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Conformational analysis of nevirapine, a non-nucleoside HIV-1 reverse transcriptase inhibitor, based on quantum mechanical calculations

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Summary

The structure and the conformational behavior of the HIV-1 reverse transcriptase inhibitor, 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b2′,3′-e][1,4]diazepin-6-one (nevirapine), is investigated by semiempirical (MNDO, AM1 and PM3) method, ab initio at the HF/3-21G and HF/6-31G** levels and density functional theory at the B3LYP/6-31G** level. The fully optimized structure and rotational potential of the nitrogen and carbon bond in the cyclopropyl ring were examined in detail. A similar geometrical minimum is obtained from all methods which shows an almost identical structure to the geometry of the molecule in the complex structure with HIV-1 reverse transcriptase. To get some information on the structure in solution, NMR chemical shift calculations were also performed by a density functional theory at the B3LYP/6-31G** level, using GIAO approximation. The calculated ^{1}H-NMR and ^{13}C-NMR spectra for the energy minimum geometry agree well with the experimental results, which indicated that the geometry of nevirapine in solution is very similar to that of the molecule in the inhibition complex. Furthermore, the obtained results are compared to the conformational studies of other non-nucleoside reverse transcriptase inhibitors and reveal a common agreement of the non-nucleoside reverse transcriptase inhibitors. The specific butterfly-like shape and conformational flexibility within the side chain of the non-nucleoside reverse transcriptase inhibitors play an important role inducing conformational change of HIV-1 reverse transcriptase structure and are essential for the association at the inhibition pocket.

Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are highly potent, relatively low in toxicity, and can specifically inhibit HIV-1 reverse transcriptase (HIV-1 RT). A number of pharmacologically active NNRTIs have been identified such as nevirapine [1], HEPT [2], TIBO [3] and alpha-APA [4]. Recently, crystal structures of HIV-1 RT and complexes with some different NNRTIs have been published [5–25]. These structures have provided a great deal of insight into the conformational flexibility of RT induced by the binding of NNRTIs. Furthermore, these crystallographic studies show that the chemically diverse class of NNRTIs have common features of binding to RT, and the similar butterfly shape of the considered inhibitors is essential for the association at the inhibition pocket [24–27]. The majority of HIV-1 RT inhibitors that act on the binding site show a pronounced dependence for their action on seemingly major changes in molecular conformation [28]. Accordingly, conformational investigations have been an important part of structure-activity relationship studies. This kind of investigation is necessary to derive meaningful information relevant to drug action because, in the case of flexible molecules, the receptor is likely to alter the solution conformation upon binding. Therefore, the relevance of information about a single preferred conformation of a drug molecule in solution to the conformational requirements of the receptor site may not be clear.

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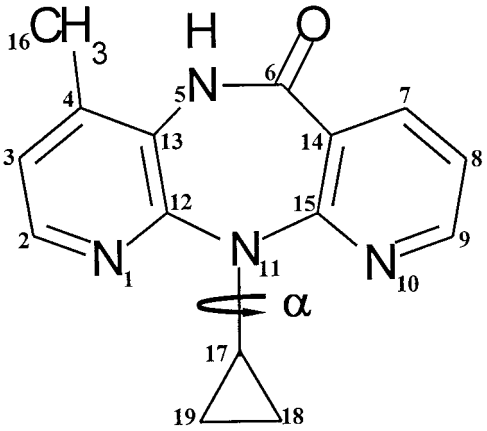
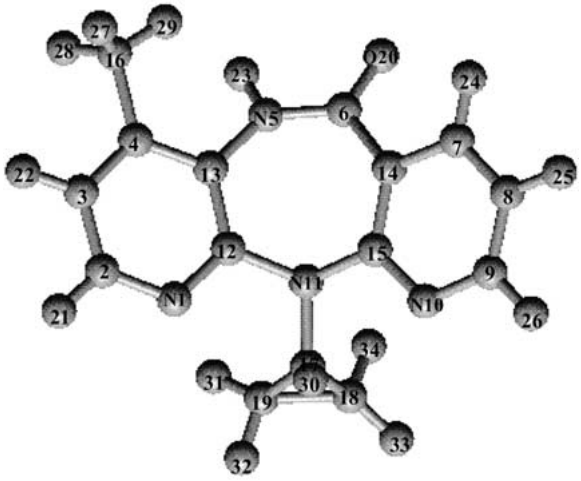


