EXERCISE III.2

A LABORATORY COURSE IN MEDICINAL CHEMISTRY INTRODUCING MOLECULAR MODELING

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Abstract: A laboratory course in medicinal chemistry introducing molecular modeling. Molecular modeling is an important and useful tool in drug design and for predicting biological activity in library compounds. A wide variety of computer programs and methods have been developed to visualize the 3D geometry and to calculate the physicochemical properties of drugs. In this paper, we describe a practical approach to molecular modeling as a powerful tool to study structure–activity relationship in drugs such as antibacterials, hormones, and cholinergic and adrenergic agents. Early in the course, the students learn how to draw 3D structures and to use them to perform conformational and molecular analyses. Thus, they may compare drugs with similar pharmacological activities by superimposing their structures and evaluating geometry and physical properties.

Keywords: molecular modeling; conformational analysis; structure–activity relationships.

INTRODUCTION

Planning and selecting educational activities in the teaching of medicinal chemistry are ever constant and necessary tasks in adapting program contents to meet the challenges of a world in permanent change. Transformations should direct the course in medicinal chemistry to favor the use of new technological resources and contribute to develop both alternative ways and the student's critical thinking. Some methodological strategies should be incorporated into the teaching of medicinal chemistry, thus promoting the teaching-learning processes.[1]

The classical structure–activity relationship (SAR) studies implied the synthesis of several, structurally related analogs to a lead compound and successive biological activity tests. After decades of SAR research, some general rules on the influence of specific structural changes on biological activity could be drawn, including the size and shape of the carbon chain, the nature and rate of substitution, and stereochemistry of lead compounds. SAR and the traditional techniques of molecular modifications are still important tools in

the search for new drugs, but they are expensive, highly time-consuming, and eventually successful.[2]

Chemical computer programs and the Web databases are important tools in the current search for and design of drugs. A series of interesting molecules can be rapidly screened as to their biological activity versus physicochemical properties. New therapeutic agents can be developed, analyzing theoretical data on structure–activity in the 3D form, obtained through recent molecular modeling techniques. In a broad definition of medicinal chemistry, relating the invention, discovery, planning, identification, and preparation of biologically active compounds, to the study of its metabolism, mechanism of molecular action, and construction of SARs, it is highly important to insert and approach topics in molecular modeling in graduation courses on medicinal chemistry.[3]

According to IUPAC, molecular modeling is an investigation of structures and molecular properties by using techniques of computational chemistry and graphic visualization aiming to obtain, under certain circumstances, a 3D representation.[4] Computer-assisted drug design (CADD) is described in many sites on the Internet, helping, through tutorials, the investigation of receptor-ligant chemical interactions and the exploring of structural factors connected to biological effects.[5] As a result, the integration of essential knowledge in organic chemistry, biochemistry, molecular biology, and pharmacology, contributes to the understanding of the mechanisms in drug molecular actions.

The laboratory course in medicinal chemistry is presented to the fifth-period Pharmacy students (30 h, 2 groups) in parallel to the theoretical course (60 h). After careful analysis of the different approaches, laboratory practices were directed to the study of the geometry and properties of drugs, enabling the students to explore the chemical and molecular basis of the drug–receptor interaction, by employing computational techniques.

More specifically, the objectives are:

(i) conformational analysis of drugs by visualizing its 3D format.
(ii) analysis of the size and shape of the pharmacophore
(iii) importance of the nature and rate of functional groups substitution
(iv) stereochemical aspects of drugs and their relation to biological activity
(v) to relate a single series of drugs trough structures and physical properties
(vi) to predict molecular mechanisms involved in drug action

Methods and computational resources employed in the drawing, accurate structural representation, and 3D visualization of drugs are initially presented in this paper; this is followed by showing the use of molecular modeling in the theoretical determination of physicochemical properties and comparison of data obtained with adrenergic and cholinergic drugs, active in the autonomic nervous system.

This approach significantly contributes both to an integration of theoretical and laboratory data in structure–activity of drugs, and to the implementation of practical courses in medicinal chemistry.

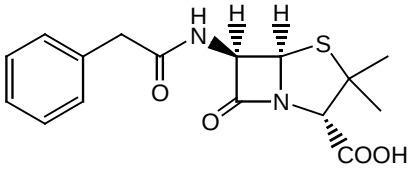
EXPERIMENTAL

Drawing, conformational, and molecular analysis of drugs

Drawing and 3D visualization

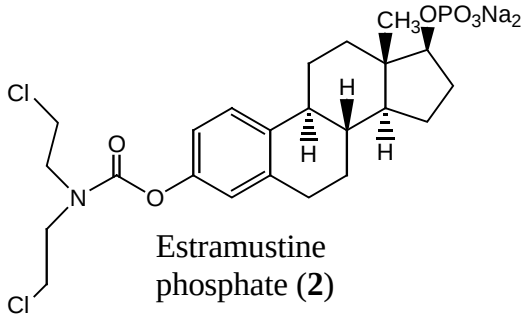
Several easily utilized programs are available for building bidimensional molecules like ChemWindow, Isis Draw, and ChemDraw. Accurate and high-quality figures and diagrams can be elaborated with the help of such programs that frequently contribute to documentation and communication in science.

The students learn the resources available in the main menu of ChemDraw[6] and Chem3D[7] and how to utilize the tool and template selection to design chemical structures. The stereochemical aspects are discussed in depth and through exercises, they are able to correctly represent the asymmetrical carbons of drugs like benzylpenicillin (**1**) and estramustine (**2**), Fig. 1. Eventually, the structures can be drawn in perspective, representing the molecules in the projections of Fisher, Newman, and Haworth.



Benzylpenicillin
(**1**)

$C_{16}H_{18}N_2O_4S$
Molecular weight.: 334.39
C, 57.47; H, 5.43; N, 8.38;
O, 19.14; S, 9.59



Estramustine
phosphate (**2**)

$C_{23}H_{30}Cl_2NNa_2O_6P$
Molecular weight: 564.35
C, 48.95; H, 5.36; Cl, 12.56; N, 2.48;
Na, 8.15; O, 17.01; P, 5.49

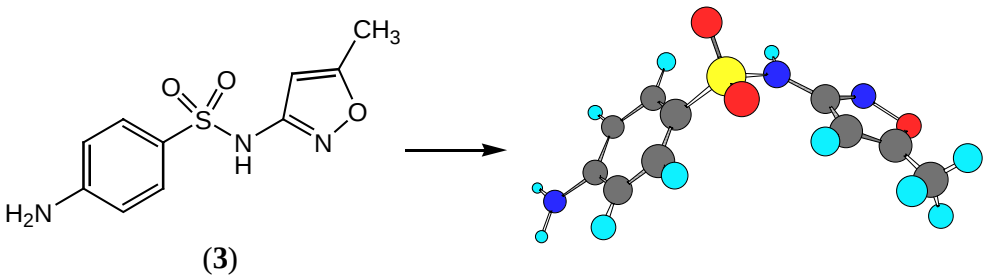
Fig. 1 Drug drawings showing relevant stereochemical features (ChemDraw).

Several molecular properties can be calculated and/or represented in some of the programs as well as the molecular formulae, molecular weights and the theoretical elementary analysis. More sophisticated programs like ChemDraw Ultra[8] can, in addition, predict ^{1}H and ^{13}C chemical shifts in NMR of chemical compounds, their freezing and melting points, log *P*, molar refractivity, and heat of formation, besides furnishing the correct chemical name (IUPAC).

The students are trained to chemically recognize heterocyclic rings, frequently present in drugs, through the use of the main menu of ChemDraw by clicking on “Edit” and “Insert name as Structure”. By introducing the English ring name in the dialog box, it is possible to visualize the corresponding chemical structure in the drawing window and the accepted IUPAC nomenclature, which helps the student build complex molecules.

In the Chem3D[7] program, drugs are three-dimensionally visualized, by the gradual building of bonds based on information on their length and position angles. More complex molecules can be obtained by alternating several of the available resources such as drawing

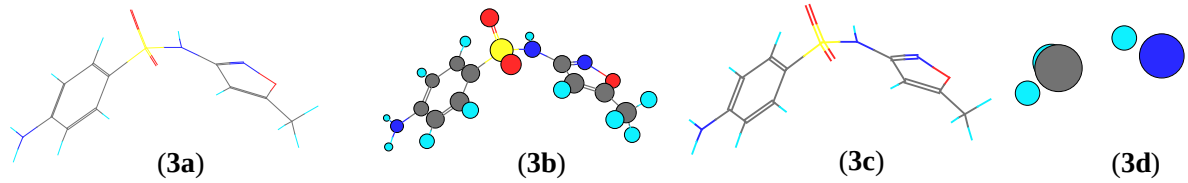
tools, whole substructures ready in the program and the dialog box, where formulae in linear representation are typed. In parallel, molecules generated in ChemDraw (“copy”) can be converted to the 3D model in Chem3D (“paste”), as shown in Fig. 2 for sulfamethoxazole (**3**).



(**3**)

Fig. 2 Conversion of the 3D sulfamethoxazole **(3)** structure into the cylindrical bond 3D display (ChemDraw-Chem3D).

