Molecular modeling of interaction between ribavirin and nucleic acids

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***Abstract*—**The ribonucleoside analog ribavirin shows the antiviral activity against a variety of DNA and RNA viruses. Ribavirin, in combination with interferon, has predominantly been applied in the treatment of hepatitis C virus infection and its potential antitumor efficacy has recently become a point of interest. The molecular structure of ribavirin was investigated by the semiempirical AM1 method, which triggered two polymorphic modifications of the antiviral drug also reported in the literature. The interactions of two polymorphic modifications of ribavirin (V_1 and V_2) with nucleic acids by the molecular mechanic and semiempirical AM1 methods were analysed. Previous experimental data pointed out that in the ribavirin – nucleic acid complexes, the 1,2,4-triazole-3-carboxamide chromophore is intercalated between the bases of the nucleic acid helix, the carboxamidic group is set outside of the helix toward the major groove and the 4-hydroxymethyl-tetrahydrofuran-2,3-diol fragment is located in the minor groove. In order to evidence the sequence specificity of the drug, some model mono- and double-stranded nucleic acid containing the bases: adenine (A), thymine (T), cytosine (C) and guanine (G) in AAAAAA, TTTTTT, CCCCCC, GGGGGG, ATATAT, CGCGCG, ATCGAT and CGATCG sequences were used. The results outline the differences in the contributions of the electrostatic and van der Waals interactions to the total binding energy and the preference of ribavirin for the binding at the sequences of nucleic acids containing adenine and thymine bases.

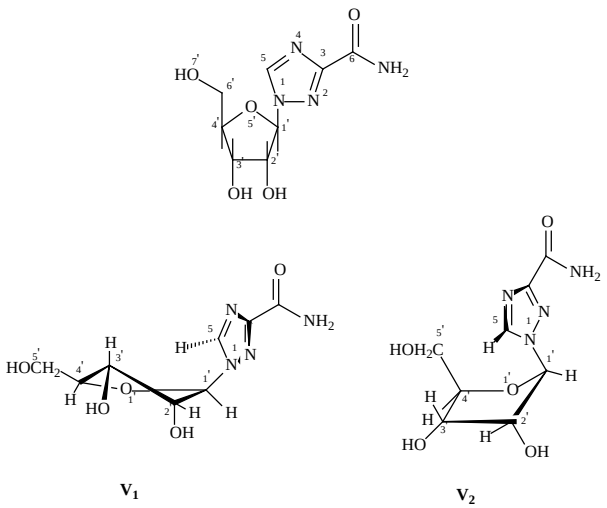
Keywords*—ribavirin; nucleic acids; molecular modeling*

I. INTRODUCTION

Ribavirin is a purine nucleoside analogue that is active against a number of DNA and RNA viruses [1]. There are numbers of proposed mechanisms of action for ribavirin. These include indirect effects such as inhibition of inosine monophosphate and immunomodulatory effects and direct effects such as polymerase inhibition and interference with viral RNA capping. Recent studies use double or triple combinations of ribavirin with other antiviral drugs, such as oseltamivir or/and amantadine in order to increase the activity against multiple virus strains in vitro [2, 3]. In addition, the antiviral drugs, such as ribavirin, are used by our research group in vitro chemotherapy to obtain of grapevine virus-free and potato virus-free plants [3].

Ribavirin has a complex structure (figure 1), comprising an 1,2,4-triazole-3-carboxamide chromophore and a ribose moiety, 2-hydroxymethyl-tetrahydro-furan-3,4-diol.

Ribavirin crystallises in two polymorphic forms V_1 and V_2. The forms V_1 and V_2 were found in slightly different conformations concerning the glycosyl bond, V_1 in „normal *anti*" and V_2 in "high *anti*" [4]. The V_1 form exhibits a glycosyl torsional angle χ (O_1'-C_1'-N_1-C_5) of 10.4° denoted as high *syn* and the ribose conformation is 3'-*endo*-2'-*exo*. The V_2 form has a χ value of 119.0° referred to as high *anti* and a 2'-*exo*-1'-*endo* ribose conformation (Fig. 1) [5]. Some inactive derivatives with substituents in 5-position (methyl, chloro) which also exist in the high *syn* forms, an lack energy minimum in the high *anti* region. Ribavirin does have a second minimum energy corresponding to the high *anti* conformation and it has been suggested that the active conformation of ribavirin at the enzyme site is the high *anti* form.