Figure 1. The chemical structure of nevirapine or 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido [3,2-b′,3′-e][1,4] diazepin-6-one and atomic numbering used in this study.



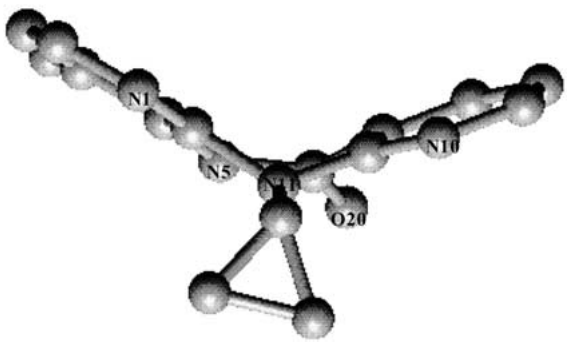


Figure 2. (a) 3-dimensional structure of nevirapine. (b) Butterfly-like shape of nevirapine.

To investigate the role of molecular structure of NNRTIs on HIV-1 RT inhibition, conformational analyses of the potent NNRTIs have been previously studied [29–32], based on quantum chemical calculations. The obtained results indicated that the NNRTIs such as HEPT and TIBO show flexibility in their structures with two pronounced energetic local minima. The energy barrier between both minima are not high, estimated to be 2–3 kJ/mol, which can be easily changed from one local minima to another. With this flexibility, the conformational change of reverse transcriptase is induced. In the present study, an attempt has been made on nevirapine or 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b2′,3′-e][1,4]diazepin-6-one (Figure 1). This inhibitor has already passed pre-clinical and clinical tests and is available on the market [33]. The structure of nevirapine together with the numbering of the atoms used in this investigation is given in Figures 1 and 2a. Particularly, the rotation of the cyclopropyl ring around the carbon nitrogen single bond (N11-C17) determine the conformational space of nevirapine and must be analyzed with respect to all energetically accessible conformations. It is found that alpha angle of cyclopropyl ring plays an important role to determine the structure of nevirapine, which has no other flexibility, and the butterfly inversion (Figure 2b) of the structure leads to a symmetry-related structure with the energy dependence on alpha.

Therefore, the structural energy minimum, corresponding to the conformation of nevirapine as obtained from X-ray investigations of the association complex, was calculated by quantum chemical calculations; semiempirical, ab initio and density functional theory (DFT) methods. Furthermore, the DFT at the B3LYP/6-311++G** level was used for the prediction of ^{1}H-NMR and ^{13}C-NMR spectra of nevirapine in order to conceptualize the structure of the molecule in solution. The detailed information about the three-dimensional structures of the inhibitor, based on theoretical investigation will be useful in structure-activity relationship studies and the design of new potent NNRTI compounds.

