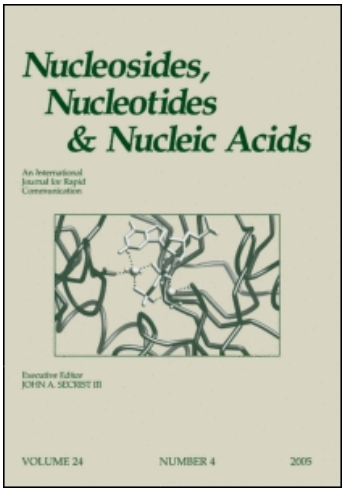

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Structural Study of Two 5-Heteroaromatic-2' deoxyuridines and Their 5-Bromine-heteroaromatic Analogues: Theoretical Conformational Analysis and NMR Experiments

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STRUCTURAL STUDY OF TWO 5-HETEROAROMATIC-2'-DEOXYURIDINES AND THEIR 5-BROMINE-HETEROAROMATIC ANALOGUES: THEORETICAL CONFORMATIONAL ANALYSIS AND NMR EXPERIMENTS.

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ABSTRACT

We have previously described that in the crystalline structure, the five membered rings of 5-thienyl and 5-furanyl 2'-dUrd have a different orientation according to the heteroatom involved (S or O). NOE difference NMR experiments were conducted in order to verify this conformation in solution. A theorical conformational analysis was further computed at the *"ab initio"* STO-3G level, to characterize the energetic profile. This comparative study realized on some 5-heteroaromatic-2'-deoxyuridines confirms the crystallographic results.

INTRODUCTION

The salvage pathway enzyme, thymidine kinase, is encoded by several herpes viruses including herpes simplex virus type 1 (HSV-1), which is usually transmitted orally and causes herpes labialis or occular disease. A number of nucleoside analogues are known that inhibit herpes simplex virus replication[1,2], and the most effective appear to share a common mechanism of action involving activities of two virus-encoded enzymes: thymidine kinase (TK) and DNA polymerase. Attempts to design antiherpes agents by a rational approach have not been very successfull till now. One of the reasons for that is the above mentioned requirement of nucleoside to be metabolised in order to exert their antiviral activity.

TK catalyses the phosphorylation of thymidine to provide dTMP, the first precursor leading to the triphosphate (dTTP) necessary for HSV-1 DNA synthesis. DNA polymerase catalyses the replication of the viral genome[3,4]. The HSV-1 thymidine kinase is particularly

tolerant to pyrimidine 2'-deoxynucleosides substituted at the 5-position of the base. Many such compounds show antiherpes activity, i.e. 5-iodo-2'-deoxyuridine (ACV), known for over 30 years. One of the most potent HSV-1 inhibitors *in vitro* and *in vivo* is *(E)* -5-bromovinyl-2'-deoxyuridine[5]. These nucleoside analogues are converted to their mono-, di- and triphosphorylated forms by viral and/or cellular kinases. These 5'-triphosphorylated forms are active intracellularly[5-7].

The replacement of the bromovinyl substituent by several 5-membered heterocycles yielded some series of selective anti-HSV-1 compounds. The 5-(2-thienyl)- and 5-(2-furanyl)-2'-deoxyuridine have been shown to have similar antiviral properties[8]. Subsequently new analogues have been synthesized[9-12]. The potent activity and high selectivity of some of these compounds make them promising antiviral agents for the treatment of HSV-1 infections.

Four 5-substituted pyrimidine nucleoside analogues are studied in this paper (figure 1): the 5-(thien-2-yl)-2'-deoxyuridine (**I**), the 5-(5-bromothien-2-yl)-2'-deoxyuridine (**III**), the 5-(furan-2-yl)-2'-deoxyuridine (**II**) and the 5-(5-bromofuran-2-yl)-2'-deoxyuridine (**IV**). Structures were obtained by X-Ray diffraction and have been reported previously[13,14]. Structural study of all compounds revealed the presence of two different conformations. Thienyl compounds have the sulfur atom in the vicinity of the carbonyl group of the uracil ring. This conformation is planar, which is probably due to the formation of a S-O interaction. On the contrary, the substituent in the 5-furanyl analogues is oriented in such a way that the oxygen atoms point away from each other.

