]**.

2.2.7.MQS de coulomb considering the equation 2:

If the operator (Ω) is adopted by the Coulomb operator, Ω (r_1, r_2)= $\frac{1}{|r_1 - r_2|}$, it provides the coulomb MQS, which represents the electrostatic repellent coulomb energy between two charge densities **[30,31]**:

$$Z_{IJ}(\Omega) = \int\int \rho_I(r_1) \frac{1}{r_1 - r_2} \rho_J(r_2) dr_1 dr_2 \quad \textbf{(7)}$$

The coulomb operator does the effect for the overlap density functions. Considering the functions of molecular density as an electron distribution in space, this expression is only for the extension of coulomb for the distribution of continuous charge, and for that reason it can be considered, in some occasion as descriptors of electrostatic potential. This operator obtains the measurement of electrostatic repulsion between electronic distributions, and is associated with electrostatic interactions **[25-29]**.

2.2.8 Euclidean distance Index considering the equation (3):

This is another typical transformation that can be defined according to the classical distance:

$$d_{ab} = \left[\sum_{j=1}^{p} (\Delta x_j)^k \right]^{1/k} \quad \textbf{(8)}$$

Where $\Delta x_j = x_{aj} - x_{bj}$ is the distance between the objects a and b, and k=2 for the definition of distance. The euclidean distance between two quantum objects A and B is defined by **[25-29]**:

$$d_{ab} = \sqrt{(x_a - x_b)^2}\,. \quad \textbf{(9)}$$

Occasionally it is expressed as: $D_{AB} = \sqrt{Z_{AA} + Z_{BB} + ZZ_{AB}}$. D_{AB} has values in the range of $[0,\infty)$ but, converges for previous cases, it has a value of zero between the compared objects, if the compared objects are identical **[25-29]**:

$$D_{AB} = 0 \quad \textbf{(10)}$$

Geometrically this index can be interpreted by the norm of the differences between the density functions of the compared objects. The index of the euclidean distance can be defined by the distance or dissimilarity index, the index can also be expressed as **[25-29]**:

$$D_{AB} = \| \rho_A - \rho_B \| = \sqrt{(\rho_A - \rho_B)^2} \quad \textbf{(11)}$$

2.2.9 Alignment method: Topo-Geometrical Superposition Algorithm (TGSA).

In this work the alignment was performed using the TGSA **[32]** method. The TGSA was proposed by Gironés and programmed and implemented by the same author. This method considers that the optimal alignment of molecules is carry out through superposition on the common skeleton, taking only into account the type of atoms and the bond of the interatomic interactions, which is the atomic number the coordination, to carry out its purpose the algorithm examines the atomic pairs of the molecules and aligns the common substructure for a series of molecules **[32]**. The method is only based on topology and geometric considerations, where the molecular topology is manifested in the way of comparing the distant bonds. In two molecules, the superposition is unique and does not depend on the type of operator chosen to provide the meaning of the similarity **[32]**.

First, molecular coordination and atomic number are necessary to indicate the performance of the program. The molecular coordination is ordered in bases according to the decrease of the atomic number, in order to determine a path for the number of hydrogens in the molecule **[32]**.

Considering that the superposition of hydrogens is not significant, and with the required computational requirements, the hydrogen atoms are not included in the process. The next step is the definition of the atomic pair, the duo is defined only if the pair of atoms in the box is involved, with their respective determinants, the duo has to be defined for each molecule, all molecules behave with each other with their respective meanings of interatomic distances, obtaining translocations. The translocations are taken within the fluctuations of the spine of the conformations produced by the presence of the different substitutions in the molecules **[32]**. This procedure always discards bons that are not common with skeletons. Once the duos are compared, the algorithm creates atomic triads by adding three atoms selected from the duos. These supplementary atoms must be in the box to later form the considered duo. In geometric terms, this generates a triangle in the plane, where the atoms occupy the vertices of the triangle, and the sides correspond to the effectiveness of the chemical box **[32]**.

The triangle obtained for a molecule is compared for the triangle obtained by the second molecule with the respective interatomic distances, and with the translational distances in the comparison duo. If the three distances of both triangles compared are similar, both triads are similarly considered and stored. The triads that do not meet the classification criteria are automatically discarded to complete the comparison, the selected triad is superimposed and the result of the molecular alignment is determined univocally **[32]**.

