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STRUCTURAL INVESTIGATIONS AND STRUCTURE-ACTIVITY RELATIONSHIPS IN A SERIE OF NEW ANTIBIOTIC 16- MEMBERED MACROLIDES

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ABSTRACT

This study is fundamental research on the structure- activity relationships in 16-membred macrolides. It is based on the molecular modelling (molecular mechanics, molecular dynamics, distribution de Boltzmann, PM3, SAR,). We defined the structural motives intervening in antibiotic macrolide properties. The compounds substituted in positions (C2, C4, C8, and C15) are the most stables and the less stable is substituted in C12. The rokitamycine which has the most elevated value of partition coefficient: 3.06. This antibiotic is lipophilic, so it has good permeability across the biological membrane, better fixation on plasma proteins and elimination by metabolic route.

Keywords: 16-membered macrolide, SAR, molecular mechanics, molecular dynamics, Boltzmann distribution.

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1. INTRODUCTION

Molecular modeling involves the use of theoretical calculation methods to determine the geometry of a molecule and evaluate the associated physicochemical properties [1-3].

The comparison of the biological activity of certain molecules and their structures has made it possible to establish in many cases correlations between the structural parameters and the properties of a molecule. Knowledge of this correlation makes it possible to develop new active molecules, with fairly good anticipation [4-6].

Macrolide antibiotics play a very important therapeutic role, due to their biological interest; the chemistry of macrolides is in increasing development [7-9].

Elucidation of the structure[10-12] of a large number of macrolides obtained revealed the existence of two parts. The first is a macrocyclic system of 12 to 40 members with several asymmetry centers with a lactone function. The second is a sugar part. The most commonly used macrolides currently are azithromycin and josamycin [13].

The majority of macrolide antibiotics currently used in the clinic were obtained by chemical modification from erythromycin and spiramycin [14]. These new macrolides have been designed specifically to combat bacterial resistance.

2. CALCULATIONS AND RESULTS

The aim of this study is to search for the preferred conformations [15-17] of 16-membered macrocycles, that's to say, the most energy-based conformations based on energetic and geometric considerations [17] by using a statistical calculation named Boltzmann distribution [18], followed by a structural comparison of the main 16-membered antibiotics.

Finally, the relationships between the structural parameters and the biological activity of these new macrolides are determined.

The investigation of the different preferential conformations of macrocycles and the structure-activity calculation was carried out by molecular modeling (molecular mechanics, molecular dynamics, quantum mechanics/PM3 and Boltzmann distribution), using the HyperChem software (7.5) [19] and CHEM 3D (8.0) [20].

The first method is considered the most suitable method for large molecules [21]. We used the

total steric energy to compare the thermodynamic stabilities of the conformers, using HyperChem software. The latter is based on the force field (MM+) of Allinger [22, 23].

The minimization algorithms used repeatedly in calculating the preferred conformation are in the following order: steepest-descent, conjugate gradient, and Newton-Raphson; the calculation procedures are stopped when the minimum energy obtained remains constant.

2.1 Conformational analysis of 16-membered macrolides

In this part of our work, we undertook a conformational study of 16-membered macrocycles, symmetric which we will designate by 16s, and asymmetric which we will designate by 16d (Figure 1). The latter constitutes the basic backbone for many 16-membered macrolide antibiotics.

Analysis of the conformations shows that the macrocycles have three structural characteristics: a diene system, α, β-unsaturated ester group and two saturated chains.

The results obtained based on the first two structural characters allow a set of conformers resulting from these calculations to be split into conformational families characterized by a given geometric specificity and an average energy [24-29].

Eight main conformational families were retained. In the type families (T2, T4, T6, T8) the two planes of the two sites (the diene, the, β-unsaturated ester function) are pseudo-parallel. For the other families (T1, T3, T5, T7) the two planes of the two sites are pseudo-antiparallel [24, 26].

By applying the Boltzmann distribution, in a gap of 1 kcal/mol the 16s macrocycle is characterized by a first favored T5 type conformer with a rate of 20.72 %, followed by the T4 type with 17.93%. While the 16d macrocycle occurs preferentially in the conformations of type T6 (Figure 1-a) with (22.69%) and type T3 (Figure 1-b) with (20.50%). The percentages of the other conformational types are recorded in Table (1).

The population rate of the preferred conformer of the 16d macrocycle is slightly higher than that of the 16s macrocycle. In an energy gap of 2 Kcal/mol, the 16d macrocycle has relatively the lowest conformational mobility, with 3 stable conformations.

