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Structure and conformational analysis of the anti-HIV AZT 5′-aminocarbonylphosphonate prodrug using DFT methods

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ABSTRACT

A comprehensive theoretical conformational analysis of the anti-HIV AZT 5′-aminocarbonylphosphonate prodrug was carried out, due to this prodrug has noticeable advantage over approved drugs AZT and Nikavir. The whole conformational parameters (χ, γ, β, α, δ, ε, τ, P, ν_{max}) were analysed as well as the NBO Natural atomic charges. The calculations were carried out by means of B3LYP/6-31G** and B3LYP/6-311++G(3df,pd) DFT levels of theory with full relaxation of all geometrical parameters. The search located at least 86 stable structures, 6 of which are within a 1 kcal/mol electronic energy range of the global minimum and 11 conformers are within a 1 kcal/mol Gibbs energy range. The global minimum with the 6-311++G(3df,pd) basis set corresponds to the calculated values of the exocyclic torsional angles $\chi = -121.6°$, $\beta = 153.0°$, $\gamma = -152.0°$ and $\alpha = -74.1°$. The results obtained are in accordance to those found in related anti-HIV nucleoside Analogs. Comparisons of the conformers with those determined in the common anti-HIV drug AZT were carried out. Several correlations and general conclusions were emphasized.

1. Introduction

The progress toward the treatment of HIV infections has steadily increased in the past 2 decades [1]. Currently, more than 20 drugs have been approved for it [2]. 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.

Many different strategies have been developed in the search for therapeutic agents against AIDS. Actually, nucleoside Analogs play a crucial role in the current treatment of cancer and viral infections as the primary components of highly active anti-retroviral therapy (HAART). They need to convert with the help of cellular enzymes, into the respective nucleoside 5′-triphosphates (NTP) [3]. The formation of triphosphates occurs in a stepwise manner and usually the first phosphorylation step, that is, the synthesis of nucleoside 5′-monophosphates, is the crucial step for NTP formation. Because phosphorylation is indispensable for biological activity, nucleoside Analogs that are poor substrate for phosphorylating enzymes are usually inactive [4]. Due to this, nucleoside Analogs lose their antiviral potency in nucleoside kinase deficient cells [5]. To by-pass this enzymatic monophosphorylation step, efforts were focused on delivery into the cell 5′-monophosphates of nucleoside Analogs [6–10]. Unfortunately, under physiological conditions, they exist as dianions and cannot cross negatively charged cell membranes [11]. Hence, it was assumed that if a phosphate moiety of mononucleotide is properly masked and became neutral, this should facilitate cell membrane penetration and increase concentration of drug inside the cell. This idea, called pronucleotide approach, triggered studies on various types of nucleotide derivatives, whose intracellular conversion to the desired nucleoside 5′-monophosphates would occur via chemical and/or enzymatic hydrolysis of the phosphate masking groups. Therefore, phosphonate-containing drugs appear as important agents for anticancer and antiviral therapy, and they are increasingly being explored in other therapeutic areas [8].

Another feature is that, the compounds containing unsaturated ribose ring structure, with lack 2′- and 3′-hydroxyl groups, belong to the most effective alternative substrates of the reverse transcriptase enzyme of HIV virus. The most common anti-HIV drug (zidovudine, AZT) has the disadvantage of its toxicity and a short half-life in the organism. Based in both features with the introduction of a H-phosphonate group into the AZT 5′ position resulted in significant improvement of its therapeutic properties (high anti-HIV activity and low toxicity) and allowed a new group of anti-HIV drugs, AZT 5′-monophosphonates. Synthesis and biological activity of these compounds have been recently studied [1,12–26] and they have been subject of several reviews [9,10,27]. One molecule in this group is AZT 5′-H-phosphonate (Nikavir®, phosphazide), which has been approved recently in Russian Federation for the prevention and treatment of AIDS [1,9,28,29]. This molecule is ca. 4 times less toxic than AZT, but one order of magnitude less effectively than AZT [9].

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In another attempt of improve the AZT efficacy and reduce the toxicity, new phosphonates have been prepared with related activity and at least ten times less toxic than AZT and Nikavir [12]. Among these molecules, Khandazhinskaya et al. [9] have emphasize on the aminocarbonylphosphonate called **18f** (or **IIf** by Jasco et al. [12], or **I** again by Khandazhinskaya et al. [1]) by its "excellent potential as an alternative to AZT". This molecule has been proved to be considerable less toxic in mice and offered an obvious advantage over approved drugs AZT and Nikavir. Thus, due to its recently great interest, it is studied in the present work. For simplicity and avoid confusions we called it here as ACP-f.

