MOLECULAR MODELING OF 1-OCTADECYL-2-METHOXYPROPYL-(N,N-DIMETHYL)-β-HYDROXY-ETHYLAMMONIUM) IODIDE ANALOGS AS POTENTIAL ANTITUMOR COMPOUNDS

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Recibido: Junio 24/98 – Aprobado: Marzo: 19/99

Keywords: Antitumor, Molecular Modeling, Molecular Dynamics, ether lipids

RESUMEN

En nuestra búsqueda de nuevos compuestos con actividad antitumoral y/o anti-HIV, estudiamos nuevos análogos del Yoduro de 1-Octadecil-2-Metoxipropil-(N,N - dimetil) - β - hidroxietilamonio, un éter lipídico de conocida actividad antitumoral y antiHIV. Se modelaron los compuestos propuestos, substituyendo la cadena hidrofóbica por cadenas similares a los lípidos de la membrana celular. Para el análisis conformacional se utilizaron cálculos de Mecánica Molecular y Dinámica Molecular. Las conformaciones de mínima energía obtenidas fueron superpuestas con el compuesto de referencia, obteniéndose una buena superposición, concluyéndose que podrían presentar propiedades similares a los éteres lipídicos en la membrana celular y por tanto son buenos candidatos para ser sintetizados.

Palabras claves: Antitumorales, Modelado Molecular, Dinámica Molecular, Éteres lipídicos.

ABSTRACT

In our search for new antitumour and/or anti-HIV compounds, we studied some new analogs to the 1-Octadecyl-2-Methoxypropyl-(N,N-dimethyl)-β-hydroxyethylammoniun Iodide, an ether lipid compound with known antitumoral and anti-HIV activity. Using Molecular Modeling techniques, we modeled the proposed analogs, changing the alkylhydrophobic chain for hydrophobic chains similar to the membrane lipids, and the reference compound. The conformational analysis was made using Molecular Mechanics and Molecular Dynamics calculations. The minimum energy conformations superimposed with the reference compound, so they could have similar action to the ether lipids in the cell membrane, we conclude that they are good candidates to be synthesized.

INTRODUCTION

Until the present there is no definitive cure for the HIV syndrome and many cancer tumors. Due to the importance of

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both types of diseases medicinal chemists continue efforts on the design and synthesis of new compounds that can prolong the life of affected people or cure in a definitive way those terrible diseases.

The ether lipids (EL) as the rac-1-octadecyl-2-O-methyl glicero-3-phosphocholine, and its analogs, have known antineoplasic (1-8) and antiHIV (9-12) activity. Their mechanism of action is not well understood. These drugs may produce permeability and fluidity of the cellular membrane causing interruption and loss of integrity. It has been suggested (13) that differences in the cell membrane lipid composition can be related to the sensibility of the EL. As a fact, the amount of membrane cholesterol influences significantly membrane effects and cytotoxicity of EL.

It has been found that synthetic EL and combined compounds of EL-AZT present potent and selective activity against the replication of HIV-1, because they inhibited the HIV-1 induced cellular fusion (12). The virus particles produced in a medium inoculated with conjugated EL-AZT compounds present impaired capacity to produce a subsequent infection.

Due to their potential activity, in the last years a lot of EL analogs have been synthesized: Cyclic analogs (1) , FAP analogs (8,14,15), N-alkylamide analogs (2), thioanalogs (4), phosphatidyl inositol derivatives (5), Et-18-OMe analogs (16). If the clinical studies actually in progress for some of this drugs show an acceptable toxicity, the great number of EL to be synthesized could create a new perspective in cancer and antiHIV therapies.

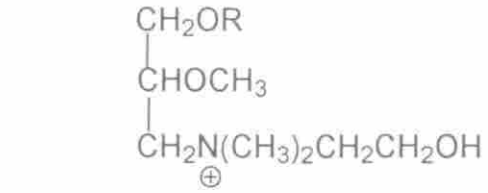
In the present work we proposed the design of new compounds using Molecular Modeling techniques trying to mimic the properties of the reference compounds.