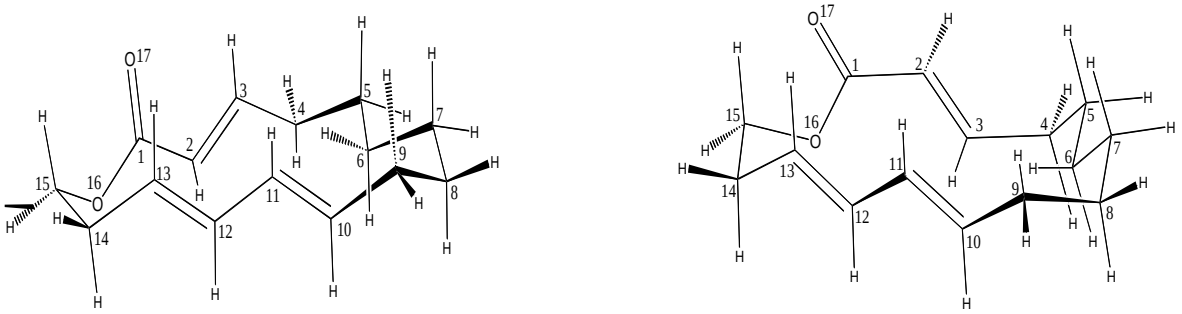
Conformational stability is linked to biological activity, i.e. the preferred conformation possessing geometric complementarity with a given receptor. It forms bonds with the latter in

order to ensure sufficient stability of the ligand-receptor complex [17].

Table 1. Energy gap and Boltzmann population for different conformational types

Macrolide	**Symmetric macrocycle 16**			**Asymmetrical macrocycle 16**		
	Type	ΔE	%	Type	ΔE	%
at 1kcal/mol	5	0.000	20.72	6	0.000	22.69
	4	0.596	17.93	3	0.417	20.50
to 2kcal/mol	6	1.292	15.14	5	1.750	14.83
	3	1.886	13.10			
Above	1	2.782	10.54	4	2.550	12.20
2 kcal/mol	7	3.303	09.28	1	4.483	07.63
	8	4.434	07.05	8	4.495	07.61
	2	4.932	06.25	7	4.623	07.37
				2	4.734	07.18

ΔE: Energetic difference to the absolute minimum %: Boltzmann Population



a) T6 ; ΔE_1 = 0.00 kcal/mol; μ = 2.66 D b) T3 ; ΔE_2 = 0.41 kcal/mol; μ = 2.64 D

Fig 1. The two preferred conformations of the 16d macrocycle

The dihedral angles of the ester and diene system are grouped in Table (2). For the values of Φ1 and Φ2 are in the majority of cases close to the value of an aliphatic system (0°or 180°). The small deviation observed is essentially due to Van der Waals repulsions between hydrogen atoms as well as to the constraint of the cycle skeleton which imposes geometric parameters to obtain the least energetic conformation [8].

For the geometry of the most preferred conformer; The α, β-unsaturated ester system has a (s-cis) conformation with a twist angle ϕ_1(O17-C2-C3-C4) = 3.1° for the 16d macrocycle and

ϕ_1(O17-C2-C3-C4) = 5.5° for the 16s macrocycle. The diene system has an (s-trans) conformation with an angle ϕ_2 (C10-C11-C12-C13) = 179.5° for 16d and ϕ_2 (C8-C9-C10-C11) = 179.8° for 16s.

2.2 Contributions of different factors to the total steric energy

The minimum steric energy calculated by the molecular mechanics method with force field (MM+), is the sum of the contributions made by the energies of elongation E(l), bending E(θ), torsion E(Φ) of Van der Waals E(VdW), electrostatic E(elect)…….. [22, 23].

$$E(ste) = E(l) + E(\theta) + E(\Phi) + E(VdW) + E(elec)\ldots\ldots$$

We will undertake an examination of their contributions and their influences on the total steric energy.

Table 2. Contribution of the different factors to the total steric energy in kcal/mol for 16d.

Macrocycle	**T6**	**T3**	**T5**	**T4**	**T8**	**T1**	**T7**	**T2**
Steric energy	10.745	11.162	12.495	13.295	15.228	15.240	15.368	15.479
E(l)	00.815	00.856	00.807	00.914	00.849	00.842	00.860	00.858
E(θ)	04.823	03.981	05.549	04.318	06.260	04.339	05.011	06.021
E(Φ)	-03.059	-02.117	-03.088	-00.252	-00.379	01.595	01.006	-00.284
E(VdW)	09.415	09.992	10.587	10.105	09.745	08.585	10.011	08.679
E(elec)	-01.521	-01.816	-01.597	-01.819	-01.547	-00.370	-01.804	-00.066

In the light of these results, we see that in the two systems the majority constraints in the total steric energy are those of Van der Waals and bending.

