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A quantum-chemical model of the inhibition mechanism of viral DNA HIV-1 replication by Iodine complex compounds

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ABSTRACT

The interaction of molecular iodine with virus DNA nucleotide is studied by ab initio RHF/3-21G method. Formation of the nucleoprotein complex of the HIV DNA, molecular iodine and the HIV-1 integrase co-factor is considered to cause the inhibition action of the integrase enzyme. Experimental data on the anti-HIV effect of the molecular iodine complex compounds and the results of calculations suggest that molecular iodine contained in iodine polymer complexes may be considered as a compound inhibiting the catalytic center of the integrase enzyme. Unlike the known integrase inhibitors, molecular iodine also changes the virus DNA structure and produces the N-I bond in the purine bases of adenosine and guanosine nucleotides.**

Keywords: HIV; Integrase HIV; Quantum-Chemical Method Ab Initio

1. INTRODUCTION

The human immunodeficiency virus (HIV) belongs to the series of retroviruses whose gene within the virion is represented by a RND molecule. After the virus penetrates a human cell, a DNA copy of the virus genome is synthesized and such virus DNA is integrated into the genome of the human cell. Both processes are produced by the virus enzymes – transcriptase and integrase [1], respectively.

A large number of agents inhibiting the activity of the HIV reverse transcriptase have been developed [2] and used for medicine production. However, the HIV reverse transcriptase is referred to the class of polymerases and most of its blockers that, to some extent, suppress the actions of this class of cellular enzymes producing significant toxic effects [3,4].

HIV integrase inhibitors have a high therapeutic effect [5] for two reasons. First, integrase is one of the key participants in the virus replication cycle [1]. Second, integrase has no cellular equivalent and, hence, the suppression of its activity should not disturb normal cellular metabolism processes [6].

HIV-1 integrase contains 288 amino acid residues and three domains can be distinguished in its structure: a short N-end domain containing 1-50 amino acid residues, a catalytic domain producing 51-212 amino acid residues and a C-end domain containing 220-270 amino acid residues.

It is typical for such family of enzymes to produce very stable complexes with a virus DNA. To be integrated, it is necessary for integrase to bind both - virus and cell - DNA molecules at the same time. The integration proceeds in two stages and begins in the cytoplasm of HIV infected cells where, upon completion of the reverse transcription of the virus DNA genome, integrase binds the virus DNA copy producing the so-called preintegration complex (PIC) which can be isolated from the HIV-infected cells [7].

A model of the integrase structure in the complex with a virus and a cell DNA was proposed in [8]. According to the model, only the catalytic domain takes part in the binding of the virus DNA, while all the three domains take part in the binding of the cell DNA.

The structure of the catalytic domain of the HIV-1 integrase is determined by the X-ray structural analysis. According to the data obtained, the catalytic domain of the enzyme in the crystal forms a spherical dimer with each monomer forming a semi-sphere. The three amino acid residues - Asp64, Asp116 and Glu152 closely located in the tertiary structure of the catalytic domain – form its active catalytic center with the active centers of each integrase monomer located on the opposite sides of the dimer sphere 35 Å apart.

The X-ray structural analysis data clearly show one Mg^{2+} ion coordinated by Asp64 и Asp116 and two water

molecules [9,10]. Based on data regarding the action mechanism of nucleotidyltransferases [11], referred to the same family as the HIV-1 integrase, it was suggested in [12] that two ions may take part in the act of catalysis, but, due to a higher conformational mobility of the catalytic enzyme, they are not coordinately bound prior to being bound with the virus DNA. With the use of the molecular dynamics method it was shown that the interaction with the virus DNA involves the integration of two Mg^{2+} ions into a stable bi-nuclear structure where the amino acid residue Glu152 simultaneously coordinates the two Mg^{2+} ions, while Asp64 and Asp116 interact only with one of the Mg^{2+} ions.