Initially, we studied previously reported structure-activity relationships of EL compounds. These results suggest that the biological activity of the EL imply an alteration of the cellular membrane surface and also of the viral coat (11).

According to the structural features of biologically active EL derivatives, we proposed the design of compounds with the following characteristics:

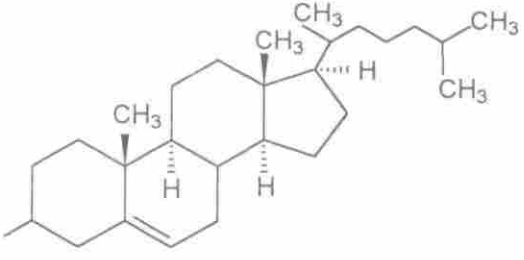
1. Ether union in C_1 of the glycerol backbone instead of an ester bond.
2. Ether bond in C_2 of the glycerol backbone.
3. Hydrophobic chains similar to cell membrane lipids (cholesterol, cholestanol and ergosterol) instead of a long alkyl chain (C-17 or C-18).
4. An inversion of the polar group present in ET-18-OMe.

The designed compounds **2**, **3** and **4** are shown in Figure 1, along with the reference compound **1**. All of them were evaluated using Molecular Modeling techniques. Molecular modeling techniques are useful tools for the rational design of new compounds. Especially, High and Low temperature Molecular Dynamics is a useful technique to explore the conformational space of compounds with a high grade of flexibility that limits their study by other computational methods and provides a fast way to obtain low energy conformations.



1: R= — $C_{18}H_{37}$

2: R=



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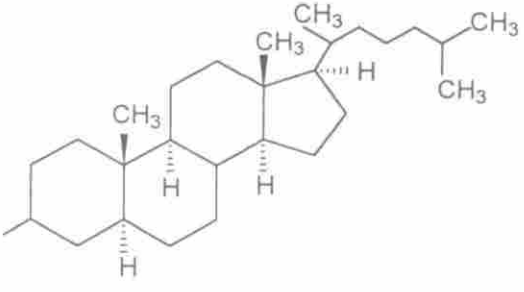

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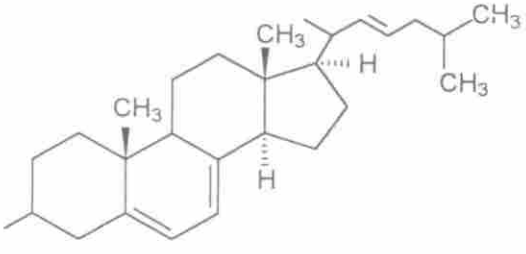

Figure 1. Structures of the reference compound, 1, and the proposed analogs compounds 2, 3 and 4.

METHODS

The compounds selected for this study are shown in Figure 1. All computations were performed on a CAChe (Computer Aided Chemistry) workstation running CAChe Scientific proprietary software, version 3.5.1.(17) Three-dimensional models of the different compounds (including the reference compound) were constructed using the molecular editor of the CAChe system. These structures served as starting points in an extensive conformational analysis of each compound used to run the Molecular Dynamics (MD) calculations.

We initially minimized each structure using Molecular Mechanics (MM) by the conjugate gradient method (18). CAChe molecular mechanics uses Allinger's MM2 force field (19). The molecular mechanics calculations were investigated in the CAChe system by minimizing the total molecular energy according to the molecular mechanics expression, that is a summation of several energy terms each one corresponding to a different type of movement or interaction:

$$E_{total} = E_{bonding} + E_{\theta} + E_{\phi} + E_{improp} + E_{elec} + E_{vdW} + E_{hb}$$

Where $E_{bonding}$, E_{θ}, E_{ϕ}, E_{improp}, E_{elec}, E_{vdW} and E_{hb} indicate bond lengths, bond angles, dihedral angles, improper torsions, electrostatic potential, van der Waals interactions and hydrogen bonding, respectively. Further, molecular mechanics calculates energies at 0 K relative to a hypothetical "perfect" geometry, rather than an absolute energy (20).