In the Chem3D program, the molecule can be drawn in different formats, such as backbone, ball and stick, and space filling by using standard length and bond angle values, Fig. 3.



(**3a**) (**3b**) (**3c**) (**3d**)

Fig. 3 Different representations of sulfamethoxazole: (**3a**) wire, (**3b**) cylinder and sphere, (**3c**) cylinder, and (**3d**) space filling (Chem3D).

Handling 3D molecular models from Chem3D[7] or Molecular Modeling Pro[9] programs can assess relevant stereofeatures of drugs, allowing information about the size, volume, and shape of the molecules.

The importance of the stereochemistry in the mechanism of action of drugs is illustrated by epinephrine (**4**) and propranolol (**5**), acting on β-adrenergic receptors, Fig. 4. Compounds **4** and **5** can be easily drawn in their active configurations, *R* and *S*, respectively, by rotating the molecules around the X, Y, Z axis and attributing according to the classical rule of Cahn–Ingold–Prelog. The configurations of the asymmetrical carbon in the side chains are apparently opposite, due to *R* and *S* nomenclature, but in comparison, the 3D representations show that the disposition and spatial orientation of the hydroxyl groups are similar, both directed to the same face. The difference in their naming, *R* and *S*, is due to the priority rule, the aryloxy group in the antagonist (**5**) has priority over the methylenamino group of the side chain, which is not the case in the epinephrine molecule (**4**).[10]

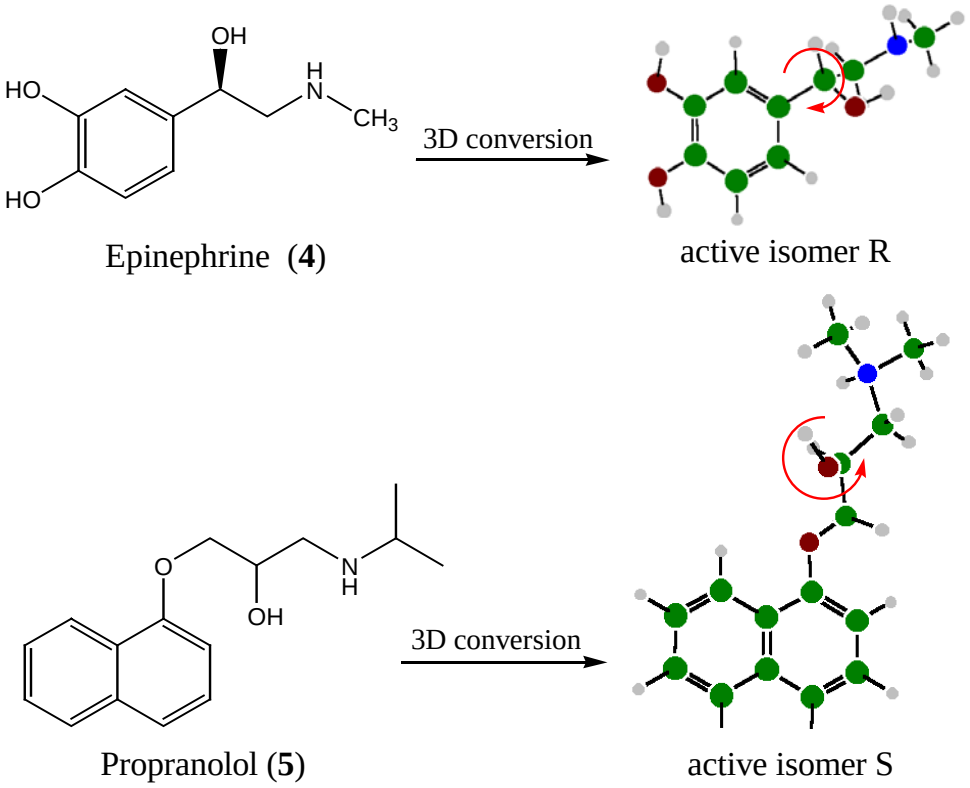


Fig. 4 Conformations of *R*-epinephrine (**4**) and *S*-propranolol (**5**) with distinct configuration descriptors (Cahn–Ingold–Prelog priority rules), but with the stereogenic center in the same spatial disposition.

The importance of the shape and size of the molecule is illustrated by *trans*-diethylstilbestrol (**6**) used to mimic estradiol, the natural hormone (**7**), Fig. 5. Comparing the interatomic distances in the natural and synthetic products, it can be seen that only the *trans*-isomer (**6**) has the desired distances between carbons containing hydroxyl groups, around 9.0 Å. The corresponding *cis*-isomer (**8**) shows distances of 5.9 Å, quite different from the estradiol molecule (8.6 Å).[11]

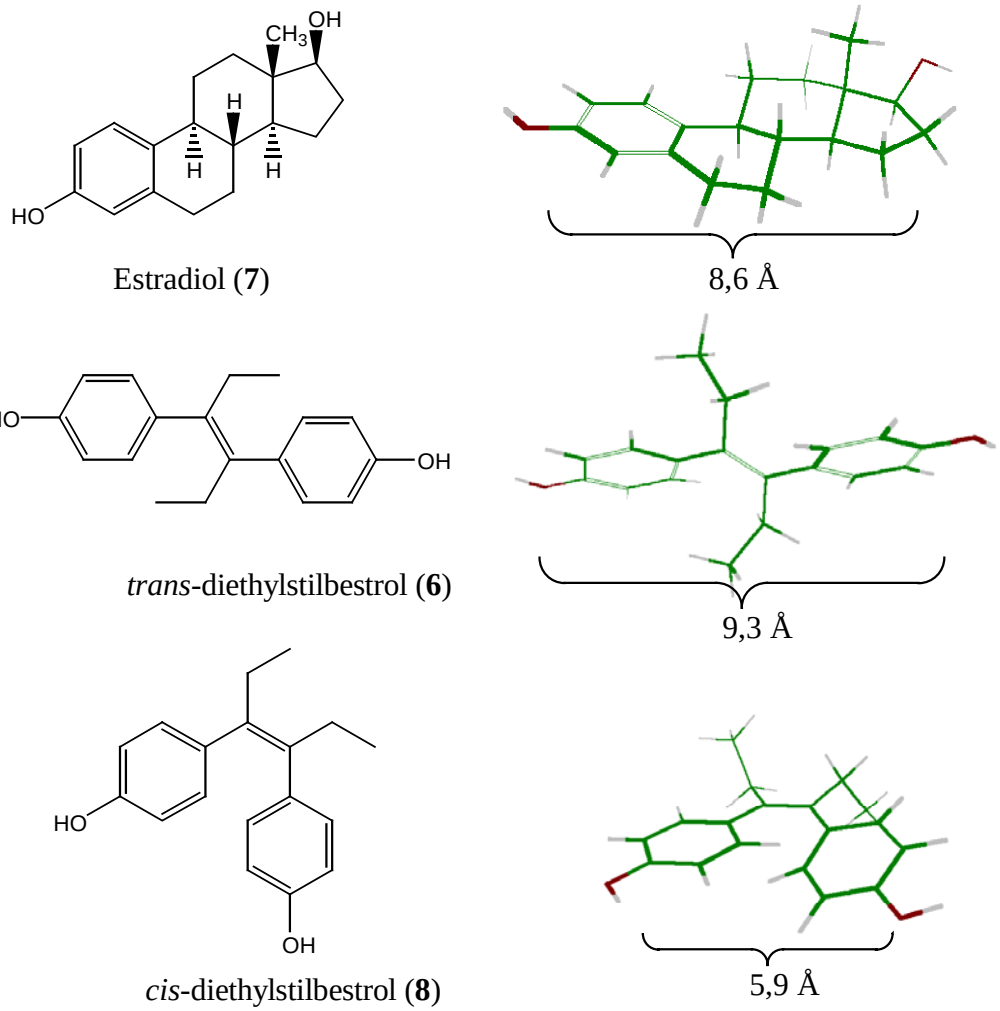


Fig. 5 Comparing molecule format and interatomic distances in hormone estradiol (**7**) and its active and inactive derivatives, respectively, *trans*-diethylstilbestrol (**6**) and *cis*-diethylstilbestrol (**8**).

Conformational analysis and minimal energy

The Chem3D program was employed for these studies, but others like Molecular Modeling Pro, Chem Site, Alchemy, Sybyl, Hyperchem, ChemX, CAChe, and Weblab Viewer are also available.

In conformational analysis of molecules, the bond rotation changes the dihedral angles and, consequently, the corresponding steric energy due to spatial overlaying of non-linked atoms and rotation torsional barriers.

The molecules drawn three-dimensionally are not necessarily in the most stable conformation. Generating a certain structure causes molecular distortions with unfavorable lengths, angles, and dihedral angles. Non-linked atoms also interact in the same spatial regions, generating steric and electrostatic repulsion. Correction of the molecule distortions may be achieved by energy minimization through two mathematical models (i) molecular mechanics or (ii) quantum mechanics. Unpredictable interactions related to superimposing molecular orbitals, electronic density distribution, or steric influence can be solved by computational methods. The optimized geometry of a molecule results from the interaction

of conformational analysis and energy minimization.[10] The method of choice for the minimization of energy is dependent both on the size of the molecule and the availability of stored data and parameters, as well as computational resources. Computer-generated molecular models are based on mathematical equations that estimate positions and properties of electrons and nuclei; furthermore, the added calculations experimentally explore the structure, producing a molecule under new perspectives.