In the paper [13] the authors offer a comparison of the inhibiting activity of a series of antiretroviral agents towards HIV-1 integrase and the *in vitro*-isolated PIC. The results of the study show that inhibitors active towards the integrase enzyme may not be active towards the PIC. The capability to inactivate a PIC was exhibited only by three antiretroviral agents: quinalizarin, purpurin, and alizarin.

There is a whole range of iodine polymer complexes with an anti-HIV effect [14-17]. For example, complexes of iodine with polyurethane polymers rapidly, within 15-20 minutes inactivate HIV-1. However, the authors of [14-18] do not propose a mechanism of inactivation of HIV. A model of the HIV-1 inhibition by molecular iodine within the framework of ab initio RHF/3-21G** method is proposed in this work. It is suggested that molecular iodine may prevent PIC formation.

The results of the study are summarized in three sections. The first section deals with the selected computational method tested on model structures. The structure and nature of the molecular iodine interaction with virus DNA nucleotides is studied by the ab initio RHF/3-21 G** method in the second section. A model of the mechanism of inhibition of the HIV-1 integrase enzyme in the nucleoprotein complex formed by the virus DNA by molecular iodine and the catalytic enzyme co-factor is proposed in the third section. Calculations were performed using the Gaussian 03 program.

2. METHOD

The purine and pyrimidine bases of nucleotides include nitrogen and oxygen hetero atoms. The formation of stable complexes of molecular iodine with compounds including such hetero atoms is confirmed by thermodynamic data [19,20] and data obtained by physical research methods (UV-spectroscopy, X-ray structural analysis) [19,21].

The RHF/3-21G**, DFT PBE/3-21G** [22] methods were used for calculating the structure and stability of iodine complexes with pyridine and quinoline isomers (**Figure 1**, **Table 1**). Charges are calculated according to the Mulliken's scheme.

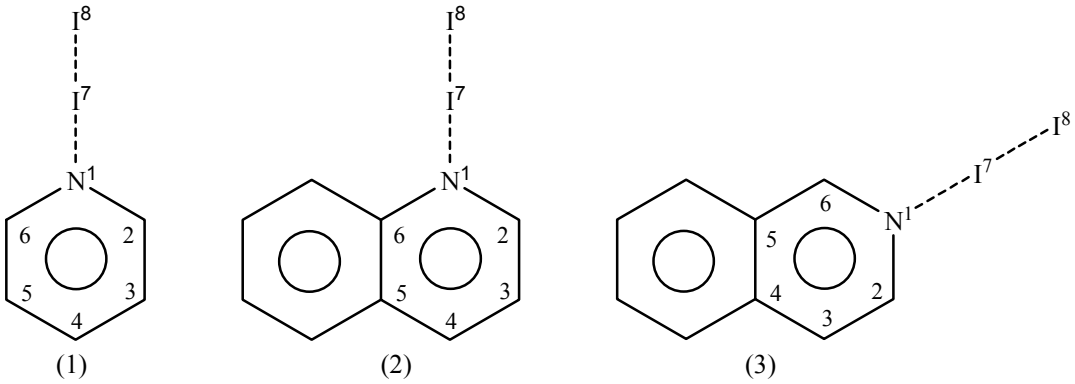

Figure 1. Molecular iodine complexes with pyridine and quinoline isomers.

Table 1. Bond length (Å), charge transfer (Δq), theoretical ($\Delta H^{theor.}$, kJ/mole) and experiment ($\Delta H^{exp.}$, kJ/mole) heat of formation for iodine complexes with pyridine and quinoline isomers.