Methods

The starting geometry of nevirapine was obtained from X-ray crystallographic data at 2.2 Å resolution of the enzyme-inhibitor complex structure (1vrt.pdb) [4, 34]. First, full geometrical optimization was performed, based on semiempirical (MNDO, AM1, PM3) method, ab initio at the HF/3-21G and HF/6-31G** level and DFT at the B3LYP/6-31G** level. These calculations were done by the GAUSSIAN98 program [35], running on an IBM/SP2 computer. The obtained geometric parameters of torsion angles was compared with the X-ray data. Second, as an examination of potential energy surface with respect to torsion angles is more informative in order to understand the conformations of nevirapine in the gas phase than just locating the global minimum, the rotational barrier bond between the nitrogen of the tricyclic system and the carbon atom of the cyclopropyl group (C15-N11-C17-C19, α) with a stepsize of 30 deg was considered and used in this study. Keeping the α angle constant, the automated geometry optimization was performed for the rest of the molecule by the individual methods. Theoretical calculations were compared to the experimental geometry, in particular to X-ray diffraction data of the reverse transcriptase and nevirapine complex, as available from the PDB (1vrt.pdb). The ^{1}H-NMR and ^{13}C-NMR chemical shifts of nevirapine were calculated by the Gauge Invariant Atomic Orbitals (GIAO) [36] method using B3LYP/6-311++G** level of theory on the structure, optimized at B3LYP/6-31G** level. Tetramethylsilane (TMS) was used as reference compound to get the chemical shifts in NMR experiment, therefore, the ^{1}H and ^{13}C NMR spectra of this compound was also calculated by the same method, described above for nevirapine. The ^{1}H and ^{13}C NMR chemical shifts of nevirapine were subtracted by those of TMS to obtain the predicted NMR spectra and compared to the experimental values.

Results and discussion

Geometrical calculations and conformational analysis of nevirapine

The conformation of nevirapine was fully optimized, based on different methods. The obtained structural parameters with the lowest energy conformation calculated by semiempirical methods and at the HF/3-21G and HF/6-31G** levels and at the B3LYP/6-31G** level were compared with X-ray diffraction data of nevirapine in the complex with RT. The CHARMM force field was used to refine the structure with resolution of 2.2 Å. The compared structural parameters obtained by each methods are presented in Table 1. The indications are that all considered methods provide sufficiently good results for the torsion angles. As expected, the standard deviations are significantly smaller for results obtained by ab initio and density functional theory calculations than for those obtained by the semiempirical methods (Table 1). There is only PM3 method that provides standard deviation comparable to those of ab initio and DFT. It is generally known that the HF/3-21G and HF/6-31G** levels lead to a good geometry close to the results of the accurate B3LYP/6-31G** level of theory.

The dihedral angles which determine the position of the cyclopropyl ring (C15-N11-C17-C19, α) are similar for all calculations and differ slightly from that obtained by the X-ray investigation. Superimposition of the lowest energy conformation calculated at the B3LYP/6-31G** level on the crystal structure of nevirapine in the complex provides an agreement (root mean squares deviation of 0.07) for the side chain, but only relatively small deviations of the positions of the cyclopropyl ring.

For the determination of the other conformational minima of nevirapine, the rotational potentials of the dihedral angles α were analyzed by semiempirical methods (MNDO, AM1 and PM3), ab initio at the HF/3-21G and HF/6-31G** levels and additionally by DFT at the B3LYP/6-31G** level. The dependence of the energy on the dihedral angle α, calculated by different semiempirical methods, is depicted in Figure 3. From this figure, it can be seen that all semiempirical methods (MNDO, AM1 and PM3) lead to almost the same conformational minimum when the dihedral angle α is equal to 218.47°. Moreover, considering the analogous diagram for the results of the ab initio calculations at the HF/3-21G and HF/6-31G** levels and DFT at the B3LYP/6-31G** level, it shows the same energy minima at $\alpha = 218.47°$. From these results, it can be concluded that the dihedral angle α is restricted to one minima at this torsion angle. Considering the position of the energy minima in more details and by comparing to the experimental values obtained by X-ray investigation ($\alpha_{exp} = 208.48°$), it can be seen that the calculated values are nearly the same as the experimental one. This is an indication for the fact that the

Table 1. Selected torsion angles of nevirapine, obtained by different methods and compared to experimental X-ray crystallographic data