To have an idea of the energetic profile, some conformational energies were computed at the *"ab initio"* STO-3G or STO-3G* levels. Some NMR experiments were conducted to further confirm the calculated conformations in solution. Crystallography, theory and NMR results are compared in this paper.

MATERIAL AND METHODS

Chemistry

Compounds used in this study are depicted in figure 1. Their synthesis[8,9], their affinity against the HSV-1 thymidine kinase and their antiviral activity (Table 1) [11,12] have been reported before. Two thienyl and furanyl compounds were compared in this paper because they present an important difference in their conformation.

Quantum mechanicals calculations

A theorical conformational analysis was realized with optimisation procedure in the framework of the RHF-LCAO-MO-SCF (Restricted Hartree Fock-Linear Combination of Atomic Orbitals-Molecular Orbital-Self Consistent Field) formalism. The *ab initio*

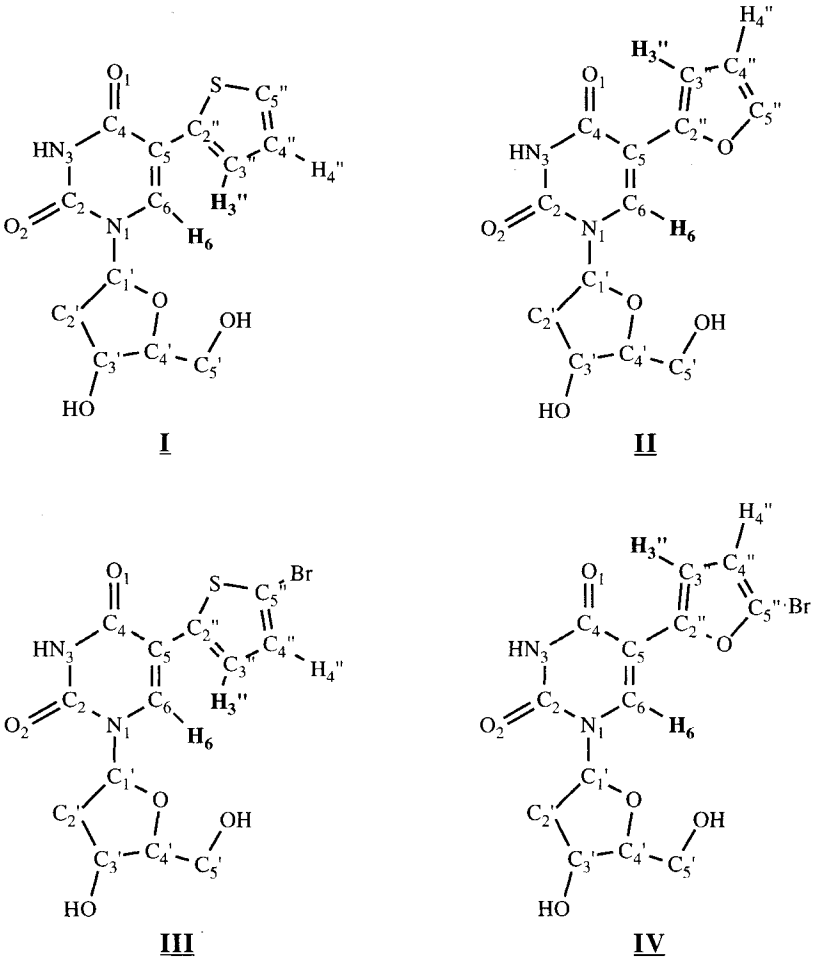

FIGURE 1 : **I**: 5-(thien-2-yl)-2'-deoxyuridine; **II**: 5-(furan-2-yl)-2'-deoxyuridine; **III**: 5-(5-bromothien-2-yl)-2'-deoxyuridine; **IV**: 5-(5-bromofuran-2-yl)-2'-deoxyuridine