These macrocycles are relatively less tense; the size of the cycle favors more anti-alignment of the methylene groups. In this case the contribution of Van der Waals is by far the most important contribution given the large number of transannular interactions created by the hydrogen atoms. The highest value for the type 6 conformer is E (VdW)=10.236 kcal/mol of the 16s system, and for the type 5 conformer E (VdW)=10.587 kcal/mol of the 16d system.

The lowest value calculated for the type 1 conformer E (VdW) = 9.257 kcal/mol of 16s, and for type 1 E (VdW) = 8.585 kcal/mol of 16d. The angular deformation term E (θ) is generally higher compared to the torsion term; this is explained by favorable torsion angles. In fact, the majority of methylene groups are in position (anti) in a conformation consisting of two parallel chains.

The elongation contribution is the least significant for all the conformers examined. They do not differ greatly from each other. This is due to the length of the links which remains practically close to those of the reference values.

In each cycle a compromise is established between the torsion, bending and Van der Waals energies, so that each of these molecules adopts the conformation corresponding to the minimum total steric energy [9,26].

2.3 Structural comparison of a typical example

We have studied in detail the structural parameters of the preferred conformation of the asymmetric 16-membered macrocycle (16d), which represents the basic core in the majority of 16-membered macrolide antibiotics [25], (Figure 2-a).

Quantitative study, after the Boltzmann distribution, gives for the preferred conformer, type 6: 22.69% of the total population of conformational types; it is followed by the second conformer, type 3: 20.50%, with the energy difference of 0.417 kcal/mol.

L' geometric study shows that the unsaturated α, β ester system has an S-CIS form with a dihedral angle ϕ_1 (O17-C1-C2-C3) = 3.1˚ d' after the calculation of molecular mechanics and 8.8˚ after the calculation of the PM3 method (Table 3).

Table 3. Selected values of distances (Angstrom)and angles (degree) of the 16d macrocycle

Distances	MM	PM3	Bending angles	MM	PM3	Torsion angles	MM	PM3
O1-C2	1.3499	1.3736	O1-C2-C3	119.112	112.656	O1-C2-C3-C4	178.273	172.193
C2-C3	1.3588	1.4787	C2-C3-C4	122.898	120.942	C2-C3-C4-C5	179.058	176.116
C3-C4	1.3431	1.3369	C3-C4-C5	123.418	122.896	C3-C4-C5-C6	118.040	123.711
C4-C5	1.5092	1.4875	C4-C5-C6	111.459	111.068	C4-C5-C6-C7	061.380	066.588
C5-C6	1.5379	1.5239	C5-C6-C7	112.363	111.727	C5-C6-C7-C8	178.992	177.887
C6-C7	1.5380	1.5205	C6-C7-C8	112.261	111.725	C6-C7-C8-C9	173.061	173.976
C7-C8	1.5356	1.5195	C7-C8-C9	114.365	112.830	C7-C8-C9-C10	066.538	072.354
C8-C9	1.5392	1.5221	C8-C9-C10	115.303	114.105	C8-C9-C10-C11	075.790	080.138
C9-C10	1.5383	1.5242	C9-C10-C11	113.579	112.736	C9-C10-C11-C12	129.862	133.854
C10-C11	1.5093	1.4871	C10-C11-C12	123.879	122.965	C10-C11-C12-C13	179.932	178.847
C11-C12	1.3436	1.3387	C11-C12-C13	122.843	121.647	C11-C12-C13-C14	179.587	174.393
C12-C13	1.3438	1.4536	C12-C13-C14	123.007	122.210	C12-C13-C14-C15	178.579	176.119
C13-C14	1.3435	1.3383	C13-C14-C15	123.661	122.269	C13-C14-C15-C16	110.822	113.375
C14-C15	1.5094	1.4727	C14-C15-C16	111.325	111.854	C14-C15-C16-O1	061.471	061.454
C15-C16	1.5347	1.5324	C15-C16-O1	108.926	110.985	C15-C16-O1-C2	095.984	097.189