In order to obtain conformations around the global minimum of the compounds a sequential search was run using the Block Diagonal Newton Raphson optimization method, relaxation factor of 1,00 and energy value tolerance of 0.001 kcal/mol.

In order to explore the conformational space we ran a Molecular Dynamics (21) simulation at 900 K for 100 ps with a time step of 1.0 fs. Solvent molecules were not included in the calculations. The Trajectory file was analyzed and conformations less than 5 kcal/mol from the minimum energy conformation were chosen. These conformations were minimized using Molecular Mechanics (18,19) and the conformation with the lowest energy was selected to perform a molecular dynamics simulation at 300 K. Then the Trajectory file was analyzed and conformations less than 5 kcal/mol from the minimum energy conformation were chosen. These conformations were minimized using Molecular Mechanics (18,19) and the lowest energy conformers were used to superimpose with the reference compound. We used the CAChe worksystem Editor for the superimposition and it was made using as reference the following atoms: the ether oxygen, the first carbon atom in the alkyl chain, C2 in the glycerol backbone and the hydroxylic oxygen in the quaternary ammonium chain. These atoms are common for all the analogs and could be implied in the interaction with the membrane.

In order to obtain the rms values of the superimposed compounds, the molecular files of the lowest energy conformations were read and superimposed in a Silicongraphics workstation running MSI/Byo-

sim proprietary software (Insight II, version 97.0) (22). The rms values are shown in Table 1

RESULTS AND DISCUSSION

It has been suggested that the EL interact initially with the plasmatic membrane, causing permeabilization and fluidization, and it has also been suggested that the membrane lipid composition influences the sensibility to the EL (13).

In order to understand the opposite effects of cholesterol and EL derivatives in the stability of the plasma membrane it is necessary to consider the structure of the plasma membrane and the type of bonds between the double hydrophilic and hydrophobic layer. The membrane has a hydrophobic center, two hydrogen bonding zones and two polar zones as is shown in Figure 2 (13). The membrane phospholipids are bonded by weak van der Waals forces in the middle of their polar groups. Consequently the fatty acid alkyl chains are coupled between them to form the hydrophobic center, but the phospholipids are really linked by the dipolar interactions between the hydrophilic groups.

Cholesterol is important to maintain the membrane "rigidity" because its beta hydroxilic groups could form hydrogen bondings with the carbonyl group present in the phospholipids, blocking their mobility. The amounts of cholesterol in the plasmatic membranes are important in the modulation of the EL fluidizing and cytotoxic effects (23,24), probably for two reasons: first, EL presents ether or

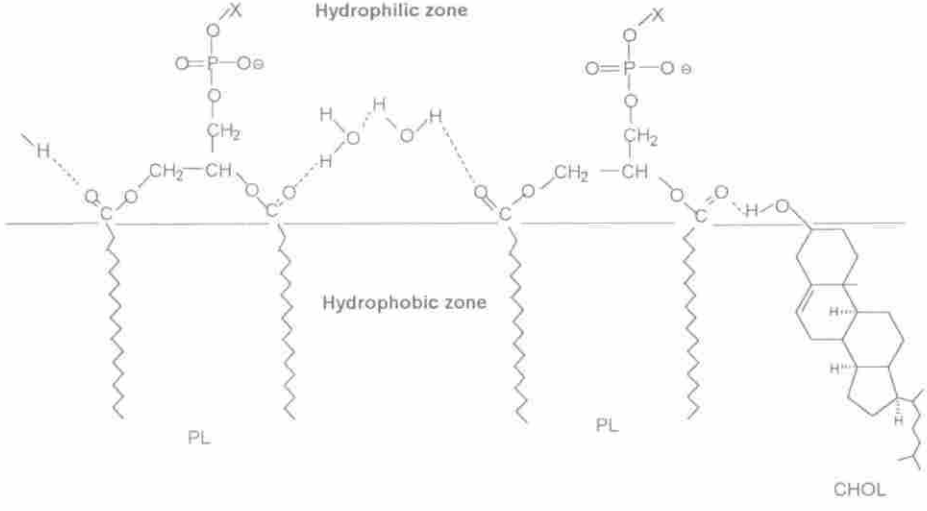

Figure 2. Diagram of the interaction mediated by hidrogen bonds between phospholipids (PL), cholesterol (CHOL) and water, in the hydrophilic core of the membrane. X is choline, inositol or serine. Interrupted lines represent hydrogen bonds[13].

