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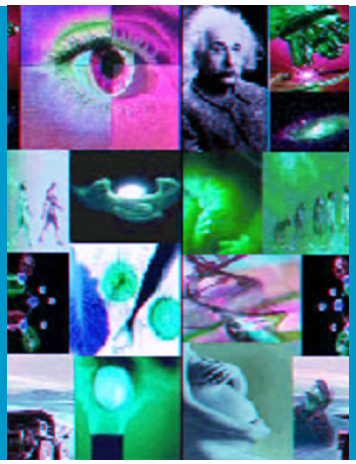

PROGRAMA FONDECYT

INFORME FINAL

ETAPA 2009

COMISIÓN NACIONAL DE INVESTIGACION CIENTÍFICA Y TECNOLÓGICA

VERSION OFICIAL

FECHA: 09/03/2011

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TÍTULO PROYECTO : 3D-QSAR MODELS FOR PREDICTION OF ANTITUMOR ACTIVITY OF EPOTHILONE ANALOGS

DISCIPLINA PRINCIPAL : OTRAS ESPECIALIDADES DE LA QUIMICA

GRUPO DE ESTUDIO : QUIMICA

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INFORME FINAL
PROYECTO FONDECYT INICIACION

OBJETIVOS

Cumplimiento de los Objetivos planteados en la etapa final, o pendientes de cumplir. Recuerde que en esta sección debe referirse a objetivos desarrollados, NO listar actividades desarrolladas.

Nº	OBJETIVOS	CUMPLIMIENTO	FUNDAMENTO
1	To determine the bioactive conformation of epothilone analogs, following an Active Analog Approach and a LBA-LCS strategy	TOTAL	
2	To perform pair-wise molecular structure superposition among active conformations by means of EOP method, in order to identify a common reference frame for 3D molecular descriptors calculation	TOTAL	
3	To calculate 3D molecular descriptors of epothilones by means of electronic structure calculations	TOTAL	
4	To discriminate whether an epothilone is active or inactive to tumor cells by meas of CA and PCA methods	TOTAL	It was determined that CA and PCA were unable to clasiffy structures.
5	To build 3D-QSAR models for prediction of the antitumor activity of epothilone analogues	TOTAL	Some models have been built, but improvement of the employed methodology is still under study.

Otro(s) aspecto(s) que Ud. considere importante(s) en la evaluación del cumplimiento de objetivos planteados en la propuesta original o en las modificaciones autorizadas por los Consejos.

Besides conformational analysis, the identification of a suitable bioactive conformation for epothilone A and its analogues was based on the analysis of 92 bound epothilone A conformations, which accounted forr both intra and intermolecular features that might play a crucial rol in determining the biological activity of epothilone analogues. This work, along with the conformational search and analysis of optimized conformations of epothilone A was published in J. Chem. Inf. Model. 2010, 50, 2176–2190. In this approach epothilone a was taken as a model compound for epothilone family.

Development of LCS-LBA strategy involved the analysis of more than 80 free and bound epothilone analogues, showing that inactive species are unable to accomplish the conformational requirements of previously proposed bioactive conformation for epothilone. This aspect is to be published in the next months, and the manuscript under preparation is attached to the present report. The study of bound epothilones to tubulin binding site has shown that intermolecular interactions responsible for the epothilone A binding are lost for most inactive analogues. The approach that employs the epothilone-tubulin interaction in the prediction of epothilones activity was not considered in the original research proposal but it has open an interesting potential area for future research.

RESULTADOS OBTENIDOS:
Para cada uno de los objetivos específicos, describa o resuma los resultados. Relacione las publicaciones y /o manuscritos enviados a publicación con los objetivos específicos. En la sección Anexos incluya información adicional que considere pertinente para efectos de la evaluación.
La extensión máxima de esta sección es de 5 páginas (letra tamaño 10, Arial o Verdana).

Specific Goal 1

To determine the bioactive conformations of epothilone analogs, following an Active Energy Approach and a LBA-LCS strategy.

1. Search for a Bioactive conformation of epothilone A

Jiménez V. A. 2010. Quantum-Chemical Study on the Bioactive Conformation of Epothilones. *J. Chem. Info. Model.* 50(12):2176-2190

A suitable bioactive conformation for epothilone analogues was proposed based on the conformational search B3LYP/6-31G(d) and geometry optimization (B3LYP/6-31G(d), X3LYP/6-31G(d), B3LYP/6-311++G(d,p) and X3LYP/6-311++G(d,p)) of epothilone A as model compound, followed by a DFT study on the binding interaction between 92 epothilone A conformers and a reduced model of tubulin receptor.