This process is repeated for the atoms and the algorithms chosen are those of the alignment that maximizes the number of atoms superimposed, minimizing the index C_{IJ}, it is used by the comparison criterion of the interatomic distances and this cost calculation with the absolute value of each difference with the composite after-location:

$$C_{IJ} : \sqrt{\frac{\sqrt{d_{II} d_{JJ}}}{d_{IJ}}} \qquad \textbf{(12)}$$

Where $d_{IJ}:\sum_{i:1}^{n_A}\sum_{j:1}^{n_B}|\ x_{i,I}-x_{j,J}|^2$, n is the number of atoms and x the molecular coordination, C_{IJ} is determined in the interval $\left[0,1\right]$, evaluating the quantification of the overlap. This indicates better the alignment when C_{IJ} it approaches unity, originating the ideal case of structural identity C_{IJ}: 1.

The TGSA method considers the molecules as rigid bodies, so there is no flexibility in the structure (nothing of rotation and vibration in the distances of the angles in the box). This is designated by the operator in the homogeneous set of molecules and does not yield good results with different molecular structures, this comes from the alignment pair that is restricted with the common recognition skeleton. In contrast, this common recognition of substructures produces a coherent alignment with chemical intuition. This procedure is simple and has low computational requirements for this reason is used in this work **[32]**.

3 DFT Based Reactivity Descriptors

Some the present authors, in several works have shown the relationship between quantum similarity and chemical reactivity descriptors **[33-44]**. In addition, the quantum similarity and DFT uses the density function as object of study, the similarity indexes specifically the Coulomb index can be related to electronic factors associated with chemical reactivity.

Using the Frontier Molecular Orbitals (FMO) and the energy gap the global reactivity indices, such as chemical potential (μ) **[45-47]**, hardness (η) **[45-47]** and electrophilicity (ω) **[45-47]**, will be calculated. These chemical reactivity indices give an idea about the stability of the systems.

The chemical potential (μ) characterizes the tendency of the electrons to escape from the equilibrium system **[44-46]**, whereas the chemical hardness (η) is a measure of the resistance of a chemical species to change its electronic configuration **[44-46]**.

$$\mu \approx \frac{E_{LUMO}+E_{HOMO}}{2} \quad \textbf{(13)}$$

and

$$\eta \approx E_{LUMO}-E_{HOMO} \quad \textbf{(14)}$$

Electrophilicity index can be interpreted as a measure of the stabilization energy of the system when it is saturated by electrons from the external environment and is mathematically defined as **[44-46]**:

$$\omega = \frac{\mu^2}{2\eta} \quad \textbf{(15)}$$

In this work, the local reactivity descriptor are the Fukui functions (equation 16 and 17, f). The equations (16) and (17) represents the response of the chemical potential of a system to changes in the external potential. It is defined as the derivative of the electronic density with respect to the number of electrons at constant external potential:

$$f_k^+ \approx \int_k \left[\rho_{N+1}(\vec{r})-\rho_N(\vec{r})\right]=\left[q_k(N+1)-q_k(N)\right] \quad \textbf{(16)}$$

$$f_k^- \approx \int_k \left[\rho_N(\vec{r}) - \rho_{N-1}(\vec{r})\right] = \left[q_k(N) - q_k(N-1)\right] \quad \textbf{(17)}$$

Where (f_k^+) is for nucleophilic attack and (f_k^-) for electrophilic attack **[44-47]**. In this sense, using the global and local reactivity descriptors is possible study the quantum dissimilarity along the molecular set.

4. RESULTS AND DISCUSSION

4.1 Statistical results of the 3DQSAR models

InTabla3 is depicted the results obtained for the descriptors (q^2) prediction and correlation (R^2) of the three models.

Please, Put Table3. Here

4.2 Alignments used in the generation of the three models 3DQSAR

As can seen in Figure 4 the alignment used to generate the 3DQSAR **A** model, in almost perfect for all structures of the geldanamycin analogs, reaching to distinguish only substituents on the carbon atom C-11 and C-17s of the macrocycle positions, as it would be expected, since the structural differences from analogues occur in these positions. On the other hand this overlap is due to this alignment was made using a template common to all the structures. Hence, that its monotony influence little successful statistical results of the 3DQSAR (see Tabla3), in addition these results are accompanied by the fact that this type of alignment is lost information relevant to the relocation of the ligands in the active site on the basis of the substituents of the macrocycle.

