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Structure-Activity Relationships/Tendencies Found in Several Anti-HIV Nicavir Derivatives Using DFT Quantum Chemical Methods

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Abstract

The molecular structure of a new group of AZT derivatives, namely, AZT 5'-aminocarbonylphosphonates anti-HIV drugs is presented by means of quantum chemical DFT/B3LYP calculations with full relaxation of all the geometrical parameters. They are reverse transcriptase inhibitors and derivatives of the nikavir nucleoside analogue used actually in clinic, but they are less toxic an offer an advantage over the approved drugs AZT and nikavir. An extensive conformational analysis is carried out and 45 optimized structures with *anti* or high-*anti* orientation were determined. The main characteristic geometric parameters of these derivatives were analyzed and compared with those calculated in other nucleoside analogues. Several structure-activity relationships/tendencies were found in these derivatives, which can help in the design of new antivirals.

Keywords: Aminocarbonylphosphonate; Nicavir derivates; Anti HIV; Antiviral agents; Reverse transcriptase inhibitors; DFT methods; Structure-activity relationship

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Introduction

Viral infections are widely recognized as an important medical problem [1]. The progress toward the treatment of HIV infections has steadily increased in the past two decades [2] and many different strategies have been developed in the search for therapeutic agents against AIDS. Human immunodeficiency virus type 1 (HIV-1) uses its own reverse transcriptase enzyme (RT) to convert its single-stranded RNA genome into a double-stranded DNA copy suitable for integration in the host cell genome.

Nucleoside reverse transcription inhibitors (NRTIs) [3], such as zidovudine (AZT), staduvine (d4T), zalcitabine (ddC), didanosine (ddI), lamivudine (3TC), abacavir (ABC), and tenofovir (PMPA), constitute the most important class of antiviral compounds for the treatment of HIV-1 infection. They are used clinically to inhibit retroviral replication and some of them in cancer therapy. About 20 drugs have been approved for this purpose [4]. The most effective alternative substrates of the reverse transcriptase enzyme of HIV virus correspond to compounds containing unsaturated ribose ring structure, which lack 2'- and 3'-hydroxyl groups. These analogues compete with the natural nucleotides (dNTPs) for binding and incorporation in the nascent viral chain and act as chain terminators because they lack a O3'H group. However, up until now, the virus has shown the ability of developing resistance mechanisms for each RT inhibitor used in the treatment of AIDS [5]. The most common anti-HIV drug AZT [6] **Figure 1** appears to cause serious toxicity problems for many AIDS patients clinically and a rapid excretion from organism requires a frequent administration of the drug. These limitations have forced to synthesize new prodrugs derivatives of AZT [7,8]. However, despite significant progress in the design of anti-HIV drugs, many problems remain. As a result, there is a critical need for more effective and less toxic therapeutics.

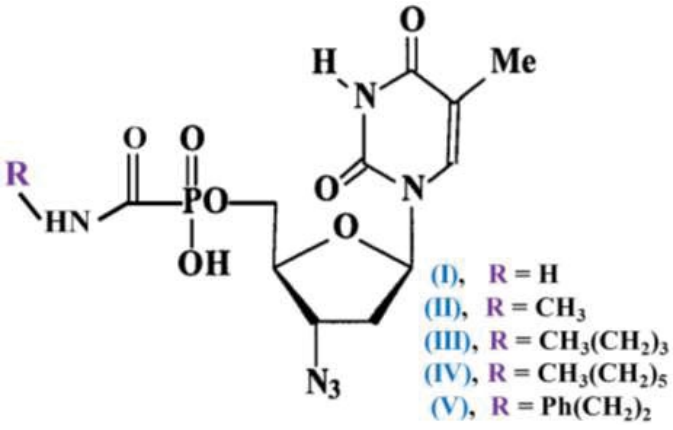
The nucleoside analogues do not exert directly antiviral activity, but they are rather prodrugs of active phosphorylated metabolites which are synthesized by action of various cellular kinases to give successively the corresponding nucleoside 5'-mono-, di- and triphosphates [9]. The 5'-triphosphate form selectively inhibits the synthesis of viral DNA catalyzed by HIV reverse transcriptase. The effectiveness of all this process is extremely low, for example, 0.3% for AZT [10]. Therefore, many efforts have been made to improve the therapeutic properties by shortening this cascade and bypassing at least the first phosphorylation step [11]. Moreover, usually the monophosphorylation step that involves cellular

nucleoside kinase enzyme, that is, the synthesis of nucleoside 5'-monophosphates (NMP), is generally considered to be the most restrictive for the nucleoside triphosphate (NTP) formation. Because phosphorylation is indispensable for biological activity, nucleoside analogues that are poor substrate for phosphorylating enzymes are usually inactive [12], and this may explain the low anti-HIV activity of some ddN compounds. The dependence on phosphorylation for activation of the particular nucleoside analogue may be a problem in cells where the nucleoside kinase activity is known to be low or even lacking [13]. A low efficiency of intracellular transformations requires the use of the preparations at high doses, which induce pronounced toxic effects.

One of the ways for improvement of the pharmacological properties of antiviral agents is design of their depot forms (latent forms), the derivatives that are subjected to chemical or enzymatic biotransformations in organism followed by release of active compounds [14]. A successful realization of this approach, in special to by-pass the enzymatic monophosphorylation step, appears with the introduction of an H-phosphate group into the AZT 5' position, which leads to a new group of anti-HIV drugs, AZT 5'-monophosphates. It is exemplified in Nikavir (NK), an anti-HIV agent [15], which is gradually hydrolyzed in organism to give AZT. This molecule of NK is ca. 4 times less toxic than AZT, with a narrower spectrum of side effects, with a slower excretion, and with slower development of drug resistance, although it is inferior to AZT in antiviral activity.

Because the toxicity of NK also remains rather high, new phosphonates of nucleoside analogues have been synthesized [7,15,16] with related activity and at least ten times less toxic than AZT and NK [15]. Others phosphate-containing drugs appear as important agents for anticancer and antiviral therapy [16,17]. Synthesis and biological activity of these compounds have been reported [14,18-20] and they have been subject of several reviews [11,15,21]. Among these molecules, the aminocarbonylphosphonate, molecule I of **Scheme 1** has special interest [15]. This molecule is noticeably less toxic, had a longer half-life in organisms and offered an obvious advantage over the AZT and NK drugs. Thus, in the present work, we have carried out a conformational analysis of this molecule and their AZT 5'-aminocarbonylphosphonates derivatives [19], **Scheme 1**, and we compare the molecular structure of the most stable conformers. These compounds can be regarded as depot forms of AZT widely used in the treatment of HIV infections, and they exhibit low toxicity in experimental animals and lack of cumulative effects [19].