The analysis of 92 stable conformations of free and bound epothilone led us to identify 10 minimum energy epothilone-tubulin complexes with relative energies below 5 kcal mol^{-1}. Within this group there are structures that contain epothilone conformers with relative energies < 5 kcal mol^{-1} in their isolated form. Structure-activity data led us to propose a new model for epothilone binding as shown in Figure 1. According to our results, the intramolecular interaction between C3-OH and the N atom of the thiazole side chain stabilizes the presumed bioactive conformation and accounts for the importance of C3-OH stereochemistry and the nitrogen heterocycle connected to the macrocycle in the biological activity of epothilones. On the other hand, the van der Waals contact between C17-methyl and C8-methyl groups accounts for the importance of C8 stereochemistry in the biological activity of epothilones. In addition, this interaction is helpful to understand why the incorporation of an ethyl group at C17 leads to a less active epothilone analogue.

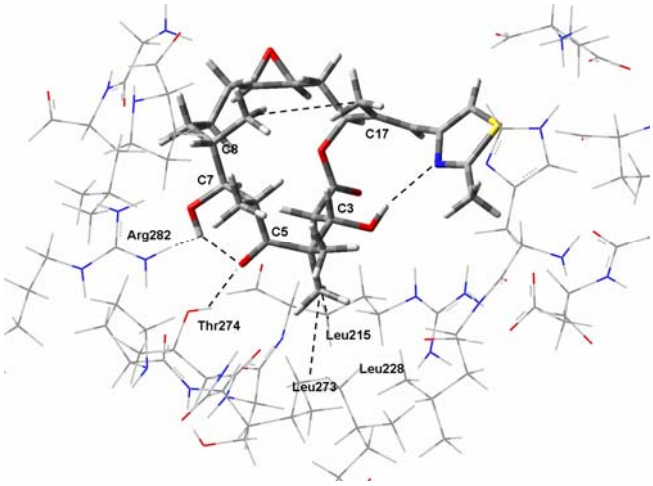


Figure 1. Structure of the proposed binding model for epothilone-tubulin complex 4. Some relevant intramolecular and intermolecular interactions are highlighted. The optimized geometry was obtained at X3LYP/6-31G(d) level of theory.

Once a suitable bioactive conformation was proposed, main efforts were oriented to obtain reliable conformational profiles and tubulin-binding energies for epothilone analogues, considering chemical modifications in specific macrocycle regions, in order to validate our binding mode for active species.

2. Validation of the bioactive conformation based on the structure of epothilone analogues and their binding interaction with tubulin binding site

Second manuscript under preparation.

Geometry optimization and conformational search on epothilone analogues

PM3 and B3LYP/6-31G(d) calculations were employed to perform a full conformational search on the structure of 87 epothilone analogues, including very active, moderately active and inactive species. Epothilone analogues were classified according to the region of the macrocycle where the chemical modification has been performed, based on the model structure of epothilone A, as shown in Figure 2.

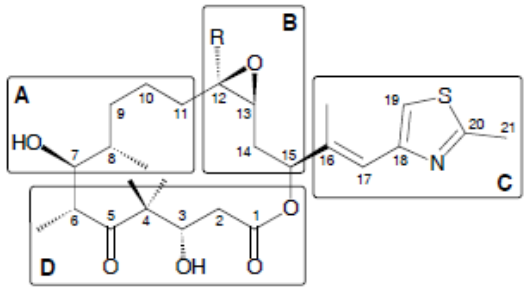


Figure 2. Regions of the macrocyclic structure considered in the present study to analyze the effect of chemical modification on the biological activity of epothilone analogues.
Epothilone A (R=H)
Epothilone B ($R=CH_3$)

It is worth to note that according to our proposed binding mode, epothilone binding occurs mainly through the interaction between tubulin receptor and epothilone regions A and D. On the other hand, region B resides essentially outside the tubulin binding pocket

Relative energy trends with the respect to the presumed bioactive conformation of each species were analyzed. It was found that for some inactive species the bioactive conformation is energetically less accessible, thus the loss in biological activity is presumed to be a consequence of the conformational changes that the macrocyclic structure undergoes as a result of chemical modification.