V_1 **V_2**

Fig. 1. Chemical structure for ribavirin and their two conformations V_1 ("high *syn*") and V_2 ("high *anti*")

Our experimental studies for ribavirin–DNA system [6] have pointed out a complex nature of the binding process. Two binding processes were highlighted: a process of the internal binding, that involve the drug intercalation between the bases from nucleic acid and a process of the external binding, that involve the drug binding to the grooves from the nucleic acid structure. It was found that the external binding prevails, the binding constant of this process being with an order of magnitude greater than the binding constant of the second process.

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In addition, the dependence of the binding constants on the ionic strength of the medium allowed the dissection of the binding free energy in electrostatic and non-electrostatic contributions. It was found that the non-electrostatic contribution prevails.

The purpose of this paper is to perform a theoretical modeling of the interaction of ribavirin with some model nucleic acids. We will focus on the following aspects:
i) The study of the electronic structure of the antiviral drug, which involves the analysis of the possible conformers in order to find the optimal conformations for the interaction with the nucleic acids, the calculation of the charge distribution and the electrostatic potential;
ii) The theoretical modeling of the drug - nucleic acid complexes in order to estimate the relative contributions to the interaction energy (van der Waals and electrostatic terms) and to get an insight on the sequence selectivity of the drug.

II. COMPUTATIONAL DETAILS

The structures of the ribavirin conformers and the sequences of nucleic acids were built within the HyperChem Release 7.5 program and optimized by the semiempirical AM1 method (parameters: SCF control of 0.01, RHF spin pairing, Polak - Ribiere optimizer, RMS gradient of 0.01 kcal/mol·Å for the ribavirin conformers and the sequences of mono-stranded nucleic acids, RMS gradient of 0.1 kcal/mol·Å for the sequences of double-stranded nucleic acids).

The calculations on the complexes of two ribavirin conformers (noted V_1 and V_2) with the sequences of mono- and double-stranded nucleic acids were performed in vacuo by both the Molecular Mechanics (MM+ force field) and the semiempirical AM1 methods. The optimization criteria for the drug – nucleic acids complexes were 0.01 kcal/mol·Å for the MM method and 0.1 kcal/mol·Å for the AM1 method.

III. RESULTS AND DISCUSSION

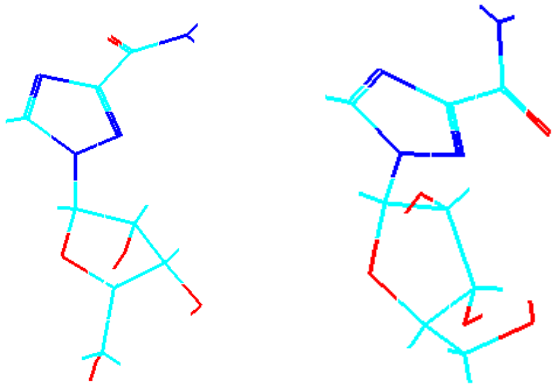
A. *Conformers of ribavirin*

After the conformational analysis, we have obtained a series of conformers presenting the features indicated in literature for the polymorphic forms V_1 and V_2. From all of the conformers obtained, they were selected two optimum conformations, with the minimum energy and corresponding to the crystallographic data presented in literature.

Table 1 shows the values of torsions angles between the binding atoms of the two rings from the ribavirin structure and figure 2 shows the molecular structures of the ribavirin conformers obtained by AM1 method.