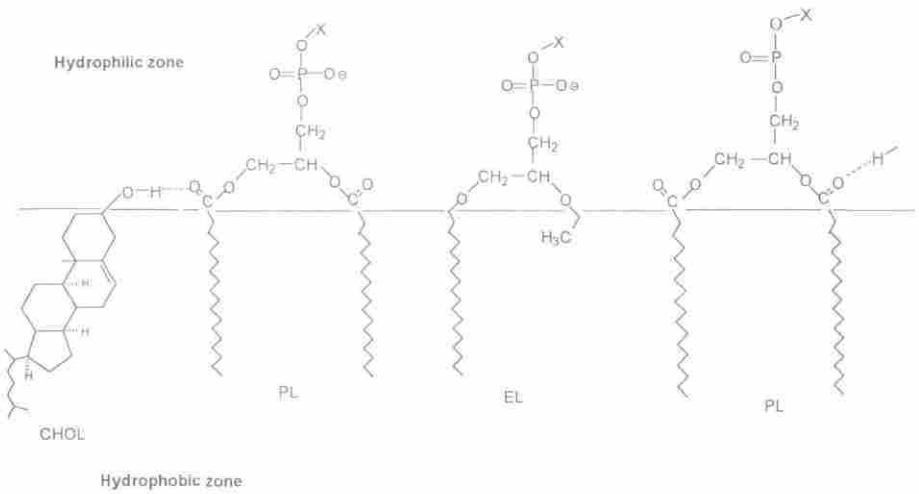

Figure 3. Diagram of the localization of an ether lipid (EL) within the membrane. The presence of EL causes rupture of the hydrogen bond net. The 1-octadecyl-2-methyl-rac-glycerophosphocholine is reported as a representative molecule of the EL family. X is choline, inositol or serine. Interrupted lines represent hydrogen bonds[13].

thioether groups instead of ester groups, so they can not form hydrogen bonds and causing "holes" in the net formed by the hydrogen bonds, and second, when the membrane has a high level of cholesterol or sphingomyelin, the EL access is kinetically and thermodynamically blocked. This kind of action is supported by experimental data showing that cells resistant to the toxic action of EL are richer in cholesterol and sphingomyelin than the more sensible cells (23). This observation means that the interaction between cholesterol and the EL compounds is due to the contrary effects in the membrane polar zone over the hydrogen bonds and justifies the fundamental role assigned to the membrane lipid composition. A model for the interaction of EL analogs in the membrane(13) is shown in Figure 3. Of course this observation does not exclude the possibility that the toxic effects caused by EL compounds could be a process with multiple steps in which biochemical and biophysical interactions play an important role.

Since the analysis of the requirements for binding to the membrane is based on the structures found in the conformational search, this step must be carried out using a technique that assures as many minima as possible in an objective and efficient manner. The search for reasonable structures of large molecules and/ or

flexible molecules benefits most from dynamics simulation because it is often impractical to compute energy maps of geometrical searches for large molecules. We used a combination of Molecular Mechanics followed by High Temperature Molecular Dynamics and minimization, which provides a fast way to scan the conformational space and has been used with good results for flexible and cyclic compounds(25-27).

The superimposition of all the studied analogs, including the reference compound is shown in Figure 4, and the best superimposition (between the reference compound and compound **4**) it is shown in Figure 5. The rms values for the superimposition between the reference compound and the studied analogs are shown in Table 1.

All the compounds studied showed a good superimposition with the reference compound, so we assume that they could have similar effects to the reference compound and that they are very good candidates to be synthesized. Their synthesis is already in progress by the medicinal chemistry group in our synthesis laboratory.