		N^1-C^2	C^2-C^3	C^3-C^4	C^4-C^5	C^5-C^6	C^6-N^1	N-I	I-I	$-\Delta H^{theor}$	$-\Delta H^{exp}$	$-\Delta q$
	I	1.33	1,38	1.39	1.39	1.38	1.33	2.68	2.74	36.12	31.30 - 35.99	0.108
RHF/3-21G**	II	1.30	1.41	1.42	1.36	1.41	1.36	2.75	2.74	31.98	30.25	0.100
	III	1.37	1.35	1.42	1.40	1.42	1.30	2.68	2.74	36.45	34.90	0.110
	I	1.36	1.40	1.41	1.41	1.40	1.36	2.57	2.80	78.80	31.30 - 35.99	0.221
PBE/3-21G**	II	1.34	1.42	1.39	1.43	1.44	1.38	2.50	2.81	79.53	30.25	0.230
	III	1.38	1.38	1.43	1.44	1.42	1.34	2.47	2.81	80.28	34.90	0.234

The X-ray structural analysis data for the complex of I_2 with methylpyridine show that R_{N-I} = 2.31 Å, while R_{I-I} ranges from 2.67 Å in a free I_2 molecule to 2.83 Å in the complex [21]. The DFT PBE/3-21G** RHF/3-21G** methods give a longer N-I bond, while the length of the I-I bond is close to the experiment.

The capability of the method to correctly reproduce the geometry of charged polycations and polyanions of iodine was tested on the structures (**Figure 2**). The RHF/3-21G** method gives interatomic distances close to the experiment [23] and correctly reproduces the geometry of the ions (I_5 (q = –1), I_5 (q = 1), I_3 (q = 1)).

The N-I bond is of a donor-acceptor nature, the formation of the I-III complexes is accompanied by a charge transfer to the I_2 molecule (**Table 1**). UV spectral data for iodine complexes with heterocyclic aromatic bases are given in [19]. The formation of donor-acceptor complexes results in a significance change in the electronic spectrum of the system: it is indicated by appearance of strong ~230 nm - 240 nm bands connected with the charge transfer from the donor to the acceptor.

The heat of complex formation calculated by the DFT PBE/3-21G** method (ΔH^{theor}) is much overestimated, while ΔH^{theor} in RHF/3-21G** is close to the experiment and correctly represents changes in the complex stability depending on the position of the nitrogen atom in the quinoline heterocycle (**Table 1**).

The presented data show that RHF/3-21G** may be used for calculations of the structure and stability of iodine complexes with purine and pyrimidine bases of nucleotides.

2.1. The Structure of Molecular iodine Complexes with Nucleotides

The intercalation of the active substance of the drug into the DNA structure is considered as a possible reason by an anti-HIV effect [24]. The interaction of the active substance of the drug with DNA results in the formation of a new structure whose geometry and stability is determined by the nature of interaction (stacking [25], a donor-acceptor interaction) between the drug and nucleotide triplets.

The RHF/3-21G** level of theory was used for studying the interaction of molecular iodine with deoxyadenosine monophosphate IV(a,b), deoxycytidine monophosphate V and deoxyguanosine monophosphate VI (**Figure 3**). In the calculations, the phosphate fragment is replaced by a hydrogen atom on the assumption that the phosphate fragment of the nucleotides should not significantly affect the donor properties of the bases.

The calculation results show that in the interaction of I_2 with nucleotides the formation of donor-acceptor complexes is energetically preferable. Coordination is

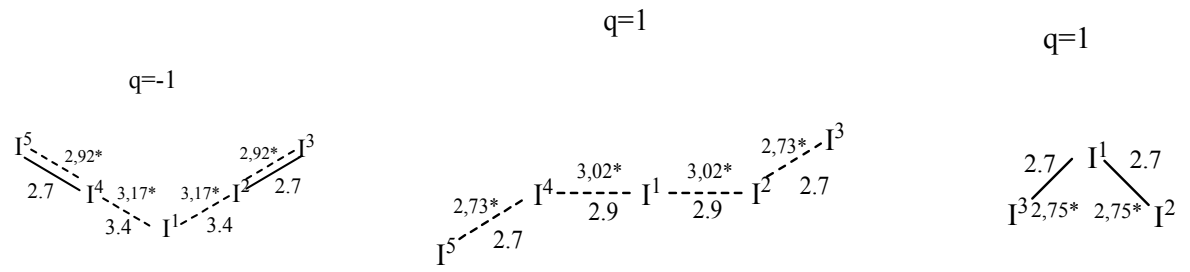

Figure 2. Data X-ray and result of calculation done by method RHF/3-21G** (*) for iodine polycations and polyanions (Å).