A. Molecular mechanics

Energy is calculated by comparing angles and distances bonds in a molecule, using values that are listed by the MM2 program. Molecular mechanic equations only consider atomic nuclei and do not include electrons in the calculations. The interactions due to stretching of bonds, angular torsional and spatial deformation are determined by the program, which also calculates the energy of the starting molecule comparing it with a standard, methane (1 KJ/mol). The program of molecular mechanics resulting in new conformations and the corresponding energy calculation modifies angles and lengths of the original atomic bonds. The program also recognizes changes leading to more stable structures with lower steric energy, but the calculations are interrupted if the variation in energy in relation to the original molecule is not considerable. Although molecular mechanics predict energy associated to a particular conformation the quantities expressed are not absolute ones, but only differences between two or more conformations.[10]

B. Quantum mechanics

In this process, properties of the molecule are calculated by equations of quantum physics, involving interactions between electron and nuclei. Electron movements are more rapid and, since they rotate independently of the nucleus, it is possible to describe electronic energy separately from the nuclear one. Some approximations based on empirical data are made in calculations by this process, which are not exact and may be executed by two methods, ab initio and semi-empiric. The first one, is applied only to small molecules, and although more precise and not needing stored data, requires ample computer memory capacity and time. On the other hand, the semi-empiric method is faster and can be used to minimize energy and optimize molecules with 10 to 120 atoms, although less accurate. Energy is calculated by the Schrödinger equation from stored parameters; MOPAC is the most frequently used semi-empiric method, subdivided into the following: AM1, MINDO/3, MNDO, MNDO-d, and PM3.[10] Figure 6 shows chlorpromazine (**9**) and indomethacin (**10**). In these drugs, the biologic effect is highly dependent on certain conformations for the receptor interactions. There is an obvious difference in spatial arrangements between non-optimized forms (**9b** and **10b**) and the corresponding (**9c** and **10c**), energetically optimized by the program MOPAC (AM1). The tricyclic system of the antipsychotic chlorpromazine (**9a**) in the planar form does not seem to react with the dopaminergic receptor, but it does in an angle of approximately 25° in the ring junction, that coincides with structure (**9c**). Indomethacin (**10a**), however, shows relevant features for anti-inflammatory activity, such as the nonplanar or perpendicular orientation of the *N*-*p*-chlorobenzylic group in relation to the indolic system.[13] Structure **10c**, closer to the bioactive conformation can be obtained by minimizing structure **10b**, relatively planar. A more detailed visualization of 3D minimized molecules can be obtained by movements around axis X, Y, and Z, with the help of the “mouse”.

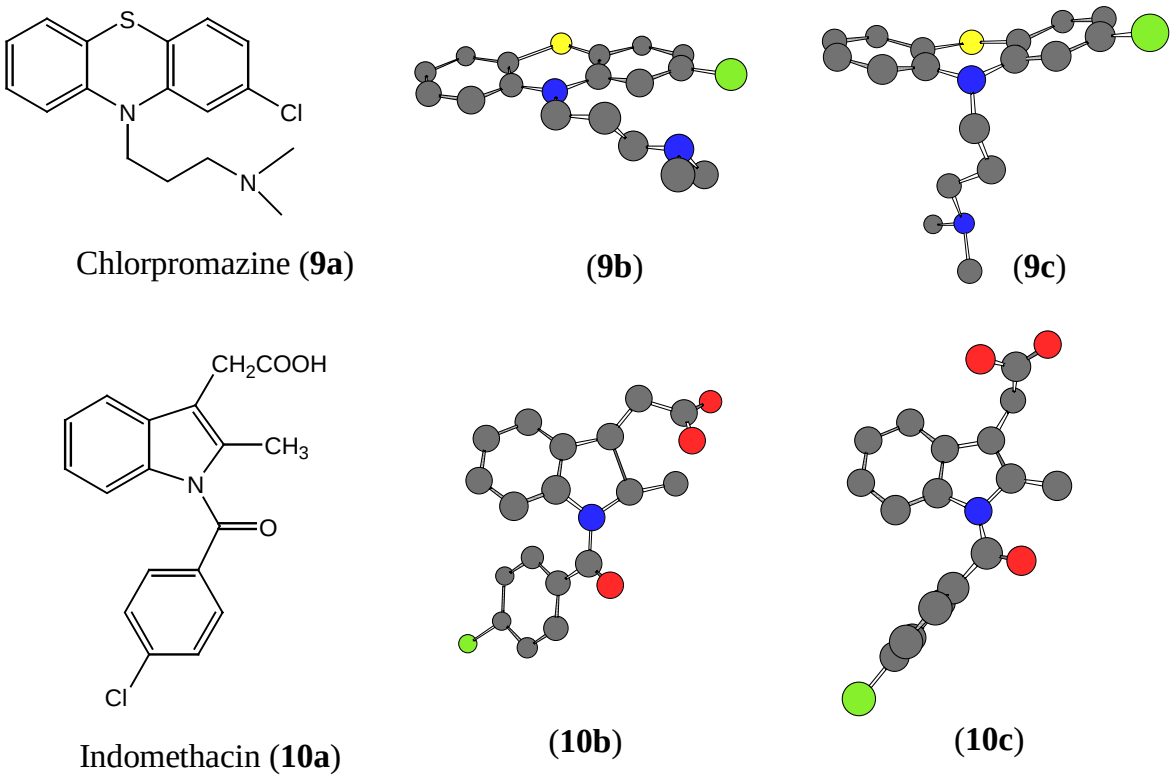
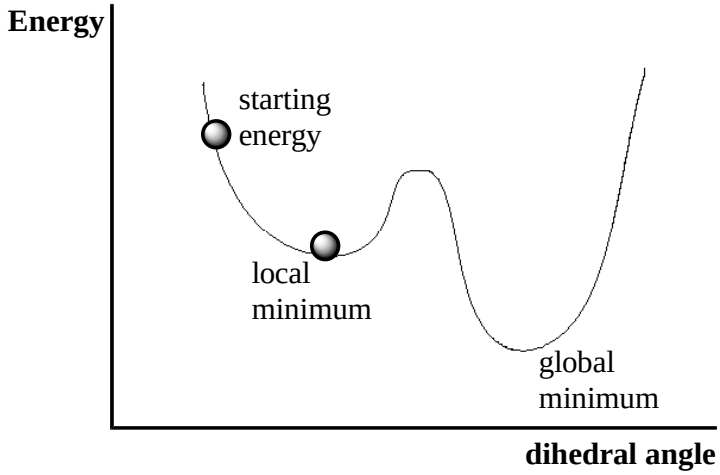


Fig. 6 Drug structures: 3D, nonoptimized, and optimized by the program MOPAC (Chem3D). Chlorpromazine (**9a**), (**9b**), nonoptimized and (**9c**) optimized; Indomethacin (**10a**), (**10b**), nonoptimized and (**10**c), optimized.

C. Molecular dynamics

It was already stated that 3D conformations are not necessarily the most stable ones and that the energy minimization process is interrupted when structure variations imply small changes in energy. Graph 1 shows that this "stable" conformation may be separated from another, even more stable that the minimizing process is unable to overcame. In this instance, the most stable conformation, with a minimal global energy, has to be identified by comparison of different conformations and the corresponding energy values.[10,14]

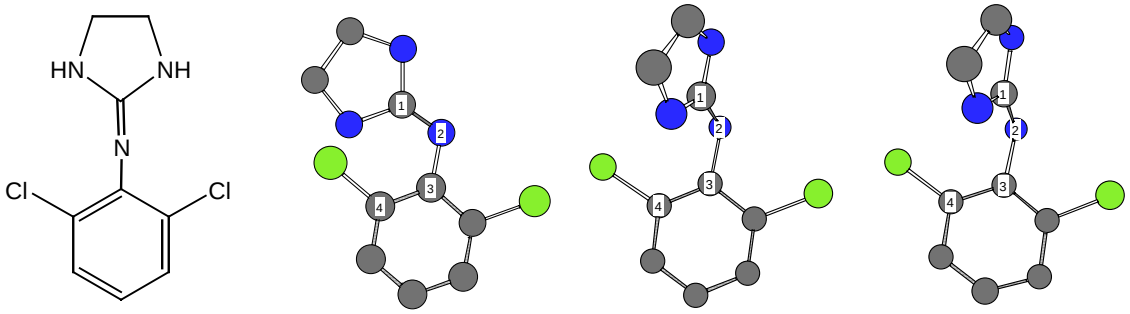


Graph 1 Local and global minimal energy, respectively, obtained by the minimization process and by molecular dynamics.