Structure and Binding energy of epothilone-tubulin complexes for epothilone analogues

B3LYP/6-31G(d) and X3LYP/6-31G(d) calculations were employed to obtain the structure and binding energies of 87 epothilone-tubulin complexes, based on a reduced model of tubulin receptor. This model was built from previous MD simulations on the conformation of free and bound tubulin (First and Second year reports). It was observed that most active species retain the intermolecular interactions found in the binding model of epothilone A, highlighting the importance of C3-OH, C5-CO and C7-OH in epothilones' binding. On the other hand, these interactions are lost for inactive species. Figures 3-5 are provided as examples of the effect of chemical modification of epothilones on the intra and intermolecular features that account for the stabilization of epothilone A-tubulin complex.

Figure 3 shows the corresponding tubulin complex containing a C3-epimerized epothilone. C3-epimerization precludes the intramolecular interaction between C3-OH and the N-atom of the heterocyclic moiety, causing a significant displacement of the heterocycle outside the binding pocket. In addition, the intramolecular interaction between C7-methyl and C17-methyls groups is apparently lost in this model.

Figure 4 displays the structure of the epothilone-tubulin complex containing a dehydrated analog in the C3-C4 region. Again, this poorly active analogue shows a loss of intra and intermolecular interactions, compared to the model compound. As previously noticed, due to C3-OH-dehydration the heterocycle moiety of epothilone is located outside the binding pocket and also, the hydrophobic contact between C4-methyl groups and Leu residues is precluded.

Figure 5 contains a representation of the tubulin complex formed by a highly active epothilone analog, modified in the C region. This structure retains both the intra and intermolecular features found in epothilone A complex and also enhances the intramolecular interaction between C3-OH group and the N atom of the heterocycle moiety (R_{H-N} 1.7Å), which was proposed to be critical to allow epothilones to fit into the tubulin binding pocket.

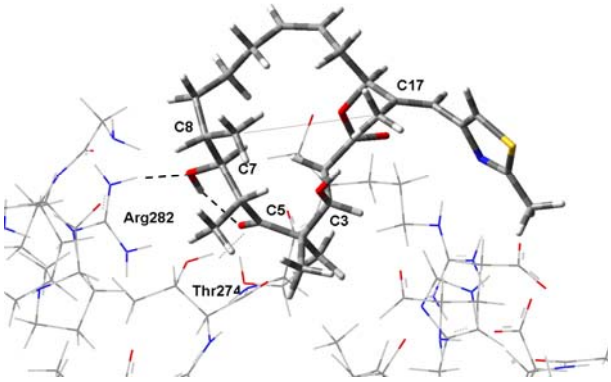
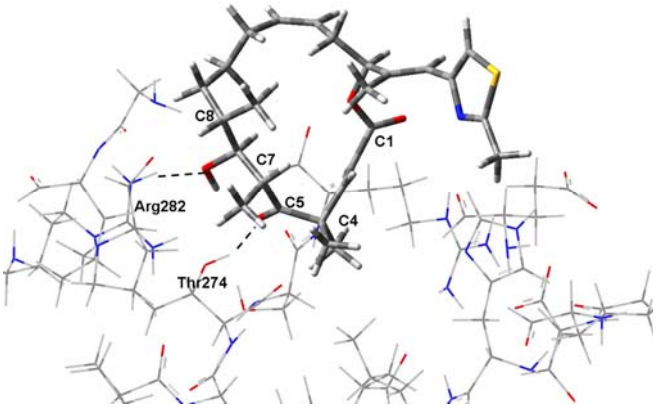


Figure 3. Optimized epothilone-tubulin complex containing a poorly active C3-epimerized epothilone analog. This graphical representation highlight the loss of intra and intermolecular interactions that account for the loss of biological activity compared to epothilone A.