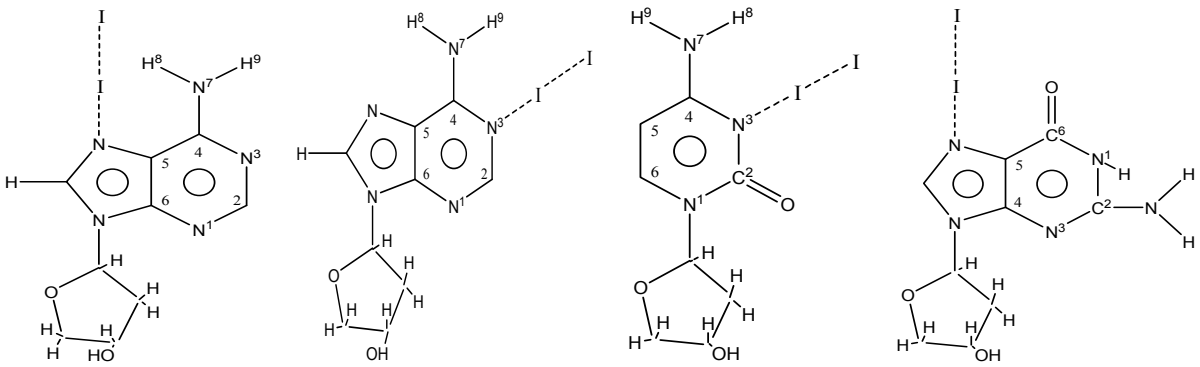

Figure 3. Molecular iodine complexes with free deoxynucleotides.

made by one of the nitrogen atoms of the purine or pyrimidine bases of nucleotides with a negative charge transferred to the I_2 molecule. The length of the N-I bond, like in the complexes of I_2 with pyridine and quinoline isomers, is in the range of 2.5 Å - 2.7 Å, and the I-I bond becomes weaker (**Table 2**).

Table 2 shows that the molecular iodine complex with cytosine is the most energetically preferable, but in the guanosine-cytidine pair the nitrogen atom of the pyrimidine base takes part in the formation of the Watson-Crick hydrogen bonds. The I-I bond is noticeably longer than the hydrogen bonds and therefore, the formation of the cytosine-molecular iodine complex should be accompanied by the destruction of hydrogen bonds in the Watson-Crick pair (**Figure 4**).

The influence of hydrogen bonds on the preferences of complex formation in the nucleotide pairs of Watson-Crick bases is studied on model structures of complimentary pairs of purine and pyrimidine bases: adenosine-thymidine, guanosine-cytosine, the sugar-phosphate fragment of the nucleotides being replaced by the CH_3-group (**Figure 4**).

The calculations have shown that in the guanosinecytosine pair iodine forms a stable complex with guanosine via a connection with the nitrogen atom of the five-membered cycle. The formation of the I_2 –cytosine complex is accompanied by a break of the Watson-Crick hydrogen bonds and, therefore, is not energetically preferable. In the complimentary adenosine-thymidine pair, the nitrogen atom of adenine which does not take part in the formation of hydrogen bonds is also the most energetically preferable atom for iodine coordination (**Figure 4**).

Table 2. Bond length (Å), charge transfer (Δq), stabilization energy ($\Delta E^{theor.}$, kJ/mole) for iodine complexes with deoxynucleotides.