AutoDock (energy categorization) and methodology hybrid AutoDock-PM6 respectively. On the other hand differences between the models B and C is the of the substituents of the macrocycle, especially those located on the C-17 and C-11 positions of the carbon atoms, as these different groups, prompting them to interact with different residues of the active site these reorganization are selected by the docking algorithm. On the other hand as noted in Tabla3 statistical q^2 descriptors and R^2 for **B** and **C** models are initially better than the model **A**, which makes us suppose that the ligand-receptor interactions help improve, that descriptors such values are reproducible, what gives these models higher degree of reliability. As is shown in Figure 5b, from alignment methodology hybrid AutoDock - PM6, shows that both the macrocycles and the common to these substituents is geared very similarly, noting the difference with the form alignment of AutoDock (Figure5 b), being evidenced by this that the methodology hybrid AutoDock-P6 reoptimiza results of docking, and therefore those of the alignment and consequently results statisticians of the 3DQSAR model (see Table3). The above issues also tell us that this methodology can be applied to studies involving the implementation of docking for a 3DQSAR model that includes information about interactions with the active site **[48,49]**.

Please, Put Figures 4 and 5. Here

In Figure 5 is depicted the alignment used for models 3DQSAR **B** and **C** respectively.

In this case the alignments were made by the superposition of the best poses obtained from

4.3 ELECTROSTATIC AND STERIC MAPS DERIVED FROM THE CoMFA

Given that the best statistical result was obtained in the model **C**, CoMFA generated from this contour map will be used in this part of the discussion. The contribution of the steric and electrostatic fields generated a R^2= 0.963 and a q^2= 0.50 for an optimal number of 7 components. On the other hand the steric and electrostatic individual contributions were of a 57.6% and 42.4 per cent respectively. Steric and electrostatic contour maps are shown in Figures 6a and 6b.

Please, Put Figure 6. Here

In Figures 6a and 6b are depicted the steric and electrostatic contours. These contours are located in a special way on positions C-11 and C-17 macrocycle, still this expected fact because these are the positions where the structural differences of the geldanamycin analogs occur.

The steric CoMFA (Figure 6a) map covers the sterically favorable contours (80% contribution) corresponding to the regions in the space where the steric volume leads to an increase in activity (green contour map), while the sterically infavorables regions (20% contribution) correspond to areas in the space where the steric volume expected decrease of activity. Map of green steric contour on the carbon atom C-17 position of the macrocycle indicates analogues with bulky substituents in this position tend to favor the activity, as it is reflected in the 3e ligands (pIC50 = 7.96) and 1f (pIC50 = 7.77) that have this peculiarity while carbon 11, the yellow outline indicates that the presence of bulky groups adversely affected activity, as noted in the 4f ligands (pIC50 = 5.77) and 8i (pIC50 = 5.57). This analysis of steric maps allows to infer that the ligands of the series **1** and **3** (are favored while to the ligands of the series **8** - **7** (Tables 1 and 2) who are disadvantaged.

On the electrostatic map (Figure 6b), the blue contours (80% contribution) indicate the regions in the space where the groups with low electron density favor activity, while the red contours (20% contribution) indicate regions where groups with high Electron density decrease activity.

The blue outlines on the C-17 position of the macrocycle indicate that the activity is favored for those ligands with substituents electroatractors in that position (ligands 1e pIC50 = 7. 62 and 3 h pIC50 = 7. 22). On the other hand the red and blue outlines on the C-11 position of the macrocycle indicate that both the electronegative and electropositive substituents can promote activity provided that they should be geared towards the contours set out on the map. This accommodates the vast majority of the ligands of this study can present any of the two conditions set out for the substituents of C-11, characteristic of this examples being ligands **1 g** (pIC50 = 7. 15) and **3d** (pIC50 = 7. 62) oriented towards the blue outline and **4b** (pIC50 = 6. 99), **3a** (pIC50 = 7. 08), **3b** (pIC50 = 6. 55) that are oriented towards the red outlines.

Maps CoMFA can be seen that the steric map is that better explains the relation structure-activity of the geldanamycin analogs, since the contours derived from this are more specific

than the electrostatic, and give a vision more clearly the type of substituents that should be on positions C-11 and C-17s of the macrocycle.