TABLE 1
Antiviral activity and affinity values for HSV-1 thymidine kinase.

compounds	HSV-1 TK IC_{50} (μM.)	HSV-1 (KOS) ED_{50} (μg/ml)
5-(thien-2-yl)-2'-dUrd (**I**)	2.4	0.45
5-(furan-2-yl)-2'-dUrd (**II**)	2.9	0.49
5-(5-bromothien-2-yl)-2'-dUrd (**III**)	3.5	0.03
5-(5-bromofuran-2-yl)-2'-dUrd (**IV**)	36	20

dUrd = deoxyuridine

molecular orbital STO-3G method (or STO-3G* in the presence of a sulfur atom), which has proven to be convenient for analysis of series of analogues[13], has been used to scan the conformational space around the C_5-C_2" bond of the four compounds. The atomic coordinates of the atoms were taken from the crystallographic data previously published[14,15]. However the deoxyribose moiety was replaced by a methyl group in order to reduce the size of the molecule and to avoid time consuming calculations. The C_3"-C_2"-C_5-C_4 torsion angle (or S-C_2"-C_5-C_4 in the case of thienyl compounds) was modified in steps of 15° from -180° to 180°. The energy was computed for 24 conformers. These computations were performed on a RISC6000 computer system using the GAUSSIAN92 program[16].

NMR

^{1}H-NMR spectra have been performed on a VARIAN unity 500 spectrometer (500 MHz). The spectra were measured in DMSO-d_6 with TMS as internal standard (δ = 0.00 ppm) at 306°K. The one-dimensional differential NOE experiments were performed with 15 sec of irradiation time using a decoupling power of 1 dB.

RESULTS

Theoretical conformational analysis

As the crystalline structure of thienyl and furanyl analogues is different, and as crystal packing influences this conformation, we scanned the conformational space around the C_2"-C_5 bond.

The energetic profile of compounds **I** and **III** is presented in figure 2. The most stable conformation (S-C_2"-C_5-C_4 ≅ -15° for **I** and ≅ 0° for **III**) corresponds to the one observed in the crystalline state (S-C_2"-C_5-C_4 ≅ -5.3° for **I** and ≅ 14° for **III**). The conformation in which S is in close proximity to O_1 is in fact 2.26 kcal.mol^{-1} more stable for **I** and 2.13 kcal.mol^{-1} for **III** compared to the conformation presenting an angle of 180°. The stability around the C_2"-C_5 bond is probably due to a S-O electrostatic interaction which leads to the formation of a pseudo-five-membered ring.

For the furanyl analogues (**II** and **IV**), the variation of total relative energy has the same profile (Figure 2). The minimum energy conformation for **II** and **IV** is again at around 0° of the torsion angle C_3"-C_2"-C_5-C_4. These results corroborate with crystallography (C_3"-C_2"-C_5-C_4 ≅ 1.8° for **II** and ≅ 5.7° for **IV**). The two oxygen atoms (of the heterocycle in the 5-position and of the carbonyl group at C_4) tend to point away from each other as much as possible. The conformation at 180° informs about an energy difference of 3.7 kcal.mol^{-1} for **II** and 4.33 kcal.mol^{-1} for **IV**, in comparison with the crystalline structures. A dihedral angle of 90° leads to a maximum in energy for all compounds.