TABLE 1. Values of torsion angles in ribavirin conformers

Torsion angles	Ribavirin V_1	Ribavirin V_2
N_1-C_1'-O_1'-C_4'	101.95°	113.08°
C_1'-O_1'-C_4'-C_5'	130.78°	-115.76°
O_1'-C_4'-C_5'-O_5'	-67.83°	102.83°

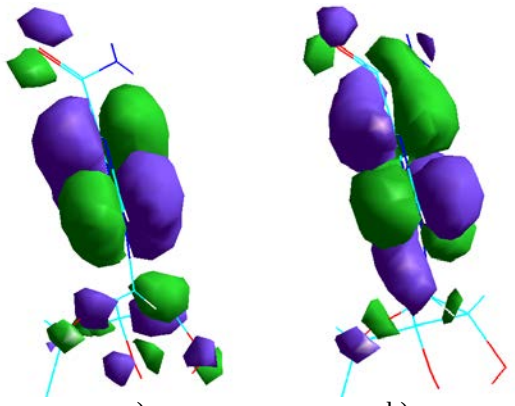


(a) Conformer V_1 (b) Conformer V_2

Fig. 2. The molecular structures of the ribavirin conformers obtained by AM1 method

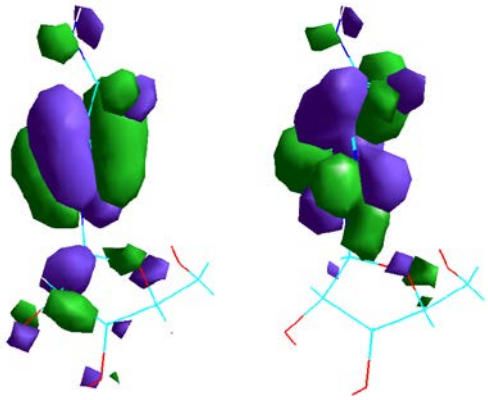
The charge distribution in the two conformers of ribavirin is similar, excepting the oxygen and nitrogen atoms from carboxamide group, the N_2 and N_4 atoms from 1,2,4-triazole group and the O_5' atom from ribose moiety. In the V_1 conformer, the smallest charge density was found at the nitrogen atom from carboxamide group and the O_5' atom from ribose moiety while in the V_2 conformer, the nitrogen and oxygen atoms on carboxamide group bears the smallest charge density.

The frontier molecular orbitals (Figs. 3 and 4) are π orbitals.



a) b)

Fig. 3. The HOMO (a) and LUMO (b) orbitals of ribavirin V_1



a) b)

Fig. 4. The HOMO (a) and LUMO (b) orbitals of ribavirin V_2

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In both conformers of ribavirin, the highest occupied molecular orbital (ε_{homo}~-10.45eV) and the first vacant molecular orbital (ε_{lemo}~-0.1eV) are preferentially localized on the heterocyclic oxygen and 1,2,4-triazole-3-carboxamide chromophore, ensuring a high superposition with the π-system of the base pairs from the nucleic acids and explaining thus the strong intercalating tendency of ribavirin conformers.

The electrostatic potential is a useful analytical tool in the analysis of chemical reactive behaviour involving the electrophilic and nucleophilic processes, as well as recognition and hydrogen bonding interactions. The electrostatic potential was calculated for each conformer of ribavirin and the minimum and maximum values for these conformers are presented in table 2.

TABLE 2. Results of AM1 calculations

Parameter	Ribavirin	
	V_1	V_2
$\Delta H_{formation}$, Kcal/mol	-139.47	-141.25
ε_{homo}, eV	-10.42	-10.47
ε_{lumo}, eV	-0.05	-0.13
V_{min}, Kcal/mol	-41.67	-36.39
V_{max}, Kcal/mol	266.53	246.01

Due to the presence of the lone pair electrons, the oxygen and nitrogen atoms are characterized by negative regions of the electrostatic potential while the rings are characterized by positive electrostatic potential. There are not significant differences considering the extension of the positive and negative regions of the electrostatic potential between the conformers of ribavirin. However, the V_2 conformer is more stable than the V_1 conformer, although the differences between them are small.

The both ribavirin conformers were optimized by both MM and AM1 methods. The optimized values of the ribavirin conformers energies calculated by the MM method were used to calculate the drug – nucleic acid interaction energy.

B. Sequences of nucleic acids

Nucleic acids are complex organic molecules that contain the genetic code for the organism. Nucleic acids act as drugs by different mechanisms, they may bind with the synthesized proteins, and they can hybridize to a messenger RNA leading to translation arrest or may induce degradation to target RNA. In this way the nucleic acids act as drug for inhibiting gene expression or protein synthesis.