The conformational analysis of anti-HIV compounds is extremely important in order to better understand their chemical structures and biological activity. This biological activity of a molecule is related to a limited conformational space and it can have a direct application in the comprehension of the structure-activity relationship and in the design of new drugs. Also an accurate knowledge of the flexibility and conformeral properties of a nucleoside would be an important help for the interpretation of drug-target interactions. For this reason, the conformers of natural and analogues nucleosides have been analyzed by different authors [22-31]. Now, a computational study of the



Scheme 1 Structural formulas of the phosphonate molecules studied.

main conformers in AZT 5'-aminocarbonylphosphonates was carried out, and compared the results with AZT, NK and the natural nucleoside 3'-deoxythymidine (dT).

Another goal of the present manuscript is to establish relationships/tendencies between structure, conformational parameters, physicochemical properties or activity of these derivatives that can help in the design of new antiviral drugs. Structure-activity relationships/tendencies have been established by us in other nucleoside analogous [32,33] and in other molecules [34]. We will attempt to determine herein, if the various geometric parameters in these compounds are correlated or interact with one another. Due to these drugs have been synthesized recently, structural studies have not been reported yet.

Computational methods

The B3LYP Density Functional method (DFT) which use the Becke Three Parameter Hybrid exchange Functionals B3 with LYP type correlation has been employed to find the equilibrium geometry in the phosphonates under study. MP2 ab initio quantum chemical method was also used in AZT and thymidine molecules. All the calculations were carried out by using the GAUSSIAN 09 program package [35]. MP2 provides accurate geometrical parameters and energy values, while DFT methods provide adequate compromise between the desired chemical accuracy and the heavy demands put on computer time and power and they are adequate for frequency calculations. Moreover, DFT methods have been used satisfactory in many studies of drugs [36-38] and related molecules [39]. Several basis set were used starting from the 6-31G(d) to 6-311++G(3df,pd), but the 6-31G(d,p) represents a compromise between accuracy and computational cost, and thus it was the base set selected for all the calculations. The B3LYP method was chosen because it is the most standardized and used today, and different studies have shown that the values obtained with this method are in good agreement with those obtained by other more cost computational ones, and it predicts vibrational wavenumbers of DNA bases better than the HF and MP2 methods [40].

The optimum geometry was determined by minimizing the energy with respect to all geometrical parameters using the gradient

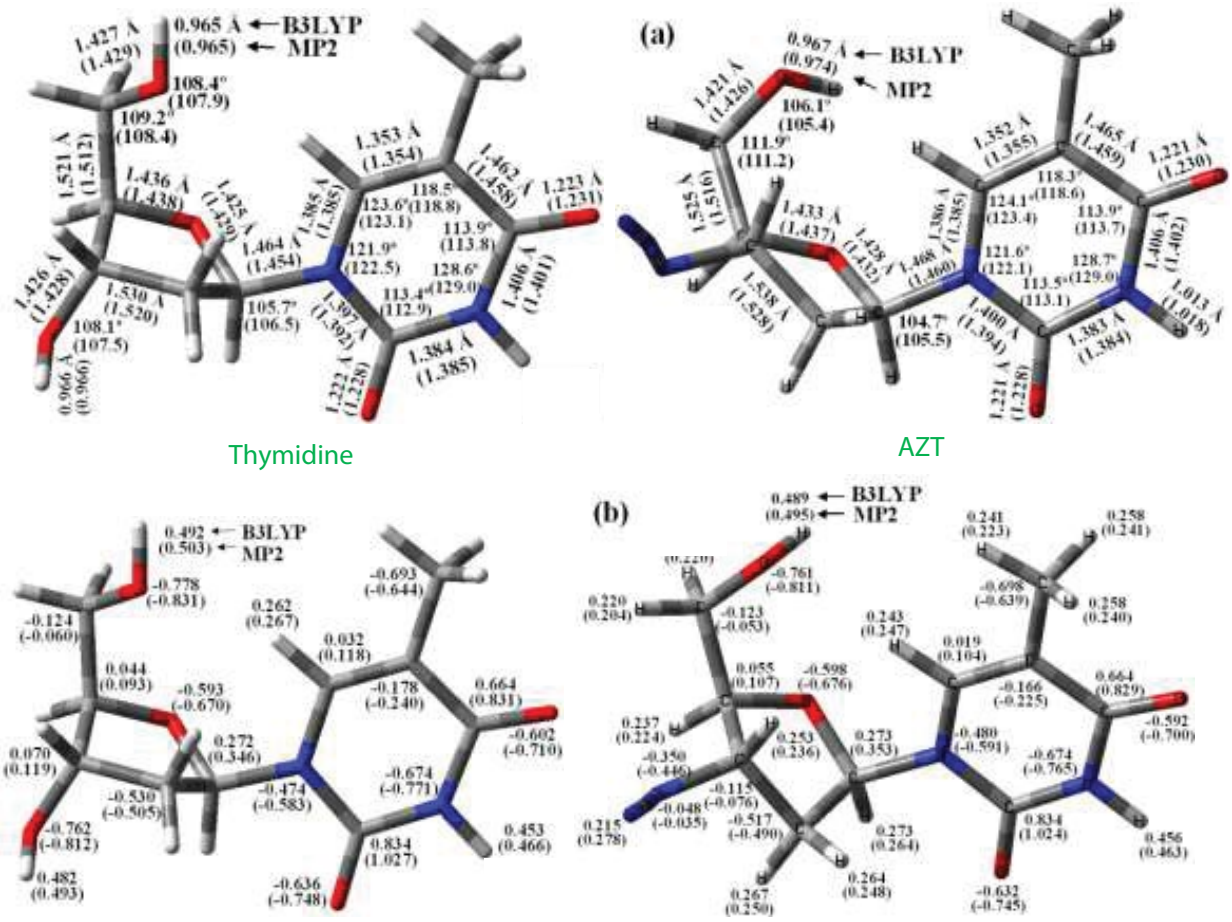


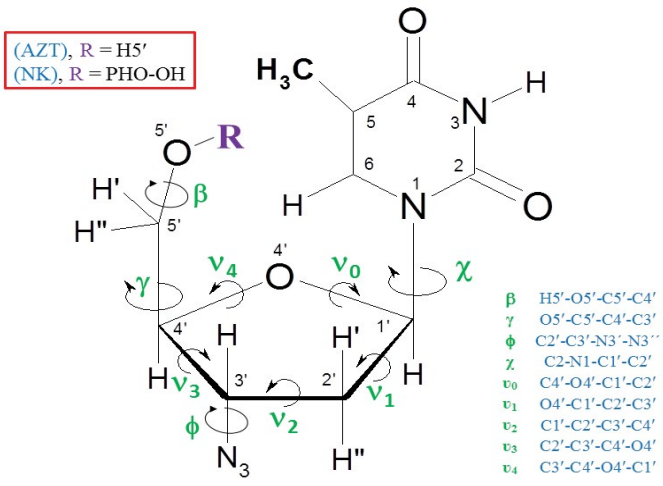
Figure 1 Differences between the most stable conformers in the *anti* form of dT and AZT at the B3LYP/6-31G(d,p) and MP2/6-31G(d,p) (values in parentheses) levels: (a) in the bond lengths, (b) in the NBO atomic charges. The calculated optimum bond lengths are in Å.