		N^1-C^2	C^2-N^3	N^3-C^4	C^4-C^5	C^5-C^6	C^6-N^1	C^4-N^7	N-I	I-I	$-\Delta E^{theor}$	$-\Delta q$
RHF/3-21G**	IV(a)	1.33	1.33	1.34	1.40	1.38	1.33	1.34	2.64	2.75	49.32	0.120
	IV(b)	1.31	1.35	1.34	1.40	1.38	1.34	1.34	2.68	2.75	46.72	0.117
	V	1.40	1.37	1.31	1.43	1.34	1.35	1.34	2.68	2.75	53.25	0.121
	VI	1.37	1.30	1.35	1.37	1.43	1.43	-	2.66	2.75	51.37	0.121

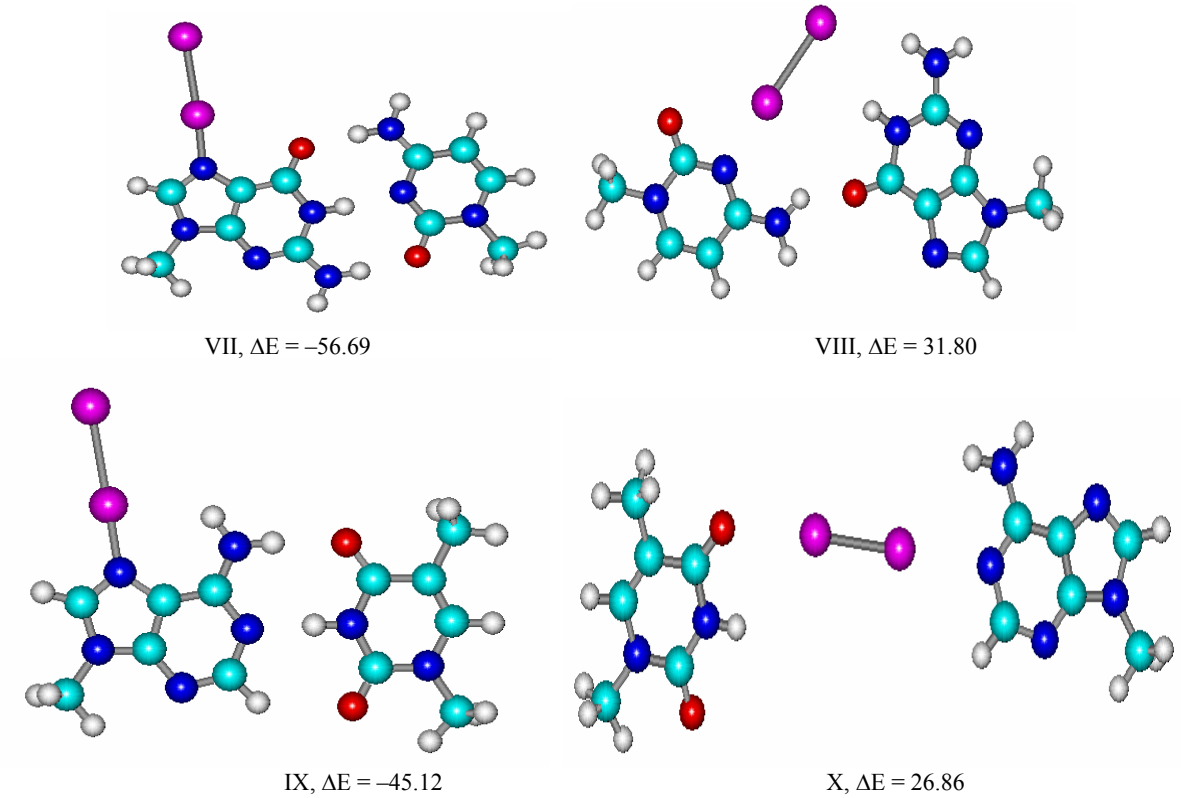

Figure 4. The influence of hydrogen bonds on the preferability of complex formation in complimentary Watson-Crick nu cleotide base pairs. ΔE (kJ/mole) is the energy of complex formation by molecular iodine and the purine and pyrimidine bases of nucleotide. Blue balls - carbon atoms, dark blue -nitrogen atoms, red- oxygen atoms, white-hydrogen atoms, violet-iodine atoms.