Tabla 1. rms values for the superimposition of compunds **2**,**3** and **4** with the reference compound 1.

COMPOUND	rms
2	0.318244
3	0.388323
4	0.278378

Using the results obtained in the present work, in the next step of our molecular modeling research work we will continue with the study of the structure-activity re-

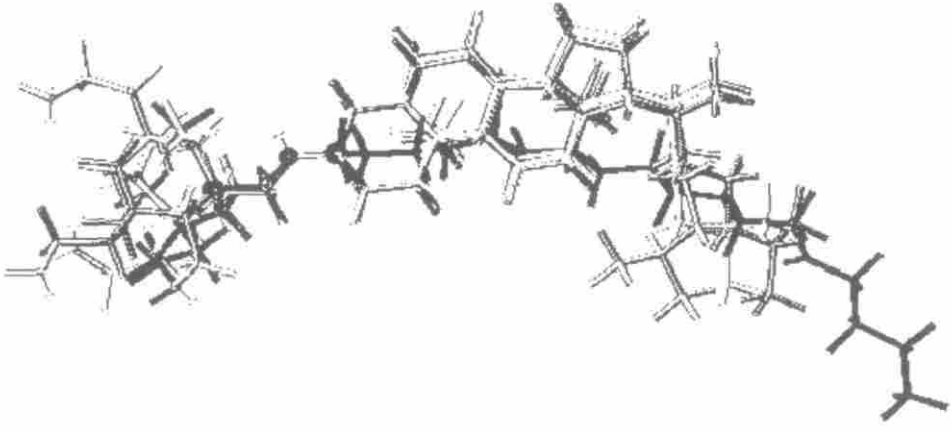

Figure 4. Superimposition of the proposed analogs with the reference compound (Reference compound in dark gray).

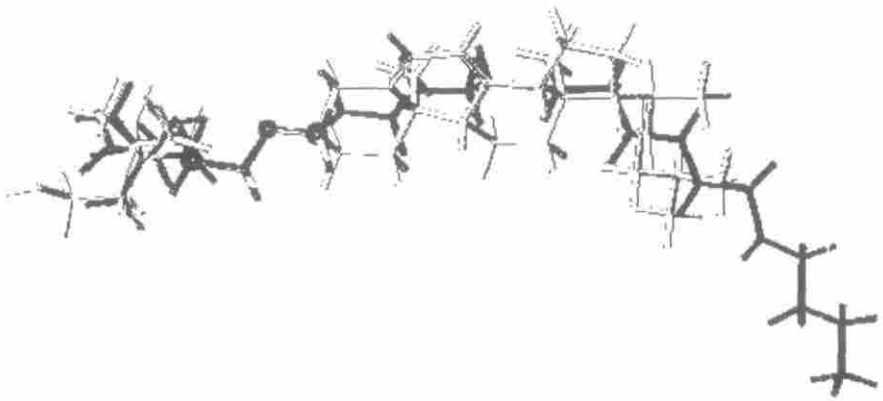

Figure 5. Superimposition of **4** with the reference compound **1** (Reference compound in dark gray).

lationships of this interesting group of compounds in order to obtain their pharmacophoric group.

CONCLUSIONS

The combination of Molecular Mechanics followed by High Temperature Molecular Dynamics and minimization was a very good method for the confomational study of the ether lipid analogs, compounds with a high grade of flexibility.

The results for the superimposition of the lowest energy conformers of each ether lipid designed with the reference lowest energy conformation is very good and suggest that they could occupy the same binding region of the reference compound. Although the validity of our hypothesis may be affirmed only when the designed compounds have been synthesized and biologically evaluated, from our results we can conclude that the studied isomers could probably show activity at the same level of the EL reference compound.

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ACKNOWLEDGMENTS

This investigation was supported by funds from the Universidad Central de Venezuela, grants IIF-0785, C.D.C.H. 06/10/3339/94, 06/10/3337/94 and CONICIT grant, S1-95000496.