technique without imposing molecular symmetry constraints. Berny optimisation under the TIGHT convergence criterion was used. Atomic charges were determined with the Natural NBO procedure. The harmonic wavenumber computations were carried out at the same level of the respective optimization process and by the analytic evaluation of the second derivative of the energy with respect to the nuclear displacement. Wavenumber calculations were performed in all the optimized conformers to asses that they correspond to real minimum. All the optimized structures showed positive harmonic vibrations only (true energy minimum).

Relative energies were determined by full optimizations at the B3LYP level using the 6-31G(d,p) basis set. For the calculation of ZPE, the wavenumbers were retainted unscaled. All quantum mechanical computations were performed on the alpha computer of the Computational Center from University Complutense of Madrid.

Definition of the characteristic conformational angles

The atomic description of AZT molecule as well as the most important exocyclic and endocyclic torsional angles is defined in **Scheme 2**, while



Scheme 2 Molecular structure and definition of the exocyclic and endocyclic angles in 3′-azido-2′,3′-dideoxythymidine (zidovudine, AZT), and in AZT 5′-*H*-phosphonate (Nikavir, phosphazide, NK) prodrugs.

those corresponding to the aminocarbonylphosphonates under study are plotted in **Scheme 3**. The conformation of a nucleoside

depends of three factors, namely: (i) the orientation of the base unit (BU) with respect to sugar unit (SU). (ii) The conformation of SU, and (iii) the orientation of the neutral phosphate unit (PU) with respect to SU. The main structural parameters that usually characterize the nucleosides [41] are the following:

(i) The glycosylic torsional angle χ(C2-N1-C1´-C2´) determines the three orientations of the base relative to the furanose ring, and denoted as the *anti* (χ ~ -120°), *high-anti* (χ ~ 180°) and *syn* (χ ~ 60°) forms. *Syn* is when the C2 carbonyl of pyrimidine lies over the furanose ring, and *anti* when this group is oriented in the opposite direction. *Anti* forms show the pyrimidine ring and the sugar residue nearly perpendicular to each other. *High-anti* forms are those generally observed in the solid state crystals, while the *anti* ones are the expected biological active and they are common for A and B DNA strands. The *syn* forms can be encountered in purine nucleosides involved in left handed (Z-form) helices of RNA and DNA.

(ii) The torsional angle (C2′-C3′-N′-N″) determines the orientation of the azide group relative to the furanose ring. A trimodal distribution is observed in the studied prodrugs, in agreement with that found by us in AZT [42] and NK [43], with values in the range ca. -60°, 60° and 180°.

(iii) The exocyclic torsional angle δ(C5′-C4′-C3′-N′) specifies the orientation of the substituents in C4′ and C3′. It has a trimodal distribution with values around 90°, 120° and 140°.

(iv) The torsional angle γ(O5′-C5′-C4′-C3′) describes the orientation of the O5′ atom relative to the furanose ring. Although a trimodal distribution is observed in AZT, NK and related prodrugs, however, in the aminocarbonylphosphonates under study only the γ_t (~180°) orientation is possible due to the steric interaction of the substituent.

(v) The exocyclic torsional angle β(P6′-O5′-C5′-C4′) shows the orientation of the 5′-phosphate group relative to the furanose ring. This ring is twisted out-of-plane in order to minimize non-bonded interactions between their substituents. The trimodal distribution is in accordance to that found in AZT, NK and related prodrugs.

(vi) The torsional angle α(C7′-P6′-O5′-C5′) defines the orientation of the hydroxyl oxygen O6′′ in the phosphate group. Although a trimodal distribution is obtained, the values in the range -130° and -140° lead to the most stable tautomers.

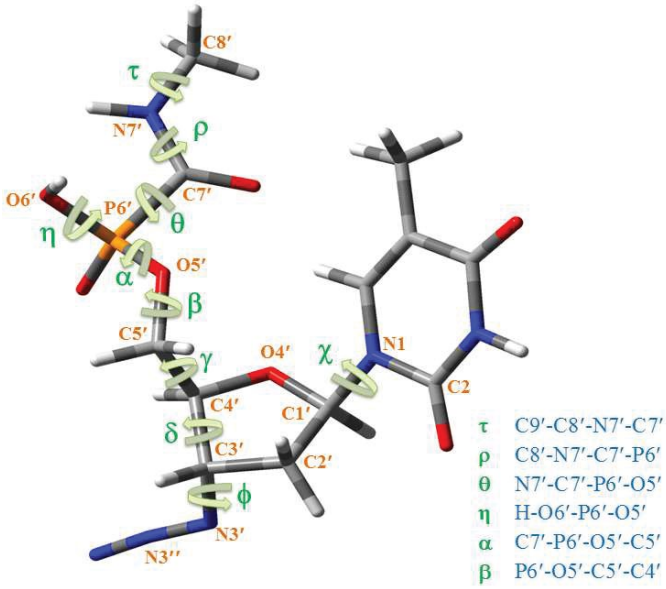
(vii) The puckering of the furanose ring and its deviation from planarity, which is described by the phase angle of pseudorotation P (0-360°) and it is defined as:

$$tgP = \frac{(\upsilon_4 + \upsilon_1) - (\upsilon_3 + \upsilon_0)}{2\upsilon_2 (\sin 36 + \sin 72)}$$

Where $_0$ - ν_4 are the endocyclic torsional angles

(viii) The maximum torsional angle (degree of pucker, maximum amplitude), ν_{max}. Its value appears correlated to the pseudorotation angle P [44]:

$$\upsilon_{max} = \frac{\upsilon_2}{\cos P}$$



Scheme 3 Molecular structure and definition of the exocyclic torsional angles in the aminocarbonylphosphonate molecule I (also called 18f or IIf [14]), with the labelling of the main atoms involved.