A remarkable feature of the nucleic acids is that in these macromolecules there are several reactive sites, uniquely displayed on the surface of the helix, depending on the nitrogenous bases succession in the nucleic acids sequences. For instance, in the minor groove of deoxyribonucleic acid, the exocyclic N2 amino group of guanine and the N3 atom of both guanine and adenine bases are particularly susceptible to the drugs action. In the major groove, the N7 atom of both guanine and adenine bases is particularly susceptible to drug action. Finally, the C4', C5', and C1' atoms of the deoxyribose in the backbone of nucleic acid double-helix are other reactive sites from the nucleic acids sequences [7-9].

Because the literature data pointed out that the intercalating drugs have a sequence selectivity for nucleic acids that does not extend beyond two or three nitrogenous base level [10-14], we have chosen some model mono- and double-stranded nucleic acids containing the AAAAAA, TTTTTT, CCCCCC, GGGGGG, ATATAT, CGCGCG, ATCGAT and CGATCG sequences. The nucleic acids sequences were constructed by the charge neutralization of phosphate groups with hydrogen atoms. The nucleic acids sequences were optimized by both MM and AM1 methods.

The results obtained by the optimization the nucleic acids sequences using the AM1 method indicate a low net charge on the nitrogen atoms and negative area for the electrostatic potential on the oxygen and nitrogen atoms, which have lone pair electrons.

The optimized values of the nucleic acids sequences energies calculated by the MM method were used to calculate the drug – nucleic acid interaction energy.

C. Complexes of ribavirin with sequences of nucleic acids

The optimized conformers of ribavirin and the optimized sequences of mono- and double-stranded nucleic acids were utilized in the optimization of the drug - nucleic acid complexes. For the optimization of the drug - nucleic acid complexes by both MM and AM1 methods, the solvent effect was not considered.

The starting structures of the drug - nucleic acid complexes were built by the docking procedure. Initially, several restraints were imposed, so that the 1,2,4-triazole-3-carboxamide chromophore to be oriented parallel to the nitrogenous bases from the nucleic acid helix, the carboxamidic group to be set outside of the helix toward the major groove and the 4-hydroxymethyl-tetrahydrofuran-2,3-diol moiety to be located in the minor groove of nucleic acids structure. After optimization of the drug - nucleic acid complexes until the required gradient, the restraints were eliminated and the complexes were optimized again.

In Figs. 5 and 6 are presented the optimized geometries for two complexes of the drug with the mono-stranded nucleic acids sequences and in Figs. 7 and 8 are presented the optimized geometries for two complexes of the drug with the double-stranded nucleic acids sequences.

In all drug - nucleic acid complexes, the formation of some intercalation complexes was observed. Initially, the nucleic acid undergoes a conformational change that leads to the obtaining of an intercalation site. In this step, the nitrogenous bases from the nucleic acid structure were separated to form the cavity in which the drug will intercalate. Then, in the second step occurs an external binding of drug at the nucleic acid sequence and in the third step occurs the drug intercalating between the nitrogenous bases from the nucleic acid structure.

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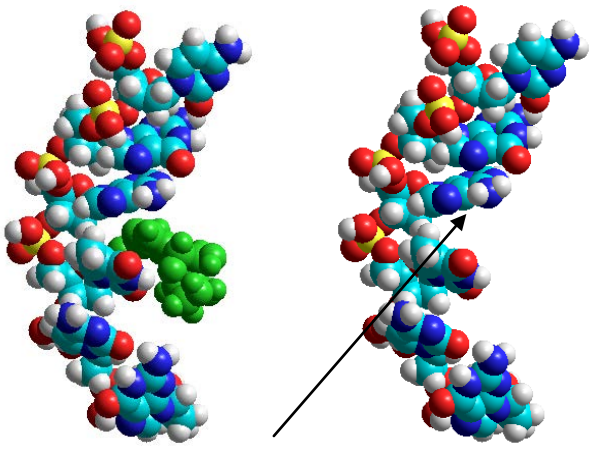
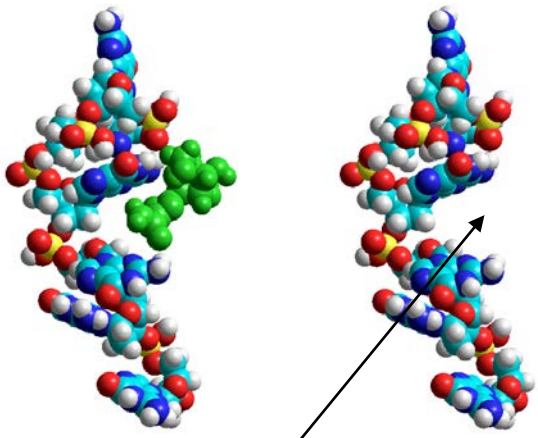