While the results of the 3DQSAR were clear they not filled expectations, because cannot be explain in all cases the behavior structure-activity of similar study. For this reason is calculating the energy of interaction (E.I) residue-ligand to observe what is happening within the site asset of Hsp90 once joined the analogs of the geldanamycin and thus to deepen the analysis of the interactions ligand (substituents) - receptor.

In the Figure 7 have been plotting the interaction energies obtained for the geldanamycin.

Please, Put Figure 7. Here

In Figure 7 are shows that the most significant values of E.I are presented with Lys58, Asp93, Lys112, waste being the most favorable to the Lys58 value. At this point we can say that these would be possible "critical" waste to analyze. On the other hand, it is expected that the interaction with the Lys58 is highly favorable because of the ease that has this waste to form a bridge of hydrogen with oxygen of the hydroxyl of the C-11 position of the macrocycle group.

The Figure 8 shows a two-dimensional picture of the interactions between the geldanamycin and the active site of Hsp90.

Please, Put Figure 8. Here

In Figure 8 can be seen and is at once evident that possible "critical" waste, interact positively with geldanamycin, that is why the results of his E.I have been significant. In this Figure shows that the Lys58 can donate a bridge of hydrogen to the C-17 methoxy group and the C-11 hydroxy group, but actually interaction is most favored with the hydroxyl group, because the methoxy group stays a bit out of the active site, making it difficult interaction with this residue contrary to what is happening with the Group hydroxy that if stays within the active site [26]. The above does not rule out that the C-17 methoxy group does nothing to the energy of interaction with this residue.

In Figure 9 have been plotting interaction energies for the composite 2 (pIC_{50}= 8.04), 3e (pIC_{50}= 7.96), 4b ($_{50}$pIC = 6.96), 6 d (pIC_{50}= 7), 8i (pIC_{50}= 5.6), 8 h (pIC_{50}= 5.57) that present high activities, media and low respectively, as well as its predecessor.

The choice of these analogs was made in order to show the changes that have the values of E.I possible waste "critical" when making modifications cellular in C-11 and C-17. In she was could corroborate observed behavior is similar to seen in geldanamycin with waste Lys58, Asp93 and Lys112, and in addition there are significant values of E.I with the Asp54, which in turn can be considered as another possible residue "critical". Interaction with the Asp54 is justified by the high electron density that has this waste, which allows it to act as bridge acceptor of hydrogen of the substituents on C-17 that are potential donors, for example the substituents possessing an amine or diamine on that position (see Tables 1 and 2)

In Figure 9, was also noted that the Lys58 and the Asp54 present a notorious gap in values of E.I with respect to the Lys112 and Asp93, which in turn means that interactions with these residues geldanamycin analogs are of high relevance, also find it interesting that those

wastes are located on positions C-11 and C-17s of the macrocycle, where precisely the substitutions occur (see Figure 8).

To verify the importance of waste Lys58, Asp93, Lys112 and Asp54 as "critical" waste it is necessary to examine the analogs of geldanamycin by family, because the structural differences will be minimal, because only varying in the substituents located at position C-17 of the macrocycle for each one of them.

Please, Put Figure 9. Here

Continuing with the analysis of the interaction energy in this occasion be jointly considered the series 11-hydroxy and 11-methoxy, since these compounds are distinguished between whether the substituent at C-11 group (see Table1). In Figures 10 and 11 have been plotting the values of the E.I for the 11-hydroxy and 11-methoxy the geldanamycin analogs. In these Figures can be observed that the vast majority of both series compounds tend to have significant E.I values with waste Lys58, Asp93, Lys112 (trend observed in the geldanamycin E.I) and Asp54, which makes us suppose that those wastes are "critical" for both series.

In Figures 10 and 11 are depicted the highest values of the E.I was presented with the Lys58 and the Asp54. It is expected the E.I for the Lys58 values tend to be taller and monotonous for series 11-hydroxy than for the 11-methoxy, since the Lys58 forms more easily bridge of hydrogen with oxygen from the Group hydroxy (the macrocycle position C-11), as does not have major complications steric or repulsive, unlike the similar 11-methoxy where such interaction is disadvantaged because the steric hindrance that presents the group methyl, but despite this in both series the atom of oxygen by effects of polarization of link holds a partial negative charge, which in turn makes it a good bridge acceptor of hydrogen (effect observed in all analogues of both series). However the values for the Asp54 are more fluctuating for both series since some analogues of these series cannot donate a bridge of hydrogen to the residue, as it is the case of the analogous 1d and 3d that lacks an atom of hydrogen in the substituent at C-17 (see Table1), hence low E.I with this residue (- 7.6577 and - 8.70502 respectively), which in turn leads to be entrenched interactions with Gly 137, Gly97, Ile96, Met98 waste compared to 1b are best. The above may be occurring because the similar 11-methoxy tend to rearranged within the active site in a way that can be interaction with the Lys58, which in turn implies that will strengthen interaction with other waste.