Molecular dynamics can be used to determine the most stable conformation. In this process, the stretching of bonds and angular alterations mimic a procedure of "heating" the molecule, where the energy barriers between conformations are overcome. An important example is the boat-distorted conformation of cyclohexane as it is minimized by this procedure. Heating the molecule by molecular dynamics generates new conformations including the most stable one, such as the chair. Clonidine (**11**), the blood anti-hypertension drug, when converted from the 2D-conformation (**11a**) to the minimized form by molecular mechanics (MM2) (**11b**) and submitted to molecular dynamics (**11c**), is a good illustration of the several spatial dispositions. It is important to note the variations in the dihedral angle of the different conformations (**11**). Structure **11c**, obtained by molecular dynamics, has the imidazolidine ring closer to a perpendicular orientation toward the 2,6-dichorophenyl group, which mimics the neurotransmitter, epinephrine, in the interactions with the α_2 receptor (Fig. 7).[15] Another more detailed and systematic procedure may obtain unidentified conformations in the Chem3D program, whereby new ones are gradually generated rotating a central bond and predetermining an angle alteration by the Newman projection. The steric energy in each conformation is determined and represented in energy versus angle graphs, to visualize the most stable ones.[10] This procedure is shown and explored during the laboratory course using acetylcholine (**12**) as a model. Three techniques are usual in the studies of the conformational properties of (**12**): X-ray crystallography, nuclear magnetic resonance, and molecular modeling.

By interacting with different nicotinic and muscarinic receptors in the autonomic nervous system, acetylcholine triggers several biologic effects. Many derivatives in different conformations of the drug have been prepared, but there is still no assurance as to the right receptor-specific conformations. It has been verified, however, that the pharmacophore group should have distinct spatial arrangements in order to interact with nicotinic and muscarinic cholinergic receptors. In this respect, the versatility of the molecule can be explained by the differences in the interatomic distances, 5.9 and 4.4 Å, between the ester and quaternary ammonium groups, respectively, for the nicotinic and muscarinic receptors interaction.[10] Interatomic distances are directly related to the

conformation of acetylcholine, that is, to the disposition of the dihedral or torsional angles of the molecule.



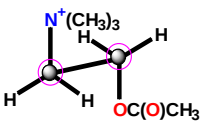
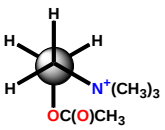
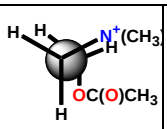
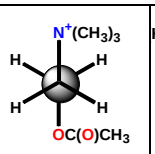
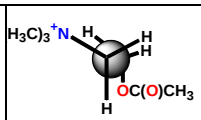
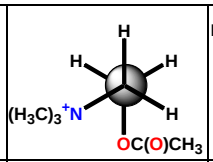
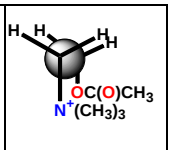
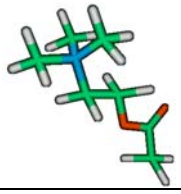
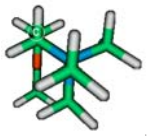
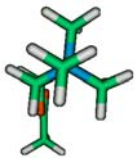
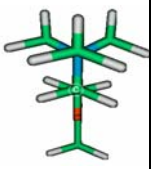
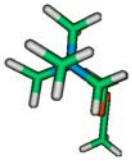
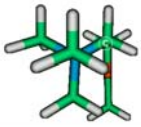
(**11**) (**11a**) τ 0.5 º (**11b**) τ 60.7 º (**11c**) τ 73.5 º

Fig. 7 Spatial representation of clonidine (**11**) showing corresponding dihedral angles (τ); (**11a**), structure drawn in program ChemDraw and converted to Chem3D; (**11b**), structure minimized by molecular mechanics, MM2 and (**11c**) structure showing systematic changes by molecular dynamics.

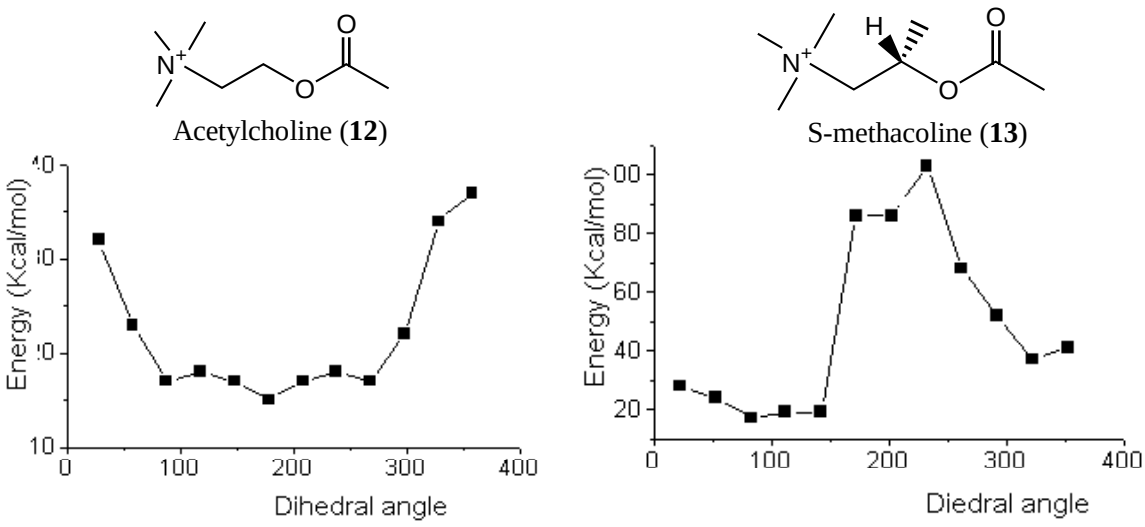
To improve the understanding of the different conformations of acetylcholine the students are trained to do the Newman projection of the central atoms of the drug, O(3)-C(4)-C(5)-N(6), in the Chem3D program and to gradually analyze the torsional angles of the molecule. The torsional or dihedral angle (τ) can be considered as the one formed by two defined planes as A-X-Y and X-Y-B of four atoms linked in the A-X-Y-B order. The projection shows the spatial relative disposition of the ester and quaternary ammonium groups.

In the main "View" menu of Chem3D, it is possible to select "Settings" and then "Movies" to manually rotate only bond C(4)-C(5) in the X-Y axis of the projection. After each 60° rotation, the dihedral angle is altered and a new conformation is generated, totaling six different conformations. At each change of the dihedral angle, the steric energies corresponding to the forms antiperiplanar (star-like) synclinal (gauche), anticlinal (gauche), and synplanar (eclipse-like) are calculated by the MM2 program, which only considers the internuclear lengths and angles, Table 1.[7] It is possible, in this experiment, to calculate the minimal global steric energy and predict the most stable preferential conformation of acetylcholine. The positive (0–180°) and negative (180–0°) rotation faces are really considered as a complete 360° movement in the X-Y axis, to facilitate graphic representation and interpretation of the results.

Table 1 Conformational analysis of the acetylcholine molecule (**12**) visualized through the Newman projection of O–C–C–N bond (Chem 3D and Weblab Viewer).

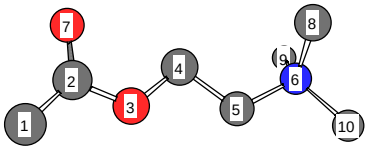
						
						
Dihedral angle	58	118	178	238	298	358
Conformations	Synclinal (+)	Anticlinal (+)	Anti-periplanar	Anticlinal (–)	Synclinal (–)	Synplanar
Energy (Kcal/mol)	23	18	15	18	22	37

The energy versus angle graphs corresponding to the different conformations of (**12**) may be constructed to interpret and compare their properties, and the steps in the changes are registered and animated. To complete the conformational analysis the students repeat the same task for *S*-(+) methacholine (**13**) (Graph 2), a muscarinic cholinergic agonist, to verity the spatial influence of the methyl group, by comparing the steric energy versus dihedral angle graphs. In the *S*-methacholine example, the stable conformation is close to a synclinal with an 80° angle. The biologically inactive isomer *R*-(–)- methacholine has its stable conformation near the anticlinal form (280°).



Graph 2 Energy variation represented as a function of the dihedral angles in acetylcholine (**12**) and *S*-(+) methacholine (**13**).