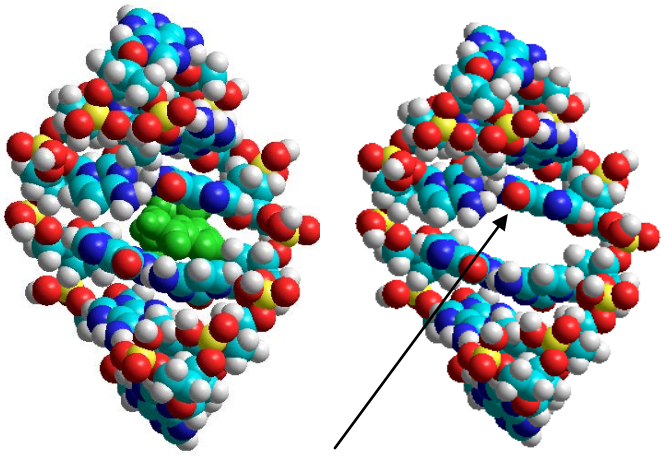
a) The intercalation site b)

Fig. 5. The optimized geometry of ribavirin V_1-CGATCG complex (a). The intercalation site of the ribavirin V_1 to mono-stranded nucleic acid sequence (b)



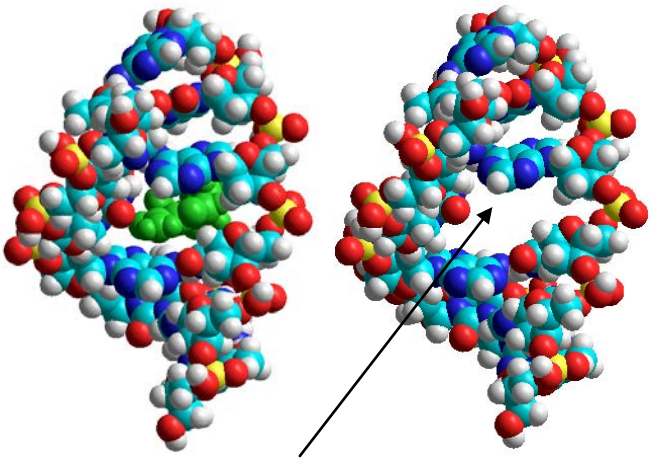
a) The intercalation site b)

Fig. 6. The optimized geometries of ribavirin V_2-GGGGGG complex (a). The intercalation site of the ribavirin V_2 to mono-stranded nucleic acid sequence (b)



a) The intercalation site b)

Fig. 7. The optimized geometries of ribavirin V_1-ATCGAT-TAGCTA complex (a). The intercalation site of the ribavirin V_1 to double-stranded nucleic acid sequence (b)



a) The intercalation site b)

Fig. 8. The optimized geometries of ribavirin V_2-ATATAT-TATATA complex (a). The intercalation site of the ribavirin V_2 to double-stranded nucleic acid sequence (b)

The energies of the drug – nucleic acid complexes were used for the evaluation of the following quantities [10, 12, 15]:

- the interaction energy:

$$E_{\text{int}\,eraction} = E_{complex} - (E_{drug} + E_{DNA})_{optimized} \quad (1)$$

- the binding energy:

$$E_{binding} = E_{complex} - (E_{drug} + E_{DNA})_{frozen\ in\ complex} \quad (2)$$

- the perturbation energy:

$$E_{perturbation} = E_{i\,nteraction} - E_{binding} \quad (3)$$

The values of the binding energies of the ribavirin conformers at the mono- and double-stranded nucleic acids sequences calculated by MM method are presented in table 3. The van der Waals (VdW) contribution to the binding energy are also included in table 3.