Please, Put Figures 10 and 11. Here

This allows us to assume that the interaction with the Lys58 is essential for series C-11 hydroxy and methoxy C-11 and that the difference between the activities of them probably lie in the ease with which gives such interaction, because the substituents of the C-17 position of the macrocycle are the same for both series and the effect they have when they interact with the Asp54 is almost the same.

In Figure 12 have been plotting the values of the E.I for 11-O-acyl geldanamycin analogs.

Please, Put Figure 12. Here

In Figure 12 is shown the trend seen in the two previous series does not follow, since most of these compounds it loses the monotony with residues Gly97 and Ile96, and the values of

the Asp54 E.I are similar to the Asp93, however retains the trend of significant the E.I values for the Lys58 like in the previous cases. The behavior observed in this series is due in particular to him volume of the substituents located at positions C-11 and C-17 of the macrocycle, then this steric hindrance conditions the formation of bridges of hydrogen with the Lys58 and the Asp54. On the other hand, as explained above the steric hindrance in the molecule induces a change involving the reshuffling of this within the active site, in such a way that these interactions can be, and that reshuffling she seeks stabilize strengthening interactions with other waste, as the case of the Gly97 and the Ile96 is series. Also in Figure12 you can see that analogues of this series despite having bulky groups in C-11 interaction with this residue remains the highest, what makes us think that the interaction with this residue is of high relevance to these analogues, and that to increase the interaction decreases the interaction with the Asp54. It is of note that the analogs that cannot donate bridge of hydrogen to the Asp54 possess low E.I as seen with similar 4e 4 d that possess a methoxy at position C-17 group, which in addition to not form the bridge of hydrogen, the atom of oxygen creates repulsion with the carboxyl group of the said waste preventing even more such interaction. In general, this series highlights the Lys58 acts as a residue "critical" and that to some extent it can affect the activity of these compounds **[48, 49]**.

In Figure 13 have been plotting the values of the E.I for the 11-ketone analogs of the geldanamycin. In this Figure you can clearly see that the E.I with the Lys58 are not significant as in the previous cases, because this time are displaced by Lys112 which happens to be the residue with the best E.I, made that it is noteworthy because C-11 is a band carbonyl that cannot easily accept the bridge of hydrogen donated by the Lys58 which would be expected. However the preference that have these analogues by the Lys112 can be occurring because the short distance and stiffness that presents the double bond to which is attached at position C-11 oxygen prevents accommodate in such a way that can be the interaction with the Lys58, which forces the molecule to rearranged within the active site and therefore to seek other interactions enabling it stabilizes is the case the Lys112, Met98 and Phe138 that in the previous installments showed a similar behavior, emphasizing the affinity for the Lys112.

On the other hand, In Figure 13 you can see that the values of the Asp54 E.I continue the trend observed in previous series, but this time as it increases the interaction with the Asp54 decreases the interaction with the Lys58, as you can see with all the analogues of this series.

Please, Put Figure 13. Here

In general, with analogues of this series you can corroborate that the formation of a weak interaction with the Lys58, forces the molecule to strengthen its interaction with other residues of the active site, in order to stabilize.

As previously mentioned these groups of compounds differ from others the geldanamycin analogs that they only have substitution in the hydrogen of the amine located in the macrocycle (Figure 2a) C-11 since the substituent at C-17 is the same for all analogues of this series. The substituents for this series are shown in Table2.

In Figure 14 have been plotting the values of the E.I for 11-amine analogs geldanamycin.