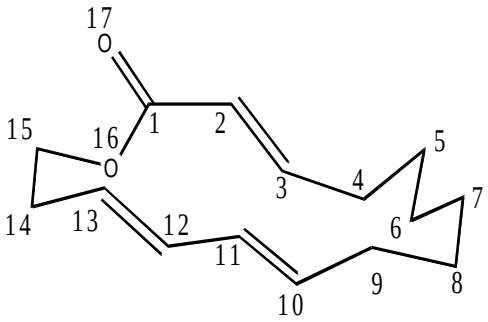
C16-O1	1.4103	1.4212	O1-C2-O17	120.776	119.531	C2-C16-O1-O17	016.661	023.057
C2-O17	1.2115	1.2171	O17-C2-C3	119.929	127.805	O17-C2-C3-C4	003.161	008.806

MM: molecular mechanics; PM3: Parameterized Method 3

The diene system has a form (s-trans), the twist angle ϕ_2 (C10-C11-C12-C13) = 179.5˚ after the calculation by molecular mechanics and 178.84˚ after the calculation by the PM3 method. The values of the distances between bound atoms are close to the reference values (Table 3). The two α, β-unsaturated ester and diene systems are perpendicular to the mean plane of the ring. We also notice that there is a similarity between the calculation results using molecular mechanics and the PM3 semi-empirical method.

2.4 Study of the effect of substituents on the basic skeleton

In order to study the role that a new substituent can play on thermodynamic and conformational stability [26-28]., we introduced a methoxy radical in various positions on the 16d macrocycle which represents in the majority of cases the basic backbone of 16-membered macrolide antibiotics. (Scheme 1).



Scheme 1

The introduction of the substituent shows that the order of the latter types is variously modified depending on the position of the methoxy in the carbon chain and the position of this relative to (CO). By comparing the lowest energies of the different positional isomers, we note that the compounds substituted in positions (C2, C4, C8 and C15) are thermodynamically the most stable then followed by those substituted in positions (C6, C7 and C14).

The most destabilizing position is that of C12 (Table 4). This is in agreement with the experimental work of Van Bambeke et al. [25,29].

Type 6 represents in the majority of cases the preferred conformer in a conformation (endo or exo). Second comes type 3 in the majority of cases.

Table 4a. Steric energies of the different configurations: substitution of C2 to C8

Position of the substitution	C2 endo	C3 endo	C4 endo	C5 exo	C6 exo	C7 endo	C8 endo
E (kcal/mol)	11.28	15.44	13.02	15.23	14.35	14.06	13.37

Table 4b. Steric energies of the different configurations: substitution of C9 to C15

Position of the substitution	C9 exo	C10 exo	C11 endo	C12 exo	C13 endo	C14 exo	C15 endo
E (kcal/mol)	14.76	15.52	16.19	17.08	14.74	13.75	11.97

The most influential position is C15 (endo) for which the probability at type 1 is of the order of 28.68%, while in the case of the unsubstituted macrolactone the Boltzmann population rate is 22.69%. Likewise, positions C12 (endo) and C13 (endo) are also influential because the preferred conformers are also in the majority with respective rates of 27.14 and 27.12%.

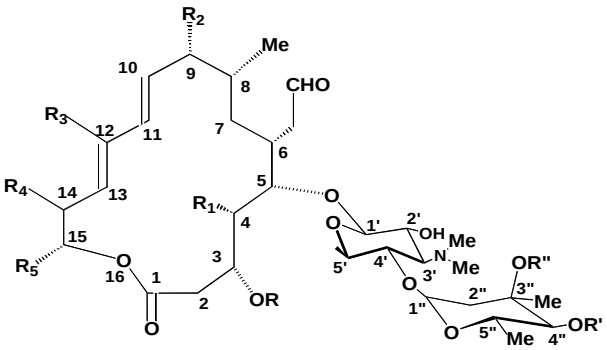
These results are in agreement with current research directions on structural modifications of macrolides, for the design of new antibiotics of this class [29].

2.5 Structural comparison of neomacrolides

These are in fact the structural elements which explain the real differences between molecules which have also been taken into account by chemists when synthesizing these derivatives .It is therefore essential to understand that all macrolides currently used clinically, with the exception of erythromycin and spiramycin, were obtained by rational and directed chemical modification from erythromycin (14 membered), spiramycin (16 membered) or other natural products of the macrolide class [29].

These macrolides are basic due to the presence of an amino function at the desosamine level and possibly an additional amino function at the C9 amino sugar level [Spiramycin I, II, III]. This results in their accumulation in acidic cellular compartments and mainly lysosomes.