The molecular dimensions of the 3D structures of acetylcholine (**12**), related to length and bond angles and torsional or dihedral angles, can be obtained in table form in an additional measurement window, to be used in conformational analysis of analog compounds, Fig. 8.[7]



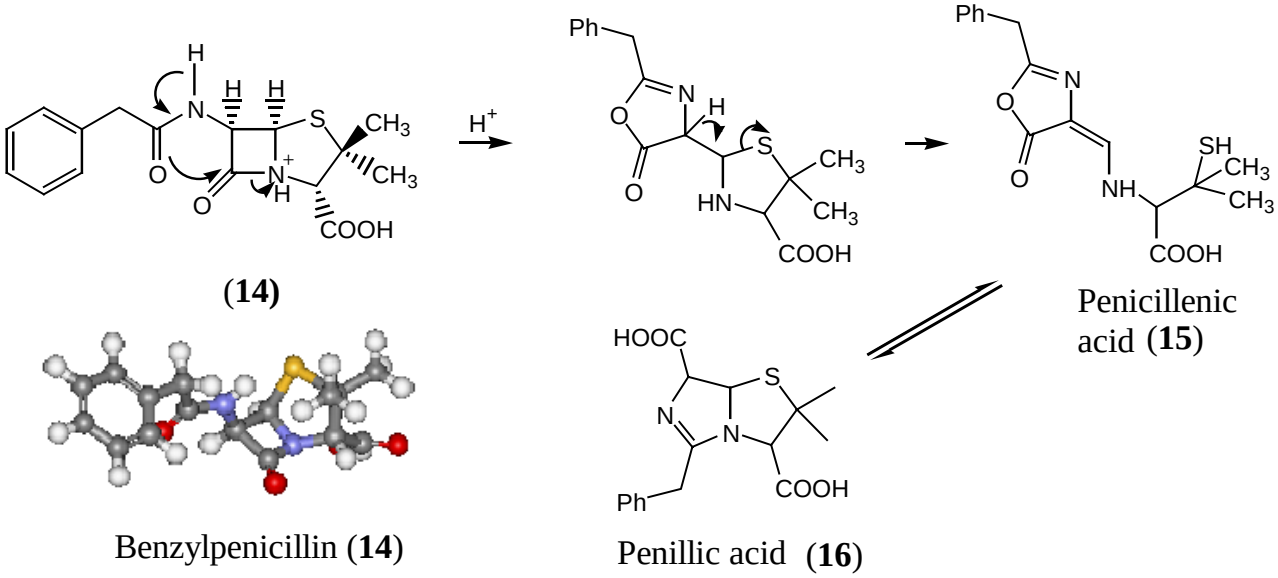
acetylcholine (**12**)

Bond lengh		Bond angle		Diedral angle	
C(1)-C(2)	1.484	C(1)-C(2)-O(3)	112.535	C(1)-C(2)-O(3)-C(4)	-179.000
C(2)-O(3)	1.388	C(1)-C(2)-O(7)	131.182	O(7)-C(2)-O(3)-C(4)	0.000
C(2)-O(7)	1.228	O(3)-C(2)-O(7)	116.282	C(2)-O(3)-C(4)-C(5)	-179.292
O(3)-C(4)	1.428	C(2)-O(3)-C(4)	115.931	O(3)-C(4)-C(5)-N(6)	179.368
C(4)-C(5)	1.533	O(3)-C(4)-C(5)	103.759	C(4)-C(5)-N(6)-C(8)	60.275
C(5)-N(6)	1.504	C(4)-C(5)-N(6)	114.492	C(4)-C(5)-N(6)-C(9)	-61.169
N(6)-C(8)	1.493	C(5)-N(6)-C(8)	110.530	C(4)-C(5)-N(6)-C(10)	179.553
N(6)-C(9)	1.493	C(5)-N(6)-C(9)	110.533		
N(6)-C(10)	1.497	C(5)-N(6)-C(10)	108.118		
		C(8)-N(6)-C(9)	109.540		
		C(8)-N(6)-C(10)	109.043		
		C(9)-N(6)-C(10)	109.035		

Fig. 8 Molecular dimensions, length, bond angles and dihedral angles in acetylcholine (**12**) (Chem3D).

Molecular properties

The reactivity of β-lactam antibiotics is essential to understand its mechanism of action and metabolism. Thus, the main objective is to predict the degradation products in certain biologic compartments and the spectrum of action in connection to the presence of additional polar groups in the molecule. A remarkable example is the analysis of the reactivity of benzylpenicillin (**14**), which undergoes intramolecular interactions in acid conditions, started by the side chain nucleophilic oxygen (amide), Scheme 1).[10] The sensitivity of (**14**) to acid is due mainly by (i) torsional tension in the β-lactam ring, (ii) the high reactivity of the β-lactamic carbonyl carbon, and (iii) the influence of the acyl group (amide) of the side chain acting through the neighboring group participation.[10]

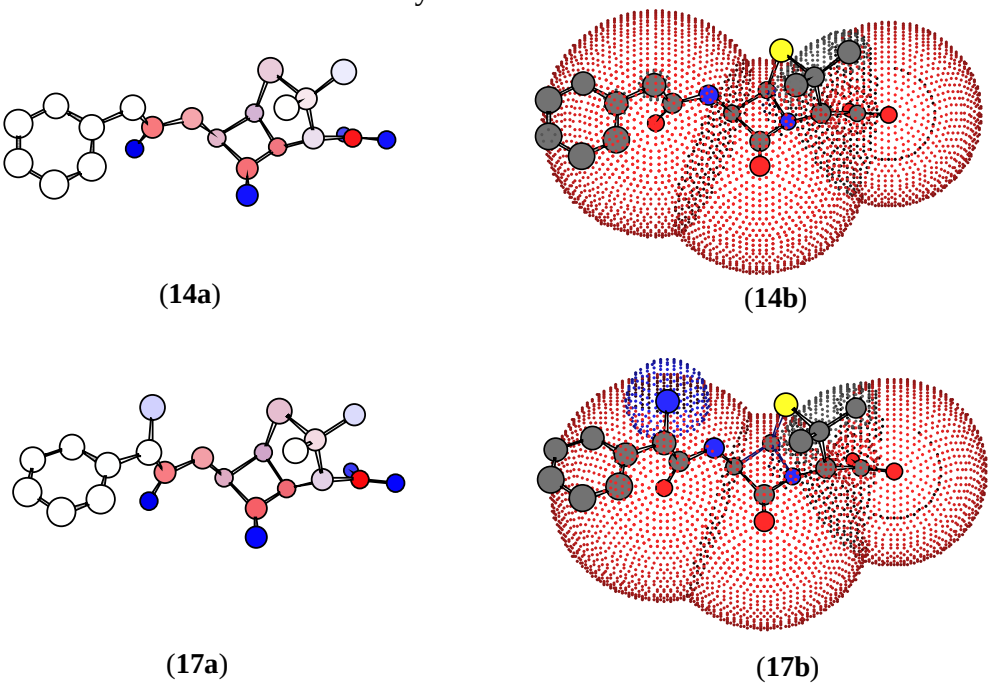


Scheme 1 Partial degradation of benzylpenicillin (**14**) in acid media.

In ampicillin (**17**), however, the extra amino group, α to the side chain amide carbonyl, withdraws electrons from the neighboring amide, and thus decreases the effect close to the β-lactam ring. In this case, the antibiotic may be used orally, and the metabolites, penicillenic (**15**) and penillic (**16**) acids will not be formed in the acid conditions of the stomach. Also, the ionization of the ampicillin polar amino group facilitates the crossing of porine channels (hydrophilic) in the bacterial cell wall and the action of the antibiotic on gram-negative bacteria.[10]

It is possible to evaluate the general reactivity of the molecule and predict pathways of degradation, like acid hydrolysis, by visualizing the partial charge of atoms in lactam antibiotics (Scheme 1). Electrons move around a molecule and are not fixed specifically to one atom. The probability of finding electrons close to electronegative atoms is higher, thus resulting in molecules with a non-uniform electrical charge. Partial charge can be quantified, and the values represent the average charge of each atom, minus the number of protons present. The higher the atomic charge, the higher the probability of bond formation with other atoms carrying a partial charge of opposite number. The extended Hückel method allows semiempiric calculations of the molecular orbital in the Chem3D program and the visualization of each atom's partial charge. A 3D molecule can have partial charges represented in three ways: atoms of different colors (blue for negative and red for positive);

size of balls representing atoms; and size of a surface represented by dots. When using dots, the size of the cloud represents the charge of the atom, red for more negative or blue for positive. In Fig. 9, benzylpenicillin is represented by structures (**14a** and **14 b**) and ampicillin by (**17a**) and the ionized form (**17b**), being the partial charge represented in **a** by the color of the atoms and in **b** by dots.[7]

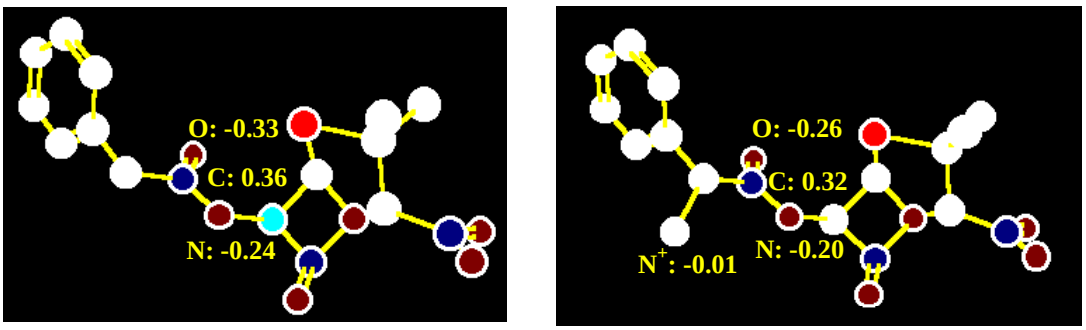


(**14a**) (**14b**)

(**17a**) (**17b**)

Fig. 9 Partial charge distribution shown in different representations of benzylpenicillin (**14a**) and (**14b**) and ampicillin (**17a**) and (**17b**, ionized form; the partial charges in structures (**14a**) and (**17a**) are represented by different colors (blue: negative and red: positive); structures (**14b** and **17b**, ionized form) are represented by dots (red: more negative; blue: positive) (Chem3D).