Please, Put Figure 14. Here

In Figure 14 are shown that the values of the Lys112 E.I are somewhat more significant than the Lys58. This is expected because the substituents on C-11, are not the most propitious to create interaction with the Lys58, because that do not possess within the substituent an atom which can accept a bridge hydrogen of the residue, followed by the volume of the majority of these substituents (Table2), which induces a reorganization of their interactions in the molecule that is to say that this search for waste that can interact better, and in turn will enable it to stabilize within the site active, as it is the case of the Lys112, Asp93, Thr184 and Asp54, the latter with special mention because as seen from Figure 15, its values of E.I are the highest, which in turn allows us to infer that in the absence of a good interaction with the Lys58, the compounds in this series are stabilized in particular to strengthen its interaction with the Asp54 and subsequent to this with the Lys112, Asp93 and Thr184. Further interaction with the Asp54 is more favored, since the substituent on C-17, you can donate a bridge of hydrogen to the residue, while the substituent is voluminous.

In general, the fall of the values of the Lys58 E.I, may be to some extent the cause of the low activity of these compounds, complemented by the considerable volume of the substituents on C-11. On the other hand, with these compounds it is shown that interaction with the Asp54 serves to give stability to the complexes, especially for those analogues of geldanamycin possessing a secondary amine in the C-17 from the macrocycle position as it is the case with this series.

Continuing with the analysis of E.I in Figure15 have been plotting the values of the E.I for the 11-oxime analogs of the geldanamycin.

In Figure 15 is depicted the E.I of the Lys112 values are higher than the of the Lys58, with the exception of the analogous 8a where such conduct is not followed as the E.I Lys58 (- 17,3721) is higher than Lys112 (- 15,6787). The behavior of compound 8a is that the atom of oxygen which is on the substituent at C-11 can accept without any steric problems bridge of hydrogen that you donate Lys58, accompanied by the fact that the substituent at C-17 is the least bulky of this series, which makes the molecule the creation of such interaction. The analogous 8b and 8d possessing the same substituent at C-11 to 8th, so would be expected that the values of the E.I with the Lys58 were similar to 8a (- 18.8069 for 8d and - 14.561 8b) and thus these were higher than the Lys112 (- 19.6651for 8b and - 18.2331 for 8 d) but this behavior is not observed for these two similar substituents at C-17 are quite bulky, which induces a change that involves strengthening the interactions with other waste, in order to compensate for the decrease of interaction with the Lys58, being the most notorious case of the Lys112 in the molecule.

Please, Put Figure 15. Here

For the rest of this series (8e-8i) is to be expected that the values of the Lys58 E.I are low, because the substituents at C-11 are voluminous (see Table1) analogues, which prevents the formation of the bridge by steric effects of hydrogen with the Lys58, since the molecule has to rearranged within the active site in a way that such interaction can be, which to some extent destabilizes the molecule and the force to strengthen interactions with other waste (Lys112**).**The above supplemented by the fact of the substituents at C-17 are also bulky.

In general, as in the previous series the fall of the values of the Lys58 E.I may be to some extent the cause of the low activity of these compounds, both complemented the considerable volume of the substituents in the positions in C-11 and C-17s of the macrocycle. On the other hand, it is possible as a result of the volume of the substituents at C-11 may look affected the interaction of the substituents of C-17s with the Asp54, because the molecule gives priority at a certain point which interaction helps you its stabilization within the site active, hence the E.I for that residue values are fluctuating.

As a complement to the analysis of the results are compared to E.I geldanamycin and the most active analogue 2 as shown in Figure16. From Figure 16 it is clear that the E.I of waste with both compounds do not have significant differences, which may at a certain time explain the increase in the activity of a 7.39 (GDM) to a 8.04 (2).

It would therefore be expected that the activities of these compounds are similar or failing that the activity of the compound 2 is slightly superior to the GDM. The above tells us it would be worth reviewing the value of the biological activity of this ligand.

Please, Put Figure 16. Here

4.4. Molecular Quantum Similarity Indices to the most reactive compounds with the reference compound GMD and reactivity Analysis: analysis of the non-covalent interactions on the active site.

To understand the 3D-CoMFA outcomes, a study using the Quantum Similarity field on the most reactive compounds is developed. Taking in account the reference compound GDM. In **Table 4** is shown the Overlap Similarity Indice using the **Eq. 21**, it indice is related with the superposition on the alignment method used. In this order of ideas, these indices allow us to make some considerations about the quatility of the superposition on the molecular set. The compound with the higher structural Similarity with the reference compound GDM is **1a** (0,9837, **Table 4**) with a Euclidean distance (1,0662, see **Table 5**). Therefore, the group ($-NH_2$) in the Compound **1a** shows a light dissimilarity in the alignment method. It result is agrees with the low steric effect to this group.