2.2. The Mechanism of Inhibition of the HIV-1 Integrase Enzyme

The spatial and electronic structure of possible structures of the active center of the nucleoprotein complex formed by the virus DNA, molecular iodine and the fragment of the catalytic center of HIV-1 intergrase was calculated (**Figure 5**).

The formation of I_2-nucleotide complexes is accompanied by the delocalization of electron density along the I-I bond and its weakening. In VII-X complexes the donor properties of the I_2 molecule are enhanced due to a negative charge transferred to I_2. The negatively charged atom of I^2, which is located far enough from the sugar-phosphate backbone of the virus DNA and, thus, is readily available, may prevent the active catalytic fragment of HIV-1 integrase from interacting with the virus DNA. Coordinating one of the Mg^{2+} ions, I_2 may become the center of another nucleoprotein complex in which molecular iodine interacts both with the virus DNS and the active catalytic domain of HIV-1 integrase, exhibiting acceptor properties with respect to nucleotides, and donor properties with respect to the Mg^{2+} ions.

On the assumption that the coordination of nucleotides of the virus DNA by molecular iodine prevents the formation of a stable bi-nuclear structure with two Mg^{2+} ions in the catalytic domain, two possible variants of Mg^{2+} coordination by carboxyl groups of the amino acid residues Asp64, Asp116 and Glu152 were considered (structures XI-XIV). In XI-XIV the hydrocarbon and amide fragments of the amino acid residues were replaced by a methyl group. This simplification of the structure of the amino acid residues Asp64, Asp116 and Glu152 may be justified by the fact that the amide fragment is separated from the carboxyl groups by several methyl groups and, therefore, does not influence their donor activity.

The calculations showed the possibility of formation of a stable nucleoprotein complex in which molecular iodine coordinates with both the purine bases of nucleotides and the Mg^{2+} ion, significantly reducing the charge on the magnesium ion (**Table 3**).

The stabilization energy of the nucleoprotein complex ΔE is calculated as:

$$\Delta E = E_1 - (E_2 + E_3 + E_{12}) \quad (1),$$

where E_1—total energy of the structures XI-XIV;