TABLE 3. MM results of drug – nucleic acid interaction

Nucleic acid	Ribavirin V_1		Ribavirin V_2	
	$E_{binding}$, Kcal/mol	% VdW	$E_{binding}$, Kcal/mol	% VdW
AAAAAA	-19,05	86,31	-17,85	69,47
TTTTTT	-21,24	79,81	-24,37	78,05
ATATAT	-23,75	71,98	-20,02	78,12
CCCCCC	-20,26	77,84	-18,77	73,73
GGGGGG	-15,25	93,38	-16,39	88,41
CGCGCG	-18,92	81,61	-17,85	88,63
ATCGAT	-14,24	80,76	-15,12	74,67
CGATCG	-14,23	83,91	-12,95	69,88
AAAAAA-TTTTTT	-27,97	78,48	-26,68	86,58
ATATAT-TATATA	-23,91	82,93	-24,39	83,81
CCCCCC-GGGGGG	-25,14	70,59	-28,27	72,56
CGCGCG-GCGCGC	-30,13	80,95	-27,11	86,09
ATCGAT-TAGCTA	-22,77	96,25	-23,24	89,51
CGATCG-GCTAGC	-20,46	79,62	-22,74	75,45

In all cases, the binding energies have negative values reflecting the drug - nucleic acid interaction. The results underline the significant van der Waals contribution (>70%) to the binding energy and, consequently, the low percentage of the electrostatic interactions, in agreement with our previous

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experimental data [6]. A slight preference for the sequences containing adenine and/or thymine bases can be noticed for both ribavirin conformers with both mono- and double-stranded nucleic acids sequences.

In table 4 are presented the values for the interaction and perturbation energies, characteristic for the inclusion processes of the two ribavirin conformers in the nucleic acids structures. It is noted that the intercalation of ribavirin conformers in the nucleic acids helix causes a small disturbance in the drug structure and a big disturbance in the structure of nucleic acids.

TABLE 4. MM results of drug – nucleic acid interaction

Ribavirin – nucleic acid complexes		$E_{interaction}$, kcal/mol	$E_{perturbation}$, kcal/mol	
			total	nucleic acid
RIBAVIRIN V_1	AAAAAA	-3,96	15,09	14,74
	TTTTTT	-9,67	11,57	10,76
	ATATAT	-16,19	7,56	6,79
	CCCCCC	-13,61	6,63	6,32
	GGGGGG	-2,71	12,54	12,12
	CGCGCG	-7,79	11,13	10,84
	ATCGAT	-12,76	1,48	0,64
	CGATCG	-13,41	0,82	0,73
	AAAAAA-TTTTTT	-25,75	2,22	1,06
	ATATAT-TATATA	-22,54	1,37	0,22
	CCCCCC-GGGGGG	-20,32	4,82	3,59
	CGCGCG-GCGCGC	-11,87	18,26	17,52
	ATCGAT-TAGCTA	-11,81	10,96	10,21
	CGATCG-GCTAGC	-15,99	4,47	2,95
RIBAVIRIN V_2	AAAAAA	-3,78	14,07	13,59
	TTTTTT	-15,88	8,49	7,81
	ATATAT	-11,31	8,71	8,11
	CCCCCC	-5,9	12,87	9,73
	GGGGGG	-2,7	13,69	12,84
	CGCGCG	-9,96	7,89	7,33
	ATCGAT	-9,67	5,45	4,61
	CGATCG	-12,04	0,91	0,44
	AAAAAA-TTTTTT	-24,39	2,29	0,55
	ATATAT-TATATA	-25,55	2,22	1,06
	CCCCCC-GGGGGG	-16,05	12,22	10,91
	CGCGCG-GCGCGC	-23,13	3,98	3,3
	ATCGAT-TAGCTA	-13,48	9,76	8,83
	CGATCG-GCTAGC	-11,38	11,36	10,34

The binding energies obtained by the AM1 method are presented in table 5.