Please, Put Table 4. Here

The compound with lowest Similarity with respect to GDM is the compound **3e** (0,6489, see **Table 4**) with a Euclidaen distance (5,1217, see **Table 5**). It compound **3e** has two substituten groups with high steric effects according to **Table 1**. The bulky substitutens can show some problems on the superposition process. The Compound **2** with higher biology activity pIC_{50}=8.04, see **Table 1** has an Overlap indice with respect to the reference compound 0,9631 with an Euclidean distance of 1,6289, This compound has two methoxy groups that are electron-donating group. This electronic considerations have strong influence in the superposition process due to that they diminish the felxibility and the free moviment in the ramifications.

With the main intention to study the electronic effects of the group substitutes, the Coulomb indices are calculated in **Table 5** on the most reactive compounds with respect to the reference compound GMD. In **Table 6** are shows the electronic similarity indices using the Coulomb Similarity to analyses the electronic effects.

Please, Put Tables 5, 6 and 7. Here

Owing to inductive effects, the basicity of the amine group might be expected to increase with the number of alkyl groups on the amine. However, correlations are complicated owing to the effects of solvation which are opposite the trends for inductive effects, accordind to the 3D-CoMFA maps. However, solvation effects also dominate the basicity of aromatic amines. For these compound, the lone pair of electrons on nitrogen delocalises into the ring, resulting in decreased basicity. Substituents on the aromatic ring, and their positions relative to the amine group, also affect basicity as seen in the **Table 1**. To understand these electronic features. In **Table 7** are shown the global chemical reactivity descriptors to the most reactive compounds.

Please, Put Tabla 8. Here

The compound with higher global reactivity descriptors is **1c** chemical potential (-4,9587 eV), hardness (5,6360 eV), softness (0,1774 eV)$^{-1}$ and electrophilicity (2,1814 eV), the reference compound GMD has chemical potential (-4,8982 eV), hardness (5,5895 eV), softness (0,1789 eV)$^{-1}$ and electrophilicity (2,1462 eV). The compound with higher biology activity **2** has chemical potential (-4,5953 eV), hardness (5,2243 eV), softness (0,1914 eV)$^{-1}$ and electrophilicity (2,0210 eV). The high hardness and electrophilicity values in theses compound may be realted with the non-covalent stabilization on the active site. Taking in account, that the electrophilicity is related with the system saturation when received electrons come from the external environment. To analyses these reactiviy details in **Figures 16, 17** and **18** are shown the Frontier molecular orbitals HOMO, LUMO and the Fukui Funtions $\left\langle f_k^- \right\rangle \approx \left|HOMO\right|^2$ and $\left\langle f_k^+ \right\rangle \approx \left|LUMO\right|^2$, to the compounds **1a**, **GMD** (reference compound) and **2**.

The compound **1a** has the higher structural and electronic similarity with **GDM** (reference compound) and the compound **2** has the higher biological activity.

Please, Put Figures 17 and 18. Here

In this compound GDM the Fukui Functions are in the same zone, these reactivity characteristic is very important due to that can be related with retrodonor character on the active site and a non-covalent stabilization.

Please, Put Figure 19. Here

The reactivity contours in this compound **2** are very similar to the compound **1a**. Therefore, theses compounds have different regions to electrophilic and nucleophilic attacks. In this order of ideas, we can see the substitution effects activators and deactivators on central ring.

5.0 Conclusion

With the data provided by the energy of interaction we could say that interactions that influence the activity of the geldanamycin analogs are those formed with waste Lys58, Lys112 and Asp54, which at this point will be "critical" waste complemented by the fact that the substituents bulky at position C-11 the macrocycle do not favors the interaction with the Lys58, and the substituents with amines secondary in the C-17 from the macrocycle position favoring the interaction with the Asp54.

Subsequently the energy profile of each group of geldanamycin analogs showed that among more active compounds have high energy of interaction with the Lys58 and the less active with the Lys112, regardless of the substituents at C-17 and that one increase in the E.I of the Lys112 or Asp54 generates consequently increased the energy of interaction of other waste and increase the values of the E.I with the Lys58 decrease the values of the E.I with the Asp54 and vice versa. The 7-series analogues differ from the rest, because substitutions occur only at the C-11 of the macrocycle (Figure 2)

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