In fact, current research directions keep the substitution for the stable positions at (C2, C4, C8 and C15) and make modifications at the level of C12. This is in agreement with our results on the effect of substitution on the basic skeleton, where we keep the substitutions in stabilizing positions and make modifications in destabilizing positions (Figure 2).



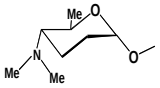
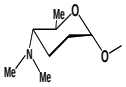
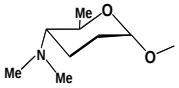
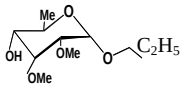
Macrolide	R	R'	R"	R_1	R_2	R_3	R_4	R_5
Spiramycin I	H	H	H	OCH_3		H	H	CH_3
Spiramycin II	$COCH_3$	H	H	OCH_3		H	H	CH_3
Spiramycin III	COC_2H_5	H	H	OCH_3		H	H	CH_3
Josamycin	$COCH_3$	$COCH(CH_3)_2$	H	OCH_3	OH	H	H	CH_3
Miocamycin	COC_2H_5	COC_2H_5	$COCH_3$	OCH_3	$OCOCH_3$	H	H	CH_3
Midecamycin	COC_2H_5	CO C_2H_5	H	OCH_3	OH	H	H	CH_3
Rokitamycin	H	$COCH(CH_3)_2$	COC_2H_5	OCH_3	OH	H	H	CH_3
Kitasamycin	H	$CO(CH_2)_2CH_3$	H	OCH_3	OH	H	H	CH_3
Tylosin	H	H	H	CH_3		CH_3		

Fig 2. Structural comparison of 16-membered

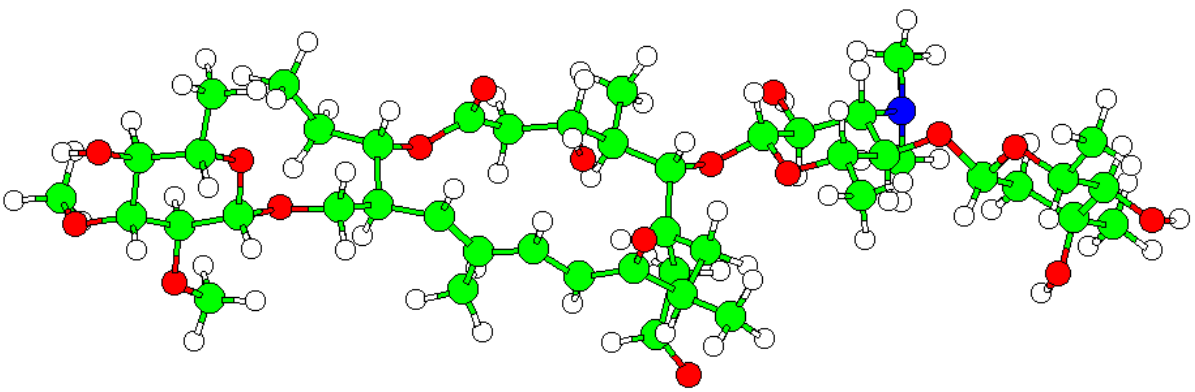


Fig 3. 3D structure of Tylosin

2.6 Study of the QSAR properties of 16-membered

In this part of the work, we were interested in the study of the lipophilicity of macrolide antibiotics at a hydrophobic/hydrophilic interface. Two parameters characterizing the hydrophobicity (lipophilicity) of a compound which are: the amphiphilic balance (hydrophilic/hydrophobic) at the lipid/water interface on the one hand and the partition coefficient (Log P) between octanol and water and on the other hand [27,30].

In fact, the compound is distributed between the two immiscible liquids depending on its affinity for one or the other of the two phases. The determination of Log P is done by a calculation using fragmentary hydrophobic constants.

The polarizability was calculated using an empirical formula by adding atomic increments following the Miller method [31], with a calculation precision of 3%.

We notice that the polarizability values are directly proportional to the volume values, but for the surfaces the order is not well respected because of the folding of some molecules and, the decreasing order of polarizability for the new macrolides at 16 links east; spiramycin III, tylosin, spiramycin II, miocamycin, spiramycin I, rokitamycin, josamycin, midecamycin, kitasamycin, (Table 5).

This order is the same for volumes. This is explained by the relationship between polarizability and volume, for relatively non-polar molecules. The latter are directly linked, because the centers of gravity of the negative and positive charges, in the absence of an external field, coincide and the dipole moment of the molecule is zero.