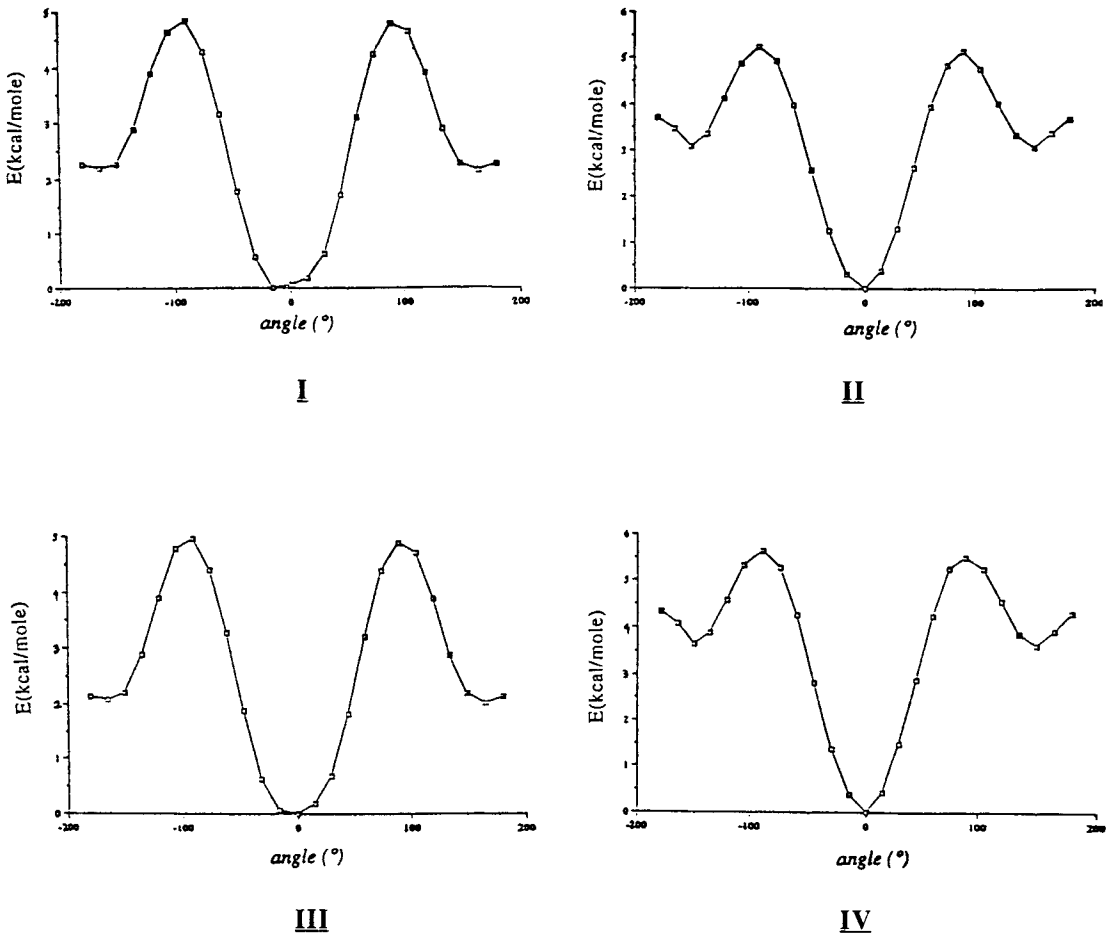

FIGURE 2 : Total energy variation (kcal.mol^{-1}) vs dihedral angle S-C_2"-C_5-C_4 (°) for **I** and **III** and vs dihedral angle C_3"-C_2"-C_5-C_4 (°) for **II** and **IV**.