The definition of other torsional angles of interest is included in **Schemes 2 and 3**. Whereas P and ν_{max} are biologically important parameters since their values are used to distinguish A- and B-conformations of the DNA and DNs, the values of $_o$ to ν_4 torsions present null interest for molecular biologists [45].

Results and Discussion

The structures of 2′-deoxyribonucleosides (DNs) have been extensively studied by experimental methods [41,46,47]: X-ray, neutron diffraction and NMR spectroscopy, and theoretical [48-51]. The results of these investigations can be basically rationalized in terms of the most preferable ranges for the characteristic conformational angles describing the primary features of the DNs geometry. However, the structures of 2′-deoxyribonucleotides (DNt) have been less studied, and we present here the DFT study of several aminocarbonylphosphonates.

Comparison of AZT, NK and dT nucleosides

The values of the most characteristic torsional angles optimized in the five most optimum stable conformers of AZT and Nikavir (NK) are listed in **Table 1**. The notation used is described in **Scheme 2**. The conformers were listed according to the increasing ΔE energy. The *syn* conformation is the most stable form found in both molecules, similarly to that found in the natural nucleoside dT. The biological active *anti* form appears closely in energy, 0.601 kcal/mol in NK and 1.050 kcal/mol in AZT. The percentage population of *anti* conformers in NK is noticeable higher than in AZT, but the percentage of the syn form is always the highest. In the natural dT the *syn* form is also the most stable. In all these molecules, the percentage of the syn form is remarkably higher than the anti form, but this anti form is the only one that can be phosphorylated by the different kinases. Therefore, in AZT, NK

Table 1 The calculated values at the B3LYP/6-31G(d,p) level of the characteristic exocyclic torsional angles (in degrees) in the four most optimum stable conformers of Nikavir and AZT prodrugs. The pseudorotational angle P and ν_{max} in degrees, and relative energies (ΔE) in kcal/mol.

confomer	Nikavir							AZT						
	χ	β	γ		P	ν_{max}	ΔE	χ	β	γ	ϕ	P	ν_{max}	ΔE
1	69.9	-98.6	66.7	115.5	35.5	28.4	0[a]	61.3	43.8	43.4	113.1	41.3	26.7	0[b]
2	65.7	104.0	41.2	105.6	51.1	34.4	0.014	61.5	43.5	44.2	99.1	44.5	26.1	0.024
3	-115.3	64.2	46.6	95.4	78.1	39.9	0.601	-129.1	68.8	61.9	95.9	31.3	33.2	1.050
4	-112.5	67.7	49.4	84.3	109.6	41.4	0.636	61.7	42.9	45.0	-53.2	45.1	26.3	1.512
5	-170.3	-84.9	-179.4	88.7	6.6	35.4	1.336	-128.1	176.5	50.2	70.9	162.9	32.5	1.556

[a]ΔE=-1455.728209 AU [42]. [b]ΔE=-963.27585 AU [41].

and in all the nucleoside analogues the percentage of compound that passes the first phosphorylation step is very small. However, in the molecule of dT it is remarkably high. Why?. One of the reasons can be found in the hydration of these molecules. Thus, the most optimum cluster of AZT with 13 water molecules is *anti* [29], as well as that obtained in dT, but in dT the H5' hydrogen of the O5'H group is in *trans* form (β≈ 180°), which is the orientation appropriate for the phosphorylation.

The difference in the molecular structure between the *syn* and *anti* optimum clusters is large enough, that we consider that in solution only one, the cluster with the *anti* orientation can enter into the enzyme cavity. Moreover, if the clusters enter into the cavity attracted through N3H and O4 and the hole to enter is something small, the length *h* [42] of *syn* clusters is higher than those of *anti*. Thus, perhaps they cannot enter into the enzyme cavity and only the *anti* form is phosphorylated [42].

In **Figure 1** collects the optimum bond lengths (in Å) and angles (in degrees) in the most sable conformer in the *anti* form calculated in the isolated state of dT and AZT molecules at the MP2 and B3LYP levels. In dT the H5' hydrogen of the –O5'H group appears in trans form (β_t ~ 180°, **Table 1**) while in AZT is in gauche + ($_+$ ~ 60°), which difficult the phosphorylation. The C3' substitution by the azide moiety in AZT does not have a significant effect on molecular conformation of the nucleoside. Thus, the molecular structure in the most stable conformer in the *anti* form of AZT is very similar to that of dT, at both MP2 and B3LYP levels. The different biological activity between AZT and dT is presumable [42] due to the presence of the azide group at the 3' position. This azide group cannot be H-bonded to water molecules and thus open clusters cannot be formed with AZT, but by contrast they are possible with dT making easy the first phosphorylation step [42].

The NBO atomic charges in dT and AZT, and at the B3LYP and MP2 levels are included in **Figure 1**. The values by MP2 appear slightly higher than by B3LYP, similar to that found in related nucleosides [23,24,27]. There is a negative charge on all the oxygen atoms slightly higher in dT than in AZT. Also the positive charge on H5' is slightly higher in dT than in AZT. This feature indicates the higher reactivity of dT than AZT.

The large values of ν_{max} indicate a flexibility of these molecules, higher in NK than in AZT, **(Table 1)**. Also there is a markedly higher negative charge on the oxygen atoms, which could be one of the factors that could explain the high activity of these prodrugs.

Conformers and energetics of the prodrugs under study

A conformational study of the aminocarbonylphosphonates under study **(Scheme 1)** in the isolated state was carried out through a rotation of the χ, ϕ, γ, β, α, θ, ρ and τ exocyclic torsional angles. A detailed collection of the most important conformational parameters of these optimized forms is included in **Table 2 (included as supplementary data)**. This Table also contains the values of the endocyclic torsional angles (ν_0 to ν_4), and the definitions of the pseudorotational parameters P and ν_{max}, that are commonly used to describe the conformational state of the sugar within the continuum of puckered states. For its symmetry (S) is used the Saenger notation [41].

Due to the nucleosides have conformational preferences by their biological targets [51] appear necessary to carry out a conformational analysis in the nucleoside analogues of the present work. Several studies has been reported [52,53] comparing the conformations of the 5'-triphosphates of AZT and thymidine bound to HIV-1 reverse transcriptase.