Figure 4. Optimized epothilone-tubulin complex containing a poorly active C3-OH-dehydrated epothilone analog. This graphical representation highlight the loss of intra and intermolecular interactions that account for the loss of biological activity compared to epothilone A



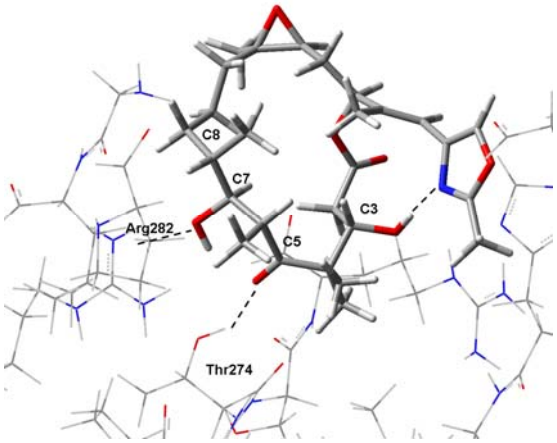


Figure 5. Optimized tubulin complex containing a region D-modified epothilone analog. The improved biological activity of this species compared to epothilone A is related to an enhancement of both intra and intermolecular interactions with tubulin receptor.

Specific Goal 2

To perform a pair-wise molecular structure superposition among active conformations of epothilones by means of Extended Orthogonal Procrustes Method, in order to identify a common reference frame for calculation of 3D molecular descriptors.

Second manuscript under preparation.

Pair-wise superposition was performed by means of the Extended Orthogonal Procrustes Method (EOP), implemented in MATLAB 7.0 scripts. This procedure led us to identify the molecular regions that undergo larger conformational changes due to structural modifications in different epothilone regions. As an example, Figure 5 shows the pair-wise superposition of epothilone analogues containing structural modification in the region A.

Conformational changes are not significant, however, epimerization at C7 and C8 was found to interrupt the intermolecular interaction with Arg282 residue, which has been reported to be critical for epothilones' activity.

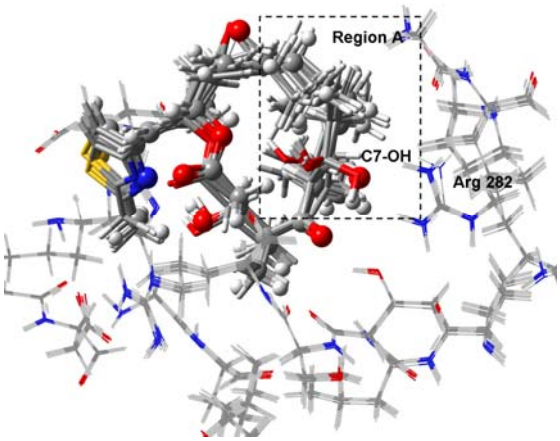


Figure 5. Superimposed conformations of bound epothilone analogues modified in the A region of the macrocyclic structure. The corresponding structure of the presumed bioactive conformation of epothilone A is shown in the ball & stick model.

Specific Goal 3

To calculate 3D molecular descriptors for superimposed bioactive conformations of epothilones by means of electronic structure calculations

Electronic structure calculations at B3LYP/6-311++g(d,p) levels of theory were performed on the optimized structures of the presumed bioactive conformations of each epothilone aligned in a common reference frame. Atomic charges were calculated as electrostatic 3D-molecular descriptors by means of Natural Population Analysis. H-bond donor/acceptor properties were accounted by calculating the interaction energy between an appropriate probe (OH group) and the donor/acceptor groups that belong to each epothilone analog (C3-OH, C7-OH, C1-CO, C5-CO). Electronic properties were described in the frame of Density Functional Theory (DFT) by means of the calculation of Fukui functions for all atoms in common along the set of epothilones under study. The relative energy of the active conformation of each epothilone analog respect to its corresponding global minimum was retained as an independent descriptor in the 3D-QSAR analysis. Shape similarity was quantified by means of comparing the Cartesian coordinates of common atoms in space.

Specific Goal 4

To discriminate whether an epothilone is active or inactive to tumor cells by means of Cluster Analysis and Principal Component Analysis applied over 3D molecular descriptors

CA and PCA protocols were programmed in MATLAB routines and tested in the set of epothilone analogues under study. As stated in the second year report, these methods were unable to discriminate between active and inactive species. Thus, the work was refocused on developing reliable 3D-QSAR models.

Specific Goal 5

To build 3D-QSAR models for prediction of the antitumor activity of epothilone analogues

Recent work focused in obtaining reliable 3D-QSAR models by means of PCR and PLS methods, to account for the antitumor activity of epothilone analogues based on 3D-molecular descriptors. Our preliminary results suggest that calculated binding energies are significant regression parameters for our models. This fact encourages the performance of more sophisticated binding energy analyses that deal with the role of explicit solvent molecules in the epothilone-tubulin binding interaction. Another important parameter appears to be the conformational energy required for each epothilone analog to adopt the suitable bioactive conformation for tubulin binding. This result is quite successful, since validates our preliminary working hypothesis about epothiilones' activity. Figure 6 shows a representation of the predicted and experimental antitumor activities obtained for our best PLS regression model.