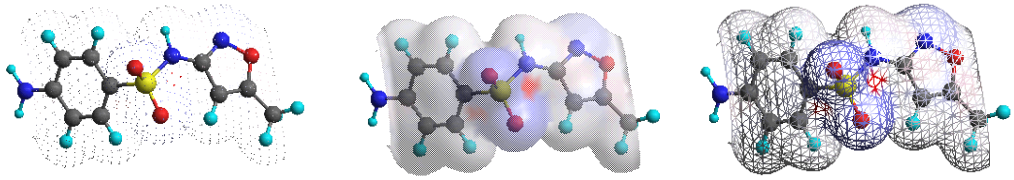
The charge differences between corresponding oxygen atoms (carbonylic, in the amide side chain), in penicillin (**14**) (–0.33) and protonated ampicillin (**17**) can be visualized by the method CNDO, in Molecular Modeling Pro.[9] The extra protonated α-amino group in ampicillin lowers the negative charge of the side chain amide oxygen, turning it less reactive in the nucleophilic attack to the carbonyl carbon of the β-lactam ring, in acid conditions. The charges in the carbon and nitrogen atoms of the amide in **17** are less altered becoming, respectively, less electrophilic and nucleophilic. Figure 10 shows the values of the atom's partial charge in the molecules of benzylpenicillin (**14c**) and ampicillin (**17c**), calculated in the Molecular Modeling Pro.[9]



(**14c**) (**17c**)

Fig. 10 Charge distribution in benzylpenicillin (**14c**) and ampicillin (**17c**) molecules represented by colors: dark blue, for strongly positive atoms; light blue, for slightly positive; brown, strongly negative; red, slightly negative and white, neutral atoms (Molecular Modeling Pro).

The values of partial charge and dipole moment may be calculated by different computational methods like the extended Hückel theory, that considers a linear combination of atomic orbitals; the CNDO involving the process of semi-empiric quantum mechanic or Del Re, which is completely empiric. The CNDO method is widely employed in analysis of the geometry and molecular properties and the calculations in the extended Hückel are used mostly in aromatic systems. Thus, the data on partial charge, shown in Fig. 10, were obtained through the CNDO method available in the main menu "Tools" in the program Molecular Modeling Pro.[9] Still some other visualization is possible, like the surface of the molecular orbital, the molecular electrostatic potential and the solvent access surface. For example, it is possible to represent the molecule of sulfamethoxazole (**3**) by the density of total charge surface, emphasizing the partial charges present, Fig. 11.



(**3e**) (**3f**) (**3g**)

Fig. 11 Total charge density in the sulfamethoxazole (**3**) structure represented by dots (**3e**), transparent surface (**3f**) and wire (**3g**) (Chem3D).

Molecular Modeling

The main issues in molecular modeling were centered in the exploring of electronic, steric and hydrophobic differences between agonist and antagonist drugs within a single therapeutic class. The programs employed in these studies were Molecular Modeling Pro[9] and Chem Site[16] (Chem SW) and all the analyzed molecules were previously submitted to steric energy minimization by the MM2 and AMBER methods of molecular mechanics, and saved in one directory for the simultaneous calculations of the desired properties. Some of the physicochemical properties are not dependent on the molecular geometry, but the optimization is necessary to calculate molecular volume and lengths, surface area, density and dipolar moment.

In the Molecular Modeling Pro, stepwise conformational analysis, described previously, is automatically and rapidly performed, allowing the rotation of one or two bonds in one single analysis. Graphs of energy versus angle, corresponding tables, and the representation of three of the most stable structure are produced in just a few seconds.[9]

At this stage of the course, superimposition of molecules with common functional groups and determination of physical parameters, possibly relevant to biological activity directed modeling studies for analysis of similarities and differences of agonist and antagonist compounds. The best superimposition was by aligning several compounds on the x-y axis, according to the program instructions.[9] Substrates were kept fixed (acetylcholine and epinephrine) and the drugs situated close to the corresponding ones, with the help of rotation tools. There are different forms to emphasize similarities and differences, like shapes and colors; in Fig. 12, acetylcholine is represented with cylindrical bonds and balls and the drugs (**13,18–21**) with sticks.

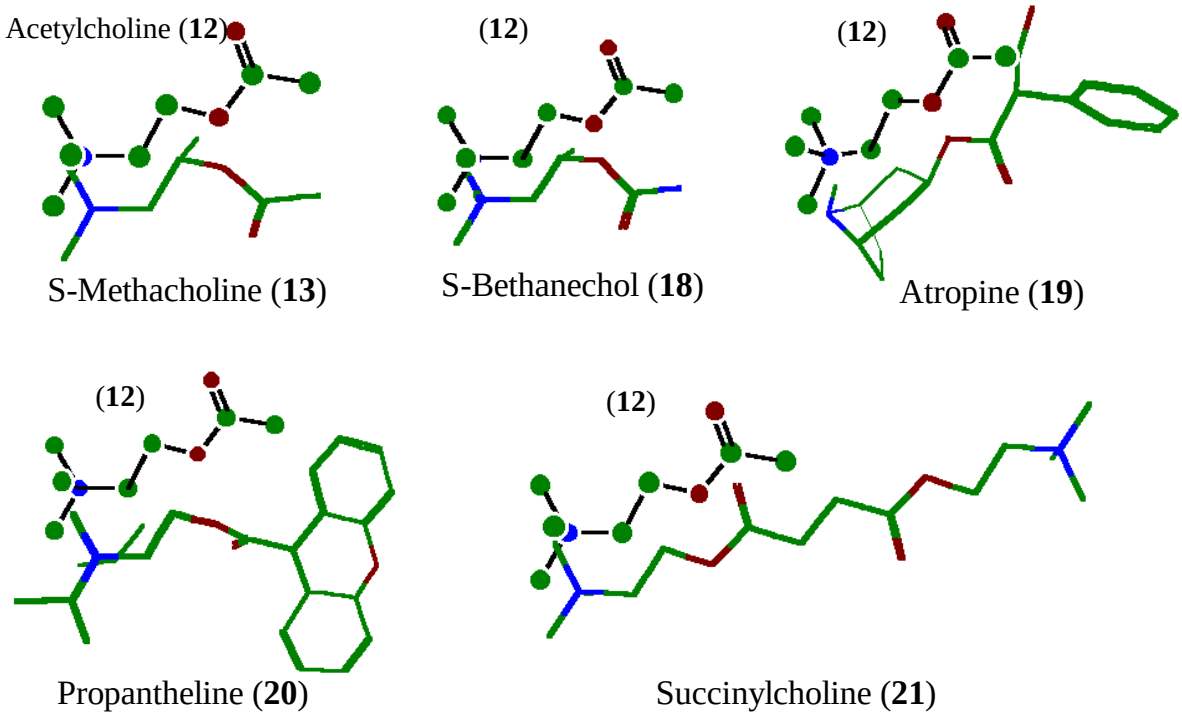


Fig. 12 Superimposing cholinergic drugs and acetylcholine (**12**), with emphasis on similarities (interatomic distances and dihedral angle) and on differences (molecular volumes). Acetylcholine (**12**) is represented by balls and drugs (**13,18–21**) by sticks (Molecular Modeling Pro).

Figure 12 shows evidence of some similarities in the superimposition of acetylcholine and agonists methacholine (**13**) and bethanechol (**18)** and antagonists, atropine (**19**), propantheline (**20**), and succinylcholine (**21**) such as interatomic distances and the dihedral angle between the ester oxygen and the side chain ammonium group. On the other hand, obvious differences are seen in the molecular size, and the predominance of bulky groups in the antagonists.

Several measurements of physicochemical parameters may be obtained as a table by applying the Molecular Modeling Pro, including interatomic distances, dihedral angles, volume, length and molecular weights, partition coefficient (log *P*), polarity, hydrogen

bonds, molecular connectivity, and dipolar moment among others. Table 2 shows some of the parameters obtained in a comparative analysis of drugs acting on the cholinergic system. The interpretation of the data contributes to a better understanding of the theoretical course showing similarities and differences between agonists and antagonists as already discussed above in the process of superimposition.

Table 2 Physicochemical parameters obtained in a comparative analysis of cholinergic acting drugs (Molecular Modeling Pro).

Cholinergic agents	Formula	Molecular volume (cm^3/mol)	Molecular length (Å)	Log *P*	Dipolar moment (Debyes)	Interatomic distance (Å)	Dihedral angle
Acetylcholine	$C_7H_{17}NO_2$	94.30	10.38	0.87	1.34	3.78	180.04
S-Methacholine	$C_8H_{19}NO_2$	104.32	10.72	1.18	1.53	3.76	149.02
S-Bethanechol	$C_7H_{18}N_2O_2$	101.15	10.81	0.33	2.88	3.77	150.56
S-Atropine	$C_{17}H_{25}NO_3$	168.55	11.05	1.52	2.28	4.03	302.10
Propantheline	$C_3H_{33}NO_3$	223.66	14.92	4.63	2.06	3.84	174.83
Succinylcholine	$C_{14}H_{32}N_2O_4$	182.05	18.94	1.05	0.07	3.75	179.99

The same comparison and measurements were made in the structural analysis of adrenergic drugs employing epinephrine (**22**) as the reference substrate in the superimposition procedure where it is represented in balls and cylinders and the drugs (**23–26**) represented as sticks, Fig. 13.