Conformers with *syn* orientation (χ: 80 ± 20°) were not included in the **Table 2 (included as supplementary data)** because they cannot be phosphorylated by its corresponding kinase. Conformers *anti* appear with χ: -142 ± 11°, while conformers *high-anti* with (χ: -171 ± 8°). In the *anti* orientation of the phosphonates under study is noted a noticeable higher χ value than in NK, with a distribution of (χ: -125 ± 14°). It can be explained by the H-bond between the amino hydrogen of the aminocarbonyl group and the O4' atom of the sugar ring. There are three conformers out of these ranges and with low χ value: -116.7° (conformer III-5), -115.7° (IV-3) and -107.2° (V-3). These three conformers have the sugar ring in the envelope $_4$E form, with the C4' atom in exo orientation, and a value of the pseudorotational angle P of ca. 55°, far from the other conformers.

For simplicity only three conformers were optimized in molecule I and nine conformers in molecule II. Due to the conformational possibilities of the butyl and hexyl chain twelve conformers in molecules III and IV were obtained. Finally, nine conformers were optimized in molecule V. Two energy criteria were considered for each conformer: the electronic energy ΔE + ZPE correction, and the Gibbs energy ΔG, the last two columns of **Table 2 (included as supplementary data)**. The conformers were numbered according to the increasing ΔE energy. With the exception of III-1, the global minimum in all the aminocarbonlyphosphonate molecules under study corresponds to a *high-anti* orientation (

~ 172°), ϕ and η torsional angles ca. 87°, γ ca. -178°, β ca. -96°, α ca. -140°, θ ca. 97°, and ρ ca. 177°. The pseudorotational angle P is ca 10° corresponding to an envelope ^{3}E form, with the C3′ atom in endo orientation. In **Figures 2 and 3**, plot the global minimum found in all the molecules studied together with the most important bond lengths and NBO atomic charges. The value of the only intramolecular H-bond observed in these molecules is also included. In the global minimum of molecules III, IV and V, the lipophilic moiety appears at one end of the molecule, which facilitates the membrane cell entrance. **Figure S1** shows the optimum molecular structure of other conformers with an intramolecular H-bond between O4′ and the amino hydrogen N7′H.

In NK molecule the *anti* orientation is stabilized by an intramolecular H-bond between the hydroxyl hydrogen of the phosphate molecule and O4′ atom. The *high-anti* orientation is also stabilized by this kind of intramolecular H-bond, but it is weaker. In the aminocarbonlyphosphonate molecules under study this H-bond is not possible, and only a weak contact (ca. 2.3 Å) appears between the carbonyl oxygen C7′=O and the hydrogen atom H6 of the thymine ring. Thus, in the molecules under study the global minimum is *high-anti,* while in NK the *anti* forms are slightly more stable than the *high-anti* ones. In NK the conformer with similar exocyclic and endocyclic torsional angles that the global minimum of the molecules under study appears in the fifth place of stability, **Table 1**. The importance of the intramolecular H-bonds in the stability of the different conformers and the intermolecular H-bonds in the drug interaction has been analyzed in other molecules [54].

In AZT the *anti* and *high-anti* forms appears stabilized by a contact between O5′ and H6 of the thymine ring, which is not possible in the molecules under study, and thus in AZT the conformer with similar torsional angles appears in the 19th place of stability of the *anti* and high-*anti* forms. These large differences in the molecular structure between AZT and NK, with the aminocarbonlyphosphonate molecules under study could be the reason of the very large anti-HIV activity between them, **Table 3.**

Values of the torsional angle η ca. 23° lead to the least stable conformers. It is due to in these conformers the hydroxyl hydrogen of the HO6′ group is not intramolecular H-bonded to the carbonyl oxygen of the C7′=O group, which reduce remarkably the stability of the conformer. With the exception of these conformers, the remaining structures differ in general very little in energy. Thus, in our calculations 41 optimized conformers were found within the electronic energy range Δ*E* and Gibbs energy range Δ*G*=0-5.8 kcal/mol related to the global minimum. If the conformers with the exocyclic angle η ca. 23° are included, this range of values of Δ*G* is higher than that reported in the natural nucleoside dT [55], 0-7.49 kcal/mol.

The values of the geometric parameters of the global minimum in the aminocarbonlyphosphonate molecules under study appear very close i.e., the substituents on N7′ little affect the molecular structure. Only small changes are observed in the substituent place. Similar feature is observed in the NBO atomic charges with very close values among the structures of global minimum i.e., the NBO charges are not affected by the different substituents in the terminal N7′H$_2$ amino group. However, the orientation of this substituent has in some conformers a noticeable influence, as in the conformers represented in **Figure S1**.

Thymine and Furanose rings

The pyrimidine ring shows a significant conformational flexibility [56-58], which represents an important source of relaxation in the molecular geometry for various inter- and intramolecular interactions [59]. According to several authors [60] the geometry of a nucleobase is almost unchanged when it is incorporated into the nucleosides, and it maintains a non-planar and non-rigid conformation. In the analysis of the most stable conformers in the molecules under study, the base heterocycle also appears to be with a small nonplanarity, in general with torsional angles lower than 2°. It is arising due to the anisotropy influence of the sugar residue on the base, the structural variability of the bases, and the weak interactions or H-bonds of the thymine base with the phosphate or aminocarbonyl moieties.

The ribose conformation can play an important role in the anti-HIV-1 activity, and differences in the ribose lead to appreciable

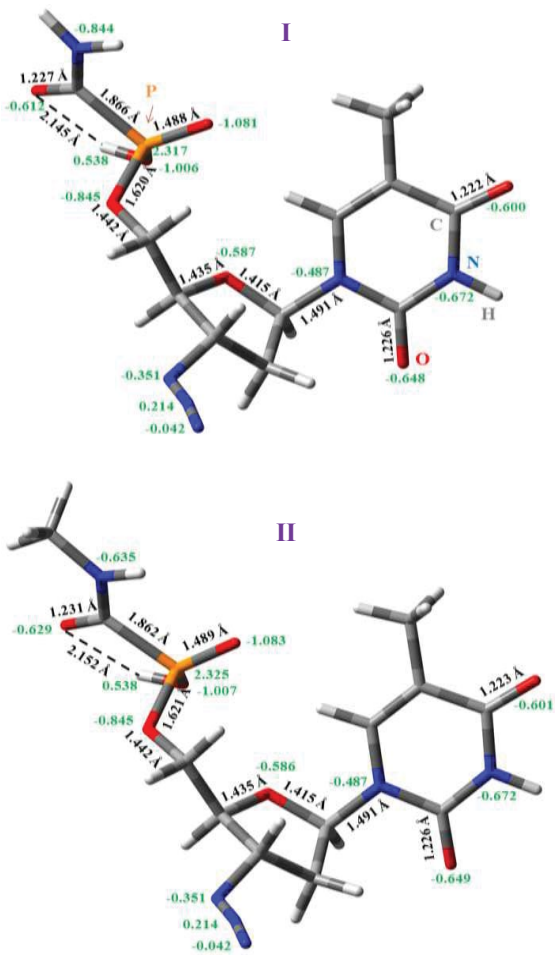


Figure 2 Optimized geometry of the most stable conformers I-1, and II-1 of molecules I and II, respectively. Bond lengths and intramolecular H-bonds in Å, and NBO atomic charges in green color.