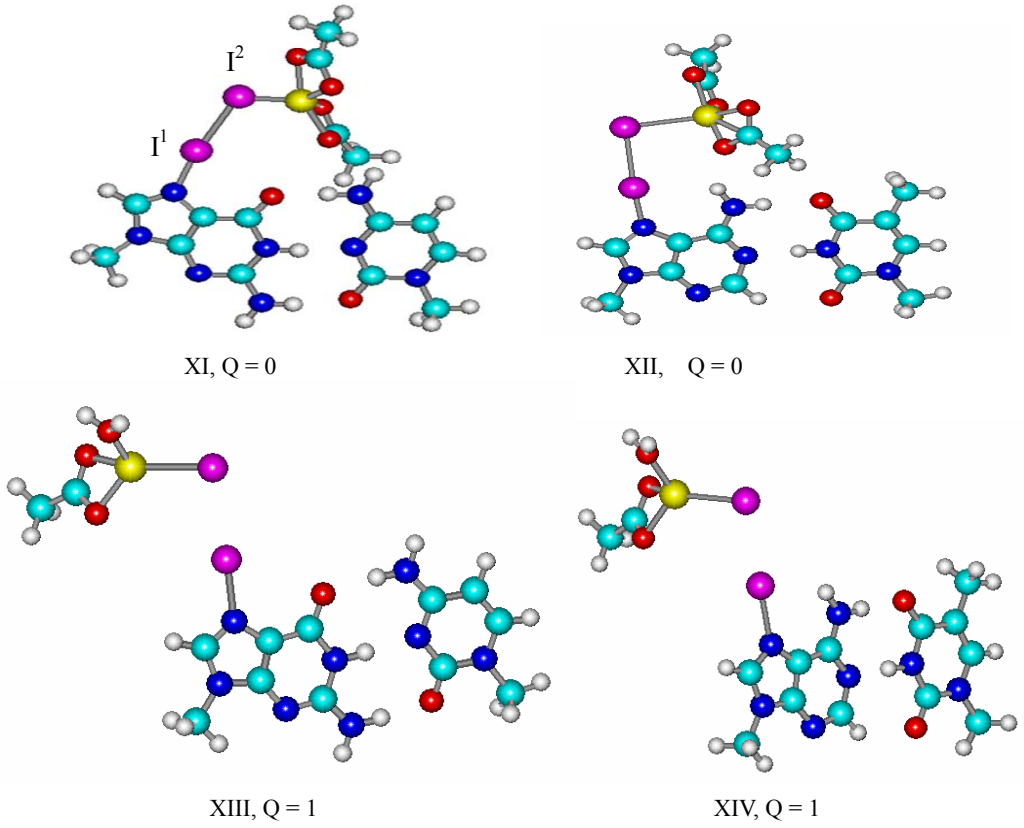

Figure 5. Possible structures of the active center of the nucleoprotein complex formed by the virus DNA, molecular iodine and the fragment of the catalytic center of HIV-1 intergrase. Blue balls-carbon atoms, dark blue-nitrogen atoms, red-oxygen atoms, white- -hydrogen atoms, violet-iodine atoms, yellow–ion Mg^{2+}.

Table 3. Stabilization energy (ΔE, kJ/mole), spatial (interatomic bond, Å) and electron characteristics of possible models of the active center of the nucleoprotein complex formed by the virus DNA, molecular iodine and the catalytic fragment of HIV-1 intergrase.

	XI	XII	XIII	XIV
–ΔE	110.07	108.35	318.69	259.70
N-I	2.42	2.53	2.15	2.13
I-I	2.81	2.79	3.12	3.21
Mg-I	2.95	2.95	2.69	2.68
Q (I^1)	0.204	0.187	0.396	0.432
Q (I^2)	–0.235	–0.186	–0.333	–0.365
Q (Mg)	0.767	0.789	0.775	0.758

*) $Q_{MgCOOCOO} = 0.885$; $Q_{MgCOOH2O} = 1.099$.

E_2—total energy of $Mg(COOCH_3)_2$ for XI,XII and $Mg(COOCH_3)H_2O$ for XIII, XIV;

E_3—total energy of the Watson-Crick pair adenosine-thymidine in XI, XIII and guanosine-cytosine in XII, XIV;

E_{I2}—total energy of I_2 molecule.

In the structures XIII, XVI the I-I bond is broken and R_{N-I} indicates that there is a new N-I bond in the purine bases of adenosine and guanosine (**Table 3**).

Among the recently developed drugs inhibiting the activity of HIV-1 integrase there are those whose inhibiting activity is connected with the formation of coordination bonds with two Mg^{2+} ions [12,26] of the catalytic fragment of HIV-1 integrase. The calculations revealed that the molecular iodine contained in iodine polymer complexes could be referred to this class of compounds, but, unlike the known inhibitors of HIV-1 integrase, I_2 also changes the structure of the virus DNA.

3. CONCLUSIONS

The computation results suggest that molecular iodine in drugs containing molecular iodine complexes may be referred to compounds inhibiting the catalytic center of the HIV-1 integrase enzyme.

It is shown that molecular iodine prevents the formation of PIC and inhibits the HIV-1 intergrase enzyme inside the nucleoprotein complex where I_2 interacts both with the virus DNA and the active center of the catalytic domain of the HIV-1 intergrase exhibiting acceptor properties with respect to the nucleotides of the virus DNA and donor properties with respect to Mg^{2+} ions.

The breaking of the I-I bond and the formation of a new N-I bond in the purine bases of adenosine and guanosine may be observed in the structure of the nucleoprotein complex.

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