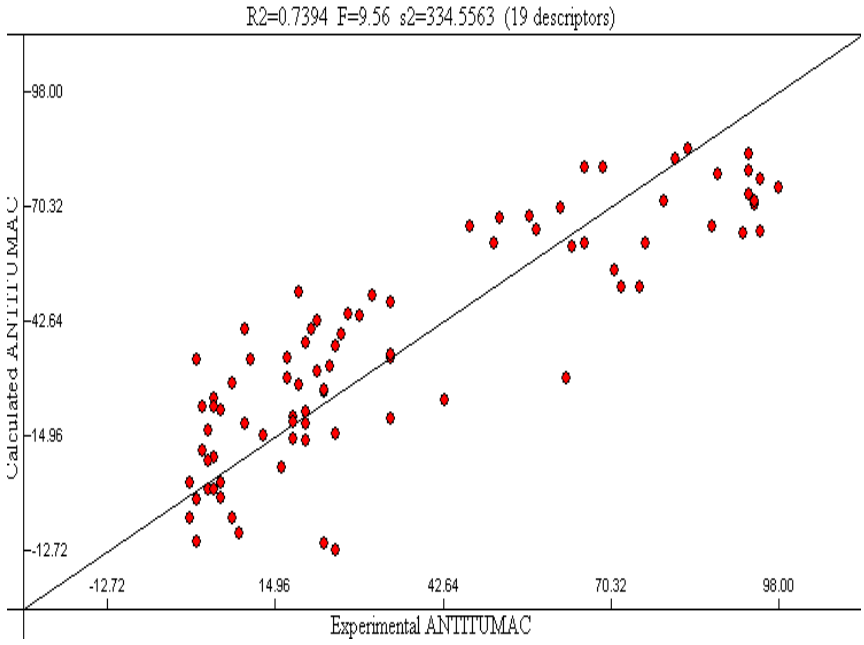
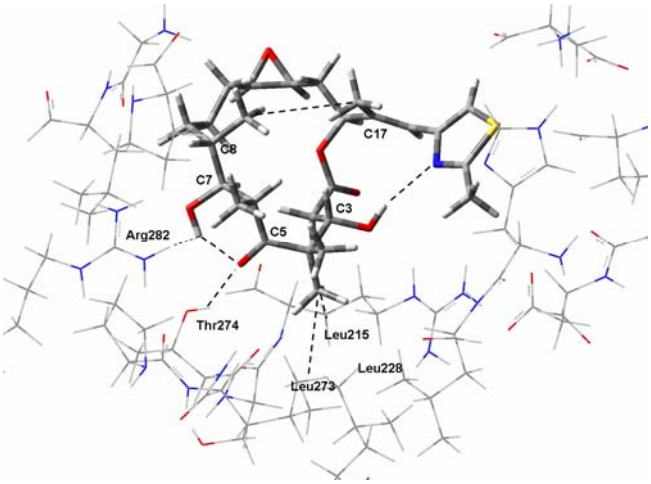


Figure 6. Representation of predicted and experimental antitumor activities for the set of epothilone analogues under study.

RESUMEN:
Describa en forma precisa y breve el tópico general del proyecto, sus metas y objetivos y los resultados alcanzados. Utilice un lenguaje apropiado para la comprensión del público no especialista en el tema. Esta información podrá ser difundida. **La extensión máxima de esta sección es de 1 página (letra tamaño 10, Arial o Verdana).**

Epothilones are a new class of macrocyclic natural products with promising properties as antitumor agents. Intensive research has been promoted in order to develop practical synthetic routes for the production of epothilone analogs, along with theoretical approaches intended to identify the structural features that determine their antitumor activity. This Project focused on the elucidation of the bioactive conformation and binding mode of epothilone analogues based on computational chemistry and 3D-QSAR modeling tools.

Our work led us to identify a suitable bioactive conformation and binding mode for epothilone analogues that account for several structure-activity data. In addition, correlation models have been built in order to account for the antitumor activity of a reduced set of epothilone analogues, based on 3D molecular descriptors.



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