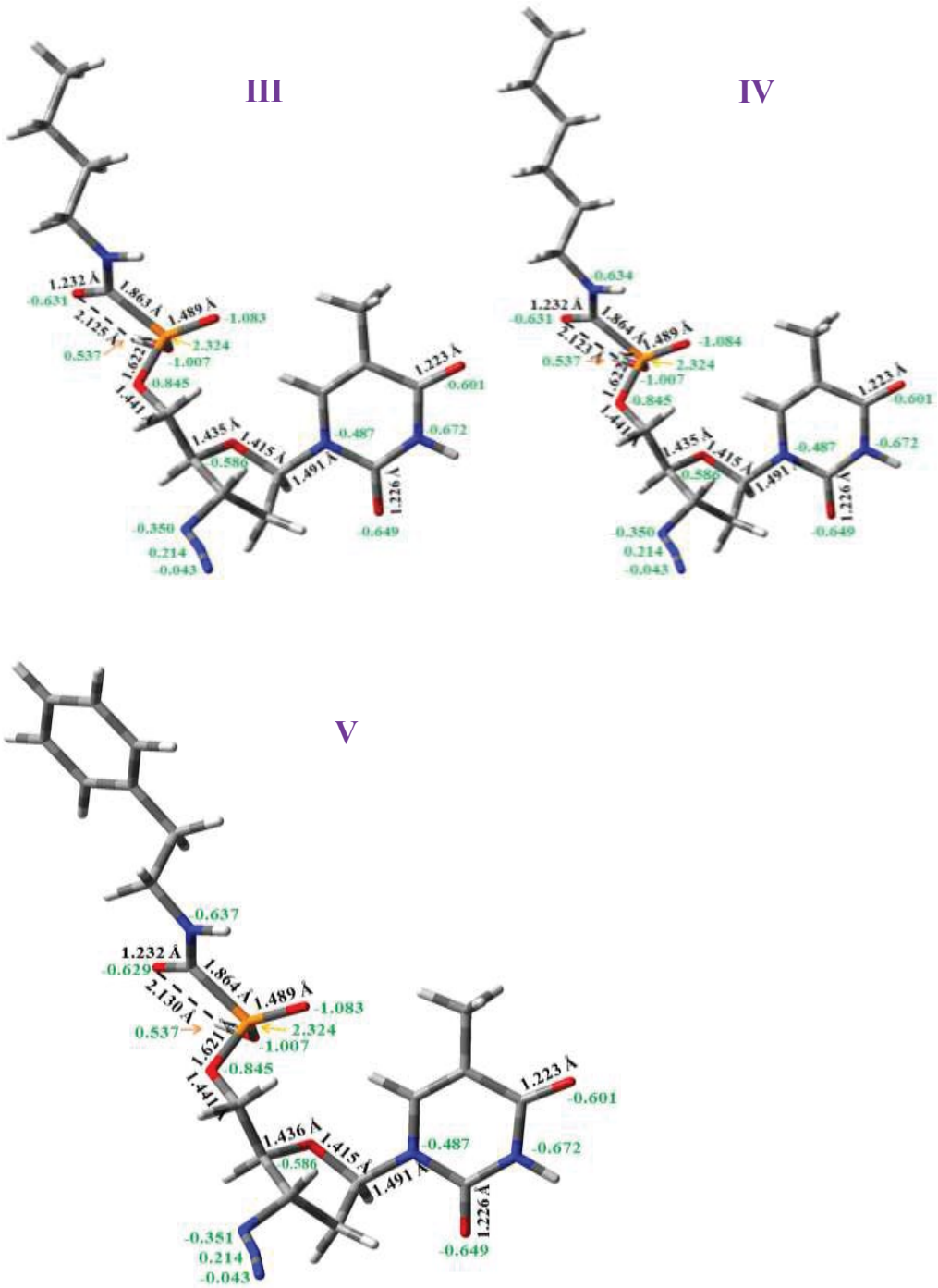


Figure 3 Optimized geometry of the most stable conformers molecules III-2, IV-1 and V-1. Bond lengths and intramolecular H-bonds in Å, and NBO atomic charges in green color.

changes in positions of the thymine ring and the C5'-OH group [61]. Thus, we have calculated the endocyclic torsional angles of the furanose ring, and they are shown in **Table 2 (included as supplementary data)**, together with the pseudorotational phase angle P, and ν_{max}. The most stable conformer corresponds to the envelope ^{3}E form (N-type), while the least stable is $_{3}E$, as it is expected. The predominant conformers are twist ^{i}T (S-type), and they are only less than 2 kcal/mol above the global minimum.

The value of ν_{max} ca. 35° is similar to that of AZT in the best *anti* form, 33.2°, but it is lower than in NK, 39.9°.

A change in the puckering mode results in a considerable deformation of the endocyclic bond angles. However, in general, the values of these angles follow the order C-O-C (~ 111°)>C-C-O (~ 106°)>C-C-C (~ 103°). The sugar ring gives specially flexibility to the nucleoside structure. The C-C bond lengths are not equal within the furanose ring, although the differences are small, and

Table 3 Anti-HIV properties of AZT and several phosphonates in MT-4 cells infected with HIV-1 [19]. The calculated dipole moments in the most optimum conformers in the *anti* orientation and at the B3LYP/6-31G(d,p) level are also included.

Compound	CD_{50} [a] (μM)	ID_{50} [b] (μM)	Selective index[c]	Dipole Moment (Debye)
AZT	80	0,037	2200	2.82
NK	180	0,131	1400	3.86
I	2700	0,60	4500	6.098
II	260	0,23	1130	6.549
III	1970	0,48	4100	6.902
IV	1500	0,11	13600	6.921
V	1080	0,20	5400	6.814

[a]Compound concentration required to reduce cell viability by 50%. [b]Compound concentration required to inhibit HIV replication by 50%. [c]Selective index=CD_{50}/ID_{50}

they follow the order: C1′-C2′>C2′-C3′>C3′-C4′. The C4′-O4′ bond length is also larger than C1-O4′. Their values essentially depend on the conformation of the sugar ring, while the C-O4′ bonds do not show such dependence [60].