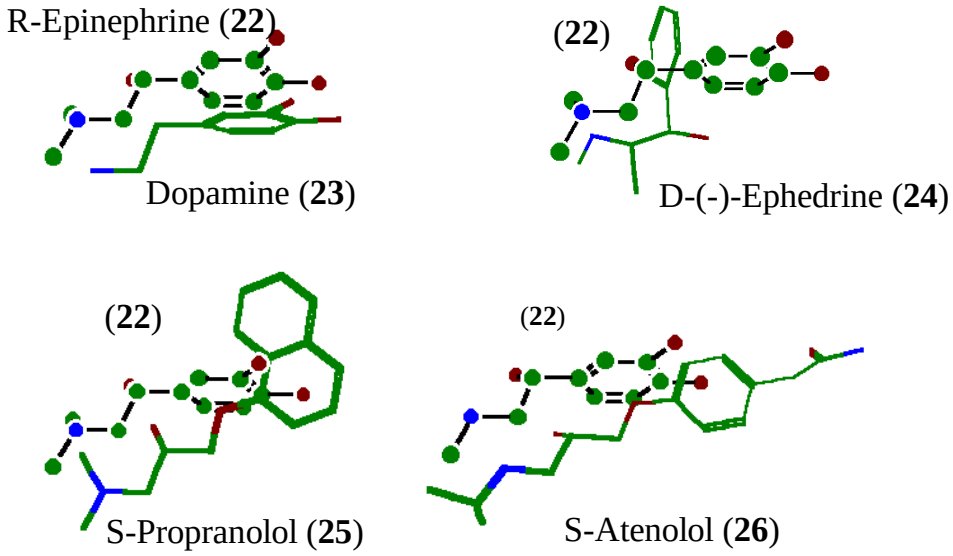


Fig. 13 Superimposing adrenergic drugs and epinephrine (**22**) with emphasis on similarities (interatomic distances and dihedral angle) and on differences (molecular volumes). Balls and sticks represent epinephrine (**22**) and sticks represent drugs (**23–26**) (Molecular Modeling Pro).

In this instance, the geometry of agonists, dopamine (**23**), and ephedrine (**24**) and antagonists propranolol (**25**) and atenolol (**26**) was compared and similarities and differences established. In this class, the similarities were in the side chains ethylamine or

ethanolamine in the agonists and oxypropanolamine in the antagonists, while the molecular volume is again a major difference between these drugs, Table 3.

Table 3 Physicochemical parameters obtained in a comparative analysis of adrenergic acting drugs (Molecular Modeling Pro).

Adrenergic agents	Formula	Molecular volume (cm^3/mol)	Molecular lengh (Å)	Log *P*	Dipolar moment (Debyes)	Interatomic distance (Å)	Dihedral angle
R-Epinephrine	$C_9H_{13}N\ O_3$	102.66	12.22	–0.60	1.39	3.84	181.66
Dopamine	$C_8H_{11}NO_2$	87.19	10.85	0.09	1.47	3.83	177.31
D-(–)-Ephedrine	$C_{10}H_{15}NO$	101.66	8.05	1.03	0.66	2.96	55.47
S-Propranolol	$C_{15}H_{21}NO_2$	146.09	12.92	2.74	2.02	3.90	179.62
S-Atenolol	$C_{14}H_{22}N_2O_3$	155.78	17.12	–0.15	2.79	3.85	181.55

Superimposition of drugs may also be used to compare lipophilicity and partial charge distribution. Figure 14 shows two illustrative examples of differences in lipophilicity of acetylcholine (**12**) versus atropine (**19**) and epinephrine (**22**) versus propranolol (**25**). Lipophilic and hydrophilic regions are shown, respectively, by blue and red or brown colors. It is also possible to observe how antagonists can be compared to the respective substrates in terms of liposolubility.

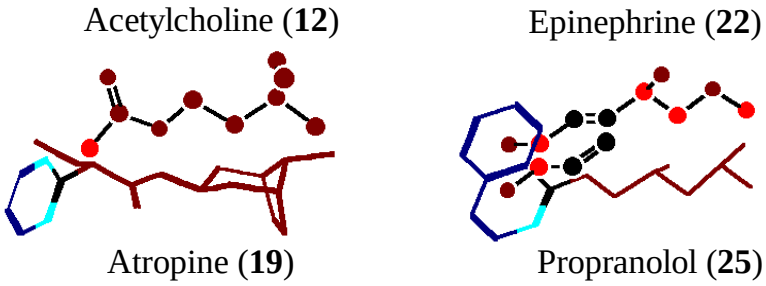


Fig. 14 Comparative lipophilicity of drugs shown in different colors: dark blue, highly lipophilic; light blue, slightly lipophilic; black, neutral; red, slightly hydrophilic; brown, highly hydrophilic (Molecular Modeling Pro).

Differences in partial charge are analyzed comparatively by superimposition and the results shown in Fig. 15 with different colored atoms in acetylcholine (**12**) versus atropine (**19**) and epinephrine (**22**) versus propranolol (**25**). Dark blue indicates a partial positive charge, light blue, less positive, red less negative and brown, a negative charge.

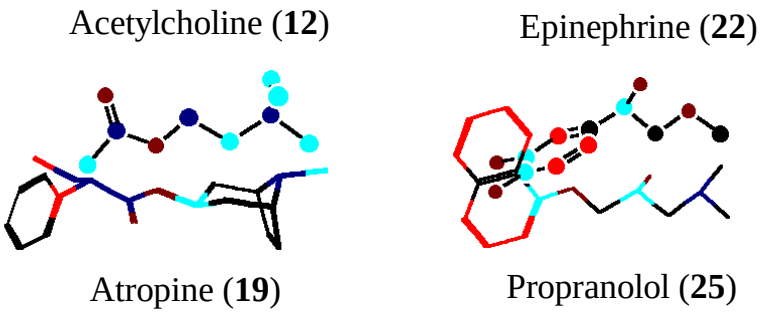


Fig. 15 Comparative partial charge distribution in drugs shown in different colors: dark blue, indicates atoms with positive partial charge; light blue, slightly positive partial charge; red, slightly negative charge and brown, negative partial charge (Molecular Modeling Pro).

RESULTS AND DISCUSSION

Drawing, molecular, and conformational analysis of drugs

The 3D structural representation of drugs in computational programs and the construction of molecular models were very important tools for the understanding of molecular and geometry features necessary to biological activity in several classes of compounds. Furthermore, this first stage of activity combined fundamental concepts of organic chemistry, such as stereochemistry, reactivity, and nomenclature for understanding the SAR of drugs.

Minimization of energy and molecular dynamics were applied to obtain stable structures, corrected for eventual distortions due to bonds with unfavorable lengths and dihedral angles. The interactions of steric and electrostatic interactions, detected in non-bonded atoms, were corrected at this stage.[10]

The variation of steric energy as a function of change in the dihedral angle of acetylcholine (**12**) provided a detailed conformational analysis and graphic visualization of the results. The absence of a stereogenic center in acetylcholine generated a graph with a symmetric curve, different from *S*-methacholine. Moreover, it was possible to visualize the most stable conformation and corresponding variation in the steric energy profile of (**12**) after the addition of a methyl group in the main chain, generating *S*-(+)-methacholine (**13**) with a recognized selective muscarinic action.

The Newman projection of the acetylcholine molecule **[$(CH_3)_3$+N-C-C-OC(O)CH_3]**, employing MM2(Chem3D[7] and Molecular Modeling[9]) and Amber (Molecular Modeling[9]) allowed visualization of the greater distance and smaller repulsion between substituent functional groups, $(CH_3)_3$N+ and OC(O)CH_3, in the 180° (antiperiplanar) and 80° (near synclinal) conformations with steric energy around 15.0 and 16.0 Kcal/mol, respectively. On the other hand, the most stable conformation of *S*-methacholine, employing the MM2 method, was close to the synclinal (80°), with a steric energy of 16.7 kcal/mol.

Data on energy, angle and interatomic distance in acetylcholine (**12**), obtained by molecular mechanics and by minimization with semi-empiric methods (MOPAC AM1 and PM3-Molecular Modeling[9]) were compared. Molecular mechanics and the AM1 method had similar results with energies between 20.3 and 22.0 Kcal; however, by applying PM3, the anticlinal (120°) and antiperiplanar (180°) conformations were the most stable with

energies 21.6 and 20.1 Kcal, respectively. In association with theoretical concepts, the students conclude that there is a high probability that drugs achieve their goals in stable thermodynamic conformations, but other factors may disturb the spatial disposition of the final structure. The acetylcholine molecule is an outstanding example of a biological drug, which may act in the antiperiplanar form (180°) on the nicotinic receptor, whereas the action on the muscarinic one is in the synclinal (~80°) or anticlinal (~120°) form, probably due to electrostatic interaction between groups $(CH_3)_3N+$ and $OC(O)CH_3$ in the molecule. This result is in accordance to the form observed for the selective muscarinic agonist, *S*-methacholine (**13**), whose bioactive conformation is close to synclinal (80°).