The polarizability of the molecule only depends on its volume, the thermal agitation of non-polar molecules has no influence on the appearance of dipole moments in these molecules, and therefore the polarizability does not depend on the temperature [8].

On the other hand, for polar molecules, the polarizability of the molecule does not depend only on the volume but also depends on other factors, namely the temperature, due to the presence of the permanent dipole [32].

We also note that the surface area and volume of distribution of these molecules are significantly higher than that of more polar molecules such as lipopeptides [17].

For these new macrolides, we found that the surface areas vary from 1055.56 to 1195.15 $Å^2$.

These macrolides have a large variation in volume, in particular tylosin and spiramycin III which have respective volumes: 2387.78 and 2353.33 $Å^3$ (Table 5).

Table 5. QSAR properties of 16-membered

Macrolide antibiotic	Surface molecular in $Å^2$	Volume molecular in $Å^3$	Polarizability in $Å^3$	Energy of hydration (kcal/mol)	LogP
Spiramycin I	1110.27	2219.04	86.72	-6.86	2.02
Spiramycin II	1151.97	2.317.74	90.47	-4.78	2.15
Spiramycin III	1159.71	2353.33	92.31	-5.24	2.78
Josamycin	1087.08	2144.40	82.01	-4.48	2.43
Miocamycin	1165.03	2323.64	89.52	-1.23	2.76
Midecamycin	1055.56	2117.29	82.01	-4.73	2.50
Rokitamycin	1106.12	2186.31	83.84	-4.88	3.06
Kitasamycin	1059.28	2056.41	78.25	-7.11	2.14
Tylosin	1195.15	2387.78	92.23	-10.06	2.24

The hydration energy in absolute value, the highest is that of tylosin (10.06 kcal/mol) and the lowest is that of miocamycin (1.23 kcal/mol). In fact, in biological environments, polar molecules are surrounded by water molecules. Hydrogen bonds are established between a water molecule and these molecules. The proton donor sites interact with the oxygen atom of water and the proton acceptor sites with the hydrogen atom. The first correspond to the complex with the strongest hydrogen bond.

These hydrated molecules dehydrate at least partially before and during their interaction. These low-energy interactions, which we observe in particular between messengers and receptors, are generally reversible [13].

Tylosin has five proton donor sites (5 OH sites; 1 OH site at the main ring (C3) and 4 OH sites at the sugar level (C2', C3", C4" and C14) and three acceptor sites (3 C=O sites at the level of the main cycle; 1 site on (C1), 1 site on (C9) and 1 site on (C6)). On the other hand, miocamycin only has one donor site (1 OH site at the level sugar (C3) and six acceptor sites (6 C=O sites; 4 sites at the level of the main cycle (C1, C3, C6, C9) and 2 sites at the sugar level (C3", C4"). This property favors the first antibiotic, not only by fixing on the receptor, but also activates it, that is to say triggers a series of enzymatic reactions. It is therefore an

agonist.

Spiramycin I has the lowest partition coefficient (Log P: 2.02), followed by kitasamycin (2.14) and spiramycin II (2.15). These three molecules are the most hydrophilic products. They have good water solubility, better gastric tolerance and effective renal elimination.

When the partition coefficient is low enough, it results in better gastric tolerance. Rokitamycin which has the highest value (3.06) is followed by spiramycin III (2.78) and miocamycin (2.76). These compounds are lipophilic. They have good permeability across the biological membrane, better fixation on plasma proteins and elimination by metabolic route (liver).

3. CONCLUSION

The calculations carried out showed that asymmetric macrocycles 16 have relatively the lowest conformational mobility, with 3 stable conformations in an energy window of 2 kcal/mol. The majority contributions to the total steric energy are those of Van der Waals and bending. The calculations also show that there is a similarity between the calculations by molecular mechanics and the PM3 semi-empirical method.

We were able to define the structural motifs involved in the antibiotic and surfactant properties of the new macrolides. Indeed, compounds substituted in positions (C2, C4, C8 and C15) are thermodynamically the most stable, and the most destabilizing position is that of C12. The most influential position is C15 for which the probability of the majority type is around 28.68%.

Spiramycin I has the lowest partition coefficient (Log P) (2.02), followed by kitasamycin (2.14) and spiramycin II (2.15). This results in better gastric tolerance. Rokitamycin which has the highest value (3.06) is followed by spiramycin III (2.78) and miocamycin (2.76). These three molecules have a significant capacity to bind to plasma proteins. Tylosin has the highest value of hydration energy (10.06 kcal/mol) in absolute value; it results in better binding to the receptor.

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