It has been suggested the possible functional roles for the azido group in enzyme binding [62], and if AZT is incorporated into the viral DNA in place of thymidine, enzyme inhibition and chain termination may be in part the result of H-bonds formed between enzyme atoms that normally bind to O3′ of the polynucleotide and a phosphate oxygen atom of the next nucleotide to be added, and N´ and N´´´, respectively of the azide group [62]. Also, a sandwiching of bases by these azido groups appears in the crystal [63]. However, we have found that in AZT and NK the azido group [42,43] does not cause significant alterations in the conformational preferences of the nucleoside in the isolated state or in water solution, and mainly changes its polarity and lipophilicity. This feature has been also observed in the aminocarbonylphosphonate molecules under study. Thus, the most stable conformers in all the molecules studied are ϕ_{g+}, but the difference in energy related to ϕ_t is only 1 kcal/mol i.e., the azide group has little influence in the stability of the structure. All the least stable conformers are $_t$.

Natural NBO atomic charges

The NBO atomic charges are plotted in **Figure 1b** for AZT and dT molecules, while for the aminocarbonylphosphonates under study they are shown in **Figure 2**. These values can be compared to those reported in nucleosides analogues of AZT [64]. The largest negative charge is on O6′ and O6′′ atoms, ca. –1*e* (where *e* is the charge of an electron). The next atom with large negative charge is O5′, ca. –0.8*e*. Phosphate group charges represent an important role in stabilization of DNt [65], and the incorporation of nucleotides to A-DNA macromolecules requires the minimum amount of deformation energy [66].

In the carbon atoms, the highest positive charge is on C2 and C4 atoms, in concordance to the high negative charge on the O2 and O4 atoms, respectively. Because O2 has slight higher negative charge than O4, C2 has higher positive charge than C4. A net negative charge appear on the C3′ atom which is necessary for anti-HIV activity. It has been reported the possibility that the azide group may not always be able to function as a terminal group, leading to one of the possible mechanisms for resistant mutations. Thus, nitrogen atoms with appropriate charges may participate in H-bonds with residues of the reverse transcriptase enzyme (RT), leading to a possible route of resistant mutations [67]. However, the values of the charge obtained in the molecules under study indicate that the charge on N3′′′ is very low and almost zero, so it is not possible the bonding through this atom. Also the charge on N3′ is low, ca. -0.35*e*, which also difficults its H-bond.

Relationships/tendencies molecular structure-activity

The antiviral properties of the phosphonates under study have been reported in MT-4 cells [19], and the values of CD_{50}, ID_{50} and the Selective Index (SI) collected in **Table 3**. The results in AZT and NK molecules are also included. These data indicate that the compounds under study inhibit the virus replication similarly to NK, and by 1 order of magnitude lower than parent AZT. However, their toxicity (except molecule II) was considerable lower than that of NK and AZT [19]. Due to the tendency is now to synthesize compounds less toxic that permit a better quality of life for the patients, the analysis of the compounds under study appear appropriated. Also a higher CD_{50} of the aminocarbonylphosphonates under study allowed better selectivity indexes.

The higher activity of phosphonate **V** and **IV** than phosphonate **III** and **I** can probably be explained [19] by the presence of a larger hydrophobic substituent in these molecules, which contributes to facilitate the penetration into cells, and thus increasing its intracellular concentration.

In the last column of **Table 3** is included the calculated dipole moment at the B3LYP/6-31G(d,p) level in the best conformer in *anti* or *high-anti* orientation. The substitution of H5′ in the structure of AZT by a phosphate group increases noticeable the dipole moment (), as it can be observed in NK molecule. The further substitution in this structure by an amino and carbonyl groups remarkably increment again the μ values, as it can be noted in the molecules I to IV. In these molecules, the slight difference in the μ values can be explained by the slight rotation of the torsional angles around the phosphate and aminocarbonyl groups.

Another objective of the present work was the possibility to establish general structure–activity relationships/tendencies.