Molecular modeling

The practice of molecular modeling was performed by using computational chemistry tools to study the features and properties of cholinergic and adrenergic drugs. In both groups, the students were able to understand and memorize structural similarities between agonist and antagonist drugs essential for receptor affinity, as well as the fundamental structural differences that often convert a substrate in a competitive inhibitor. Theoretical studies on the SAR and the pharmacophore groups of drugs acting on the autonomic nervous system, were complemented by the structural comparison of substrate and its corresponding agonists and antagonists in programs of molecular modeling, Figs. 12 and 13.[9,16]

Superimposing compounds and comparatively measuring volumes and molecular lengths in agonists and antagonists showed maintenance of some structural properties for the complementary interaction on the receptor and the outstanding presence of bulky groups in the antagonists, which contributes to hydrophobic additional interactions in regions close to the receptor. In these practical exercises, the students concluded that in the autonomic nervous system, the larger volume of antagonist drugs causes a steric hindrance and, as a result, blocks the interaction substrate-receptor. In agonist and antagonist cholinergic drugs, the interatomic distance between ester oxygen and the quaternary (or tertiary) ammonium group is about 3.7–4.0 Å, and the higher affinity to the receptor is dependent on this structural feature. The complementary distance between amino acids in the receptor linking sites could be the same to facilitate the interaction with the ligant. Thus, Thr192, and Asn382, which have hydrophobic and hydrogen bonding with acetylcholine's ester group, and Asp105, Trp378, and Tyr381 linked to the quaternary ammonium group (or protonated tertiary) through ionic and cation π interactions, should be separated by distances of the same order, Fig. 16.[18]

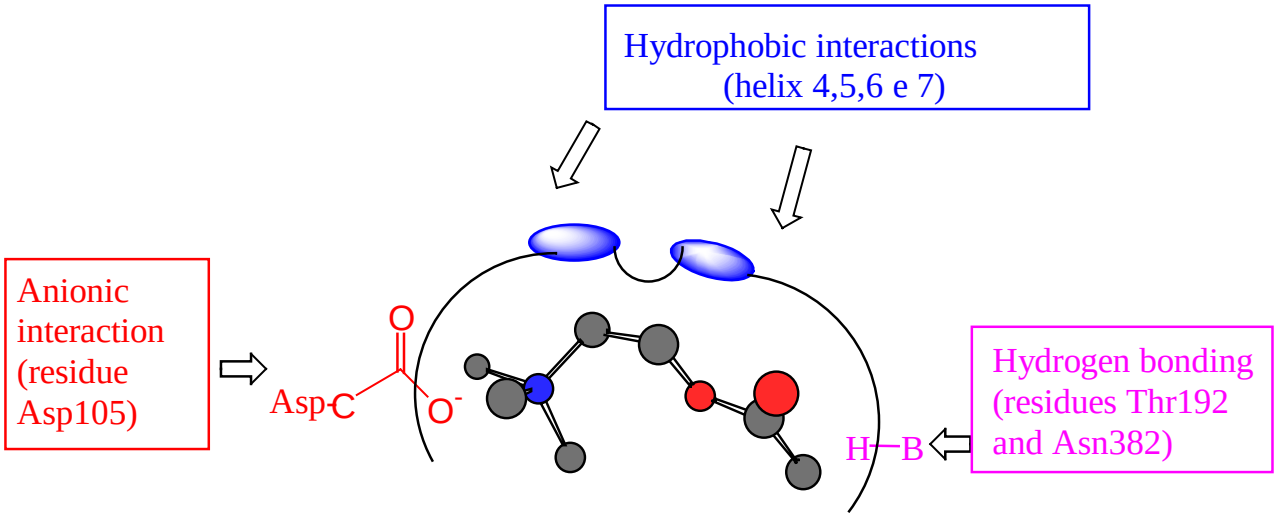


Fig. 16 Interactions between a hypothetical muscarinic receptor and the neurotransmitter acetylcholine (**12**) in hydrophobic, anionic, and hydrogen-bonding regions.

With the exception of ephedrine (**24**), the interatomic distances in adrenergic drugs, between the side chain amino group and the aromatic ring carbon directly bonded to the side chains, ethylamine (dopamine, **23**), ethanolamine (epinephrine **22**), and oxypropanol amine(propranolol **25** and atenolol, **26**), varied around 3.8–3.9 Å. The side chain of ephedrine is greatly distorted by the presence of the methyl group, but for the majority of agonists and antagonists there is a high side chain geometrical similarity. In the adrenergic receptor, the distance between Asp113 and Phe290 is similar; these amino acids, respectively, interact by ionic linkage to the protonated amino group, and by hydrophobic bonding to the aromatic ring of the ligand, Fig. 17.[10]

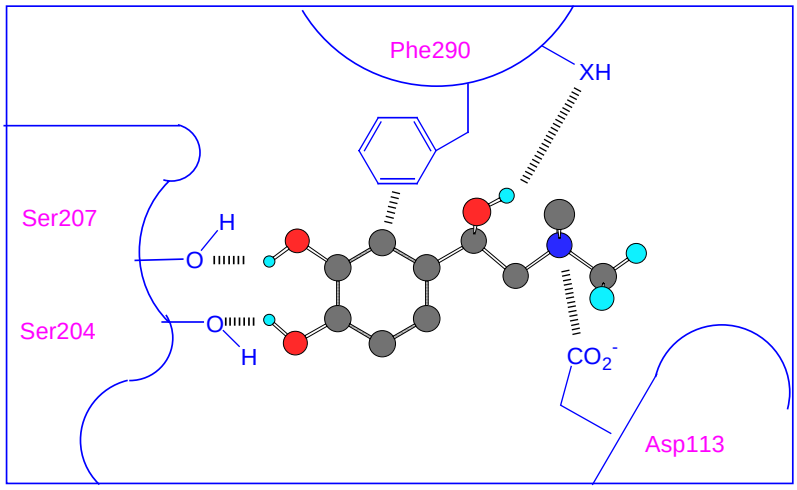


Fig. 17 Interactions between a hypothetical adrenergic receptor and epinephrine (**22**) in hydrophobic, anionic, and hydrogen-bonding regions.

In the cholinergic antagonists propanteline (**20**) and succinylcholine (**21**), atoms O–C–C–N had dihedral angles similar to the ones in the acetylcholine substrate, in its more

stable antiperiplanar conformation (around 180°). On the other hand, agonists *S*-methacholine (**13**) and *S*-bethanechol (**18**), with a methyl group in the main chain, had a different conformation with a 150° or 90° dihedral angle and smaller steric energy. The difference (80°) shown in the conformational analysis indicates that the angle, in Table 2, could be related to the local minimal energy obtained during the MM2 minimization process. Adrenergic drugs, with flexible side chains linked to the aromatic ring, showed in most examples, dihedral angles close to 180° between the hydroxyl oxygen and the amino function (O–C–C–N) in the side chain.

The dipolar moment, calculated for adrenergic and cholinergic drugs, translates the polar character of several molecules, since it represents the summation of vectors of polarizing forces affected by the functional groups present. Theoretical calculation can be made by several methods available in Molecular Modeling Pro, like the modified Del Re that considers the additional contribution of *pi* bond, or PEOE (partial equalization of orbital electronegativity), able to consider *sigma* and *pi* bonds and MPEOE related to an improved PEOE. Values shown in Table 2 are the calculations obtained by MPEOE. A stronger polar character was shown in cholinergic drugs, such as succinylcholine (**21**), a nicotinic cholinergic antagonist, in relation to atropine (**19**) and propanteline (**20**), muscarinic cholinergic antagonists. Agonist methacholine and acetylcholine had similar polarities, with intermediary values in relation to the two classes of antagonists.

Values of log *P* or oil/water partition coefficients, determined for fragments present in the molecule were characteristic of cholinergic drugs. The bulky groups of muscarinic antagonists contribute to the higher lipid solubility, and, consequently, higher log *P* values for atropine (**19**) and especially for propanteline (**20**). Agonist bethanechol (**18**) showing a carbamate group had a lower log *P* value, indicating its higher water solubility. Adrenergic drugs had log *P* values apparently not correlated to agonist or antagonist effects; propanolol (**25**) and ephedrine (**24**) were more soluble in lipids than atenolol (**26**) and dopamine (**23**).

CONCLUSIONS

The students successfully executed the laboratory practices in molecular modeling. In the early stage of the course, the resources of several programs were demonstrated (Chemdraw[6], Chem3D[7], Molecular Modeling Pro[9] and ChemSite[16]) through tutorials and written handouts. At first, there was some difficulty in relating obtained data and biological activity, but with the increasing integration of the theoretical course and the laboratory practice, the understanding improved considerably. The reports submitted during the course confirmed the students' maturity, improved knowledge, and good scientific communication.

In an internal survey, 86 % of the students answered that programs were adequate for learning the subject; when asked about the difficulty level, 53 % considered that it was compatible to the contents of the course; 28 % gave partial answers.

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