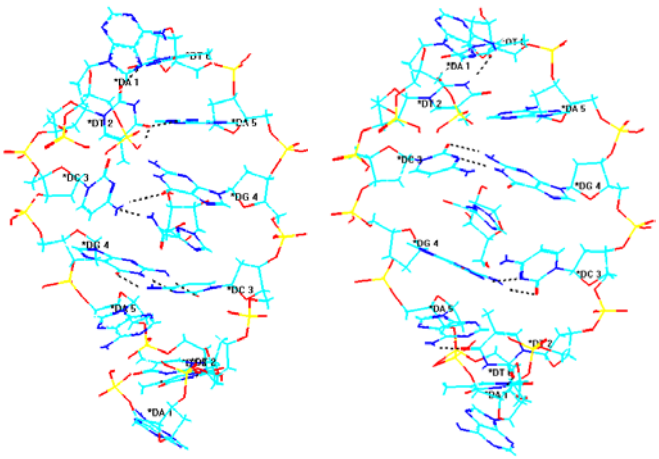
TABLE 5. Results of drug – nucleic acid interaction obtained by AM1 method

Nucleic acid	$E_{binding}$, kcal/mol	
	Ribavirin V_1	Ribavirin V_2
AAAAAA	-119,15	-102,68
TTTTTT	-21,89	-20,81
ATATAT	-20,54	-24,96
CCCCCC	-26,31	-8,31
GGGGGG	-22,95	-23,64
CGCGCG	-27,07	-13,54
ATCGAT	-16,69	-10,01
CGATCG	0,94	2,44
AAAAAA-TTTTTT	-98,73	-97,87
ATATAT-TATATA	-18,03	-9,96
CCCCCC-GGGGGG	-20,48	-16,66
CGCGCG-GCGCGC	7,96	-9,37
ATCGAT-TAGCTA	0,74	3,76
CGATCG-GCTAGC	16,11	-19,13

A slight preference for the nucleic acids sequences containing adenine and/or thymine bases can be noticed for both ribavirin conformers.

They were found a lot of features of the dyes and drugs intercalation [10-17] between the purine and pyrimidine bases from the nucleic acids structure, namely: the distortion in nucleic acid structure by the angle opening of the phosphate groups for allowing the dyes/drug intercalation, the lengthening of the helix by approximately 3.4 Å which causes a conformational change of some sugar moieties involved, the increase in the distance between nitrogenous bases at the intercalation site level.

Although some features of the intercalation are found in the ribavirin - nucleic acid system, however there is a remarkable difference, determined by the "accordion type" motion (specified in the model Lerman [16]) that occurs with the breaking of some hydrogen bonds between the purine and pyrimidine bases from the nucleic acids structure. In addition, the formation of new hydrogen bonds between ribavirin and the nitrogenous bases from the nucleic acid sequence at the intercalation site level was observed.



a) b)

Fig. 9. Hydrogen bonds in ribavirin V_1 (a), respectively ribavirin V_2 (b) - ATCGAT-TAGCTA complexes

CONCLUSIONS

The results of the molecular modeling points out that the complexes of ribavirin with nucleic acids are stabilized mainly by van der Waals forces involving the 1,2,4-triazole-3-carboxamide chromophore and the nitrogenous bases from the nucleic acids structure and that the electrostatic term brings a minimal contribution (<20%) to the binding energy. For both ribavirin conformers, a slight preference for nucleic acids sequences containing adenine and/or thymine bases was found. As a result of the ribavirin – nucleic acid interaction, only the nucleic acids structure is significantly perturbed, the structure of the drug being practically unchanged.

The theoretical calculations in the ribavirin – nucleic acid system predict an increase of the distance between the adjacent nitrogenous bases at the intercalation site level.

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The turning of the polynucleotidic helix is produced and the "accordion type" motion takes place determining a breaking in the hydrogen bonds between base pairs from double-stranded nucleic acid structure.

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