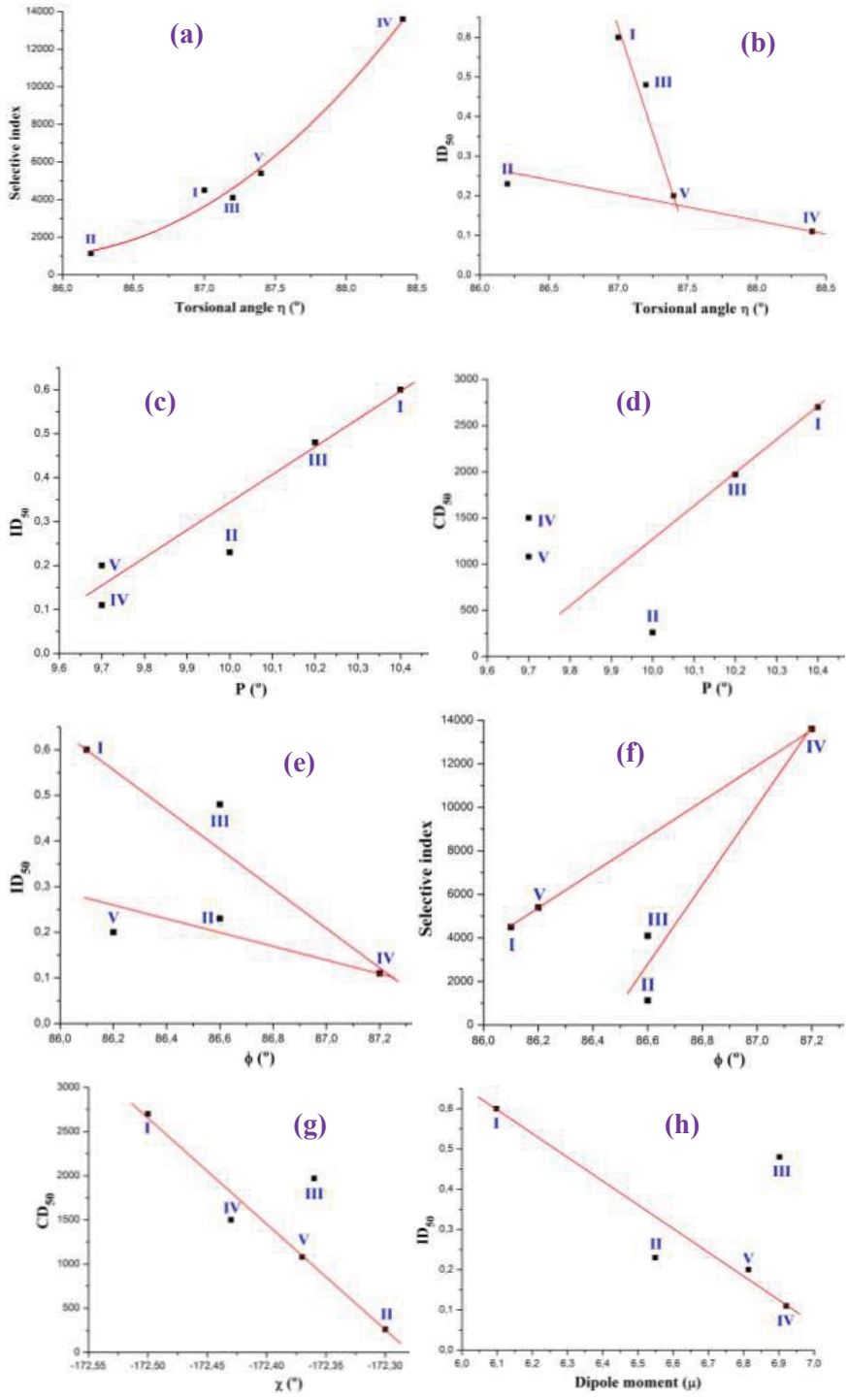


Figure 4 Relationships/tendencies observed in the aminocarbonylphosphonates molecules under study. (a) Between the selective index CD_{50}/ID_{50} and the torsional angle η. (b) Between the compound concentration required to inhibit HIV replication by 50% (ID_{50}) and the torsional angle η. (c) Between ID_{50} and the pseudorotational P angle. (d) Between the compound concentration required to reduce cell viability by 50% (CD_{50}) and pseudorotational P angle. (e) Between ID_{50} and the exocyclic torsional angle ϕ. (f) Between the selective index and the torsional angle ϕ. (g) Between CD_{50} and the exocyclic torsional angle . (h) Between ID_{50} and the dipole moment (in Debye).

Although the anti-HIV activity depends of many factors not considered in our computations, however, several relationships/tendencies can be observed as they are shown in **Figure 4**. In several graphs only a tendency can be observed rather that a linear relationship, perhaps due to the activity depends also of other parameters. Analyzing the different Figures was noted the following:

(i) The best relationship appears with the torsional angle η. A

slight rotation of the hydrogen of the hydroxyl O6'H group to higher values leads to a remarkable increment in SI. The relation is polynomic, (**Figure 4a**). This increase in SI is mainly due to a decrement in ID_{50}. The tendency to decrease ID_{50} with a slight increment in η is plotted in **Figure 4b**. Compound II appears to have a lower η value that it is expected. The increase in the value of η is due to a strengthening in the O6'-H···O7' intramolecular H-bond. Compound IV has the shorter H-bond, 2.123 Å, while compound II has the largest one.

(ii) Low concentration of ID_{50} is required in compounds with small furanose pucker P, (**Figure 4c**). The same relationship and trend but with CD_{50} was observed by us in other NK derivatives [43]. However, in our compounds the relation with CD_{50} is not well, (**Figure 4d**). Correlation with ν_{max} was not observed.

(iii) A slight opening of the torsional angle ϕ, which determines the orientation of the azide group, appears to be something related to a lower ID_{50} concentration, (**Figure 4e**). The lowering in ID_{50} leads to an increment in SI, (**Figure 4f**). The tendencies are not the same for all the compounds and it is something tentative.

Summary and Conclusions

A DFT study of several aminocarbonylphosphonates derivatives of NK prodrug is presented and discussed. The geometries and values shown here appear to be the most accurate to date. Comparisons with AZT, NK and dT provide support for the quality of our results. The most important findings of the present manuscript are the following:

(1) A conformational analysis through the rotation of the exocyclic torsional angles χ, ϕ, γ, β, α, θ, ρ and τ was carried out in five aminocarbonylphosphonate compounds. 45 stable conformers in the *anti* and *high-anti* orientation were identified at the B3LYP/6-31G(d,p) level. These conformers failing into the 0-9 kcal/mol Δ*G* or Δ*E*+ZPE energy range. A geometry investigation of the pseudorotation phase angle P, and maximum amplitude of puckering $_{max}$, was carried out.

(2) With the exception of the *syn* forms, the global minimum in all the aminocarbonylphosphonates compounds under study corresponds to a high-*anti* orientation and with values of the ϕ and η torsional angles ca. 87°, γ ca. -178°, β ca. -96°, α ca. -140°, θ ca. 97°, and ρ ca. 177°. The pseudorotational angle P is ca 10° corresponding to an envelope ^{3}E form. By contrast, in NK molecule it is in *anti* orientation due to it is stabilized by the intramolecular H-bond O6'-H···O4', as well as in AZT due to in this molecule it is stabilized by a contact between O5' and H6 of the thymine ring.

(3) The nature of the substituent on the terminal $N7H_2$ amino group has little effect on the molecular structure and NBO atomic charges. However, the orientation of this substituent in several conformers has a remarkable influence in the geometry and charges of the molecule due to intramolecular H-bonds/contacts through the aminocarbonyl group. The phosphonate substituent increases the dipole moment μ of the AZT molecule, and the value is twice incremented with the aminocarbonyl substituent.

(4) In NK the number of N-type conformers is almost twice that S-type. However, in the aminocarbonylphosphonate molecules under study it is reverse. The most stable conformer is N-type, which is the orientation appropriated for a better interaction with the reverse transcriptase enzyme. The value of ν_{max} similar to that of AZT, and other parameters indicate the flexible nature of the molecules under study.

(5) The oxygen atoms of the phosphate moiety have the highest negative charge, i.e., they are the most reactive. The values of the charge of the azide group are very small, which appear difficult to participate in H-bonds with residues of the reverse transcriptase enzyme (RT). The azide group has also little influence in the stability of the structure.

(6) Several structure-activity relationships/tendencies were observed that can help for developing other aminocarbonlylphosphonate drugs with higher anti-HIV activity and lower toxicity. For this purpose, compounds with high dipole moment, large η and ϕ torsional angles, and small P values lead to a low ID_{50} concentration. A decrement in the negative value of χ, and small P values also leads to a decrease in the CD_{50} concentration.

The study of the molecular structure and physico-chemical properties may help to understand the behavior of this new class of prodrugs with reduced cytotoxicity and improved antiviral potency. Good comprehension of the parameters investigated here could be important for developing drugs with high anti-HIV activity and low toxicity.

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