Studies have been carried out with the aims of establish the relationship between structure, conformational features or physicochemical properties and activity of the nucleoside Analogs. Thus, it has been reported that anti-HIV-1 activity depends on ribose conformation [30], and differences in the ribose lead to appreciable changes in positions of the thymine ring and the C5′—OH group [31]. From our understanding would be interesting to analyse the different conformational possibilities for ACP-f, and compare the results with the single AZT nucleoside analog and with the nucleoside natural thymidine (*T*). 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 Analogs nucleosides have been analyzed by different authors [32–44], and we have reported in previous works the effect of the hydration on the two most stables conformers of D4T [41] and AZT [42], and in their tautomeric forms [45]. Now, an extensive theoretical study of the conformational preferences in ACP-f has been carried out with full relaxation of all geometric parameters, in an attempt to gain insights into molecular features responsible for activity. We will attempt to determine herein, if the various geometric features in ACP-f are correlated or interact with one another. We are also interested in whether alternative forms of hydrogen bonding make significant contributions to the conformational behavior of ACP-f. Opportunities appear for hydrogen bonding involving the thymine moiety with the oxygen atoms of the phosphonate chain. By the novelty of this drug, structural studies have not been reported yet.

2. Computational details

Calculations were carried out using the B3LYP density functional method (DFT), implemented in the GAUSSIAN 03 program package [46]. The UNIX version with standard parameters of this package was used. DFT methods provide adequate compromise between the desired chemical accuracy and heavy demands put on computer time and power. Moreover, DFT methods have been used satisfactory in many studies of drug design [33,37,41,42,45,47–49]. Several basis set were used starting from 6-31G** to 6-311++G(3df,pd), but the 6-31G** represents a compromise between accuracy and computational cost, and thus it was the base set selected as reference for all of the calculations. The B3LYP method was chosen because different studies have shown that the data obtained with this level of theory are in good agreement with those obtained by other more cost computational method as MP2 and it predicts vibrational wavenumbers of DNA bases better than HF and MP2 methods [50–54]. Moreover, because of the high size of ACP-f, MP2 calculations with the 6-31G** basis set was not possible for memory computer problems in the alpha computer of the Computational Center from University Complutense of Madrid, in which all quantum mechanical computations were performed.

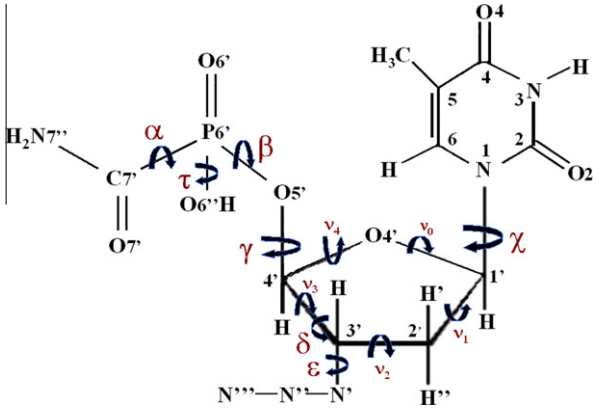
The 3D Potential Energy Surface (PES) of this molecule was determined by rotation of the torsional angles χ (glycosidic bond), γ (C4′—O5′ bond), β(O5′—P bond) and α(P—C7′). These dihedral angles simultaneously held fixed at values varying between 0° and 360° in steps of 60° in a first study, as we have carried out on D4T molecule [55]. All other geometrical parameters were relaxed during these optimisations. 86 optimized geometries were obtained in this step by minimizing the energy with respect to all geometrical parameters without imposing molecular symmetry constraints. Berny optimization under the TIGHT convergence criterion was used. Atomic charges were determined with the Natural NBO procedure [56,57].

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 nuclear displacement. Wavenumber calculations were performed on all optimized conformers to confirm that they corresponded to local minima. All optimized structures showed only positive harmonic vibrations (local energy minima). Relative energies were obtained by adding zero-point vibrational energies (ZPEs) to the total energy. For the calculation of the ZPEs, the wavenumbers were retained unscaled. The ΔG values were sums of electronic and thermal free energies.

3. Results and discussion

3.1. Definition of conformational angles

Following the Saenger's notation [58], the atomic descriptions of this molecule as well as the most important exocyclic and endocyclic torsional angles are defined in Scheme 1. The conformation in ACP-f can be characterized by the following seven important structural parameters: (i) the glycosylic torsional angle, χ (O4′—C1′—N1—C2), which determines the two orientations of the base relative to the furanose ring, denoted as the *anti* and *syn* conformations; (ii) the torsional angle δ (O5′—C4′—C3′—N′) which describes the orientation of the O5′ atom relative to the furanose ring; (iii) the exocyclic torsional angle γ (P—O5′—C4′—C3′) which shows the orientation of the 5′-phosphonate group relative to the furanose ring. This ring is twisted out-of-plane in order to minimize non-bonded interactions between their substituents; (iv) the exocyclic torsional angle β (O6″—P—O5′—C4′) describing the orientation of the hydroxyl oxygen O6″ in the phosphonate moiety; (v) the torsional angle α (O5′—P—C7′ = O7′) defining the orientation of the carbonyl oxygen O7′ in the phosphonate moiety; (vi) the torsional angle ε (C2′—C3′—N3′—N3″) which determines the orientation of the azide moiety relative to the furanose ring; (vii) The torsional angle τ (O5′—P6′—O6″—H) describing the orientation of the hydroxylic group. Finally, (viii) the furanose pucker *P* defined in the bottom of Table 1.



Scheme 1. Molecular structure and definition of the exocyclic and endocyclic angles in ACP-f prodrug.