NMR study

The observation that the thienyl and furanyl 5-membered ring is oriented differently according to the nature of the heteroatom involved (S or O) prompted us to further study the conformation adopted by the compounds in solution. The four compounds were therefore submitted to a 1-D Nuclear Overhauser Enhancement (NOE) study to test if there is an interaction between H_6 and H_3" atoms, at least for the thienyl analogues (Figure 1). In fact, if the 5-membered ring at C_5 is oriented as to put the heteroatom (S or O) on the same side as the O_1, then H_3" would itself be in proximity of H_6. This would give rise to a

TABLE 2
NOE enhancements (%) observed for H_6 in DMSO for the different compounds studied, upon irradiation of H_3''.

compounds	H irradiated (ppm)	increase of H_6(%)	increase of H_4''(%)
5-(thien-2-yl)-2'-dUrd (**I**)	H_3'' (7.40)[a]	7.8	11.7
5-(furan-2-yl)-2'-dUrd (**II**)	H_3'' (6.86)	< 0.3	8.5
5-(5-bromothien-2-yl)-2'-dUrd (**III**)	H_3'' (7.21)	6.8	b
5-(5-bromofuran-2-yl)-2'-dUrd (**IV**)	H_3'' (6.83)	< 0.4	8.1

dUrd = deoxyuridine

a H_5'' (7.45) is also partly saturated; b H_4'' (7.15) is also partly saturated upon irradiation at H_3''

significant NOE effect on H_6 upon irradiation of H_3'' or vice-versa (Table 2). On the other hand, if the ring is oriented otherwise, H_3'' would then be too far from H_6 to give rise to a non observable NOE effect.

The 90 MHz NMR spectra of these compounds have in fact been published already before[2], and the assignments given there are nicely confirmed here in the 500 MHz spectra. Especially the important differentiation between H_3'', H_4'' and H_5'' was now firmly established not only by the different coupling pattern for H_4'' (in **I** and **II**), but also by H,C-heterocorrelation experiments. The corresponding ^{13}C chemical shifts, at least for the furanyl compounds, are characteristic and well distributed among each other and thus easily assigned. For the thienyl derivatives, the NOE result itself is of diagnostic value for the peak assignments. Table 2 shows the NOE data obtained for the different compounds studied upon irradiation of H_3''. This clearly indicates that the thienyl compounds also adopt preferentially in solution a conformation where H_6 and H_3'' are close to each other. For the furanyl derivatives the conformational equilibrium is shifted almost completely to that conformation where H_6 and H_3'' are relatively far away from each other, and show almost no significant NOE interaction anymore. The existence of significant NOE enhancements between H_6, H_2' and H_3' further indicates that the orientation around the glycosyl bond shows a pronounced preference for anti conformation, as is usually observed for pyrimidine nucleosides.

DISCUSSION

It is clear from the results that a NOE effect could be detected only for both the thienyl compounds and not for the furanyl derivatives. This indicates that the five-membered ring

containing the heteroatom S is oriented so as to make possible an S---O interaction (about 2.8 Å) with O_1, that leads to a shorter distance than a normal van der Waals contact (3.35 Å).

A theoretical study on thiazole nucleosides[17,18] showed that this interaction results from an attractive electrostatic interaction between the positively charged sulfur and the nucleophilic oxygen. The positive charge on the sulfur atom is produced by the electron-withdrawing environment of the conjugated ring. Donation of electrons to the π system of the ring leaves a significant net positive charge on the sulfur.

This kind of intramolecular close contact was shown to be not uncommon during a systematic search in the crystallographic Cambridge Structural Database[18], which suggests that sulfur-nucleophile attractions are an important class of nonbond intramolecular interactions.

In conclusion, NMR experiments as well as a theoretical conformational analysis of the 5-heteroatomic-2'-dUrd in solution confirm the crystallographic observation. A S---O interaction stabilises the thienyl substitutent in the five position, forcing this substituent in a planar pseudo-five membered ring. On the contrary for the furanyl compounds, the substituent in the 5-position is oriented in such a way that the oxygen atom of the pyrimidine moiety and of the furanyl ring are pointing away from each other. The most stable conformation is again planar.

These results suggest that both type of compounds (5-thienyl and 5-furanyl substituted 2'-deoxyuridines) are bond in a different conformation to the herpes simplex virus type **I** thymidine kinase and that the structure-activity relationship of the furanyl compounds is different from that of the thienyl compounds.

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