	X-ray[a]	Semiempirical			*Ab initio* (HF)		B3LYP
		MNDO	AM1	PM3	3-21G	6-31G**	6-31G**
Torsion angle (deg)							
O20-C6-C14-C7	30.0	46.2	34.8	26.4	21.1	24.1	21.2
C12-N11-C17-C19	68.7	74.7	78.2	76.9	69.6	73.2	71.2
C12-N11-C17-C18	136.8	143.3	147.4	144.9	137.7	142.0	139.9
C14-C15-N11-C17	168.3	147.3	154.1	159.2	154.8	157.0	159.9
C13-C12-N11-C17	200.1	210.6	206.6	205.9	208.3	206.9	205.4
C15-N11-C17-C19	208.5	216.4	211.3	213.2	220.6	216.4	217.4
C15-N11-C17-C18	276.6	248.9	280.5	281.2	288.7	285.1	286.1
C6-N5-C13-C4	144.4	149.2	144.6	130.5	135.8	131.1	134.8
N5-C6-C14-C7	212.9	228.7	217.5	204.0	202.2	204.1	201.1
C15-N11-C12-N1	238.8	246.4	249.9	246.2	237.6	243.4	237.9
C12-N11-C15-N10	126.7	112.3	111.7	119.3	127.4	122.1	127.9
SD[b]		12.5	8.47	8.3	9.0	8.3	7.7

[a]Data obtained from refs. [4] and [34] with resolution of 2.2 Å.
[b]Standard deviation (SD) $= [\Sigma(x_{Cal.} - x_{Expt.})^2/n - 1]^{1/2}$.

conformation of nevirapine in the inhibition complex is rather close to its energy minimum conformation.

All possible alpha conformations were calculated*, including the inverted butterfly-like shape. The energy barrier of all conformations indicated that there is only one minimum at the alpha torsion angle equals to 218.47 deg which is possible to have two different conformations by the flipped conformation of one another. However, the conformation of nevirapine in the complex structure is found to be equivalent with the one energy minimum investigated in this study. The conformation that the two pyridine rings pointed down is very high energy and not possible to exist.

Calculated ^{1}H-NMR and ^{13}C-NMR chemical shifts of nevirapine

In order to get more information on the geometry in solution, the NMR spectra of nevirapine were calculated for the structural minimum and compared to the experimental ^{1}H-NMR and ^{13}C-NMR chemical shifts [37, 38] which reported that the error on chemical shifts is about 0.05. For the calculations, the recent tests of NMR computational methods [36, 39, 40] are limited to ^{13}C and other heavy atoms. The errors for ^{1}H-NMR chemical shifts calculated at the HF/3-21G level was reported to be one order of magnitude lower than ^{13}C errors at the same level of theory [36]. The NMR calculations typically benefit from and accurate geometry and a large basis set such as at the B3LYP/6-311++G(2d,p) level of theory [39]. Moreover, for larger basis sets, errors for ^{1}H chemical shifts consistently could be less than 1 ppm and sometimes less than 0.10 ppm [40]. Therefore, in this study, the chemical shifts were calculated at the B3LYP/6-311++G** level and used the corresponding B3LYP/6-31G** level for optimized structure. The comparison of chemical shifts between experimental and calculated ^{1}H-NMR and ^{13}C-NMR are presented in Tables 2 and 3 and also plotted in Figures 4 and 5, respectively. The correlation coefficients of the plot, $r^2 = 0.9974$ for ^{1}H-NMR and $r^2 = 0.9988$ for ^{13}C-NMR, show an excellent agreement between experimental and predicted chemical shifts. The comparison of the experimental and calculated chemical shifts favors the presence of a conformation in solution which is consistent with the optimized geometry at a dihedral angle α of about 218.47 deg. As expected, it is found in this study that there is well agreement between chemical shifts obtained by B3LYP/6-311++G** level, except the ^{1}H chemical shift of H23 (Figure 2a) atom attached to nitrogen atom of 7-membered ring. As this proton is an acedic proton, thus, hydrogen bonding can strongly influence on the electronic environment of this proton with oxygen of dimethyl sulfoxide solvent [37], and hence, it is not possible to predict the chemical shift of this proton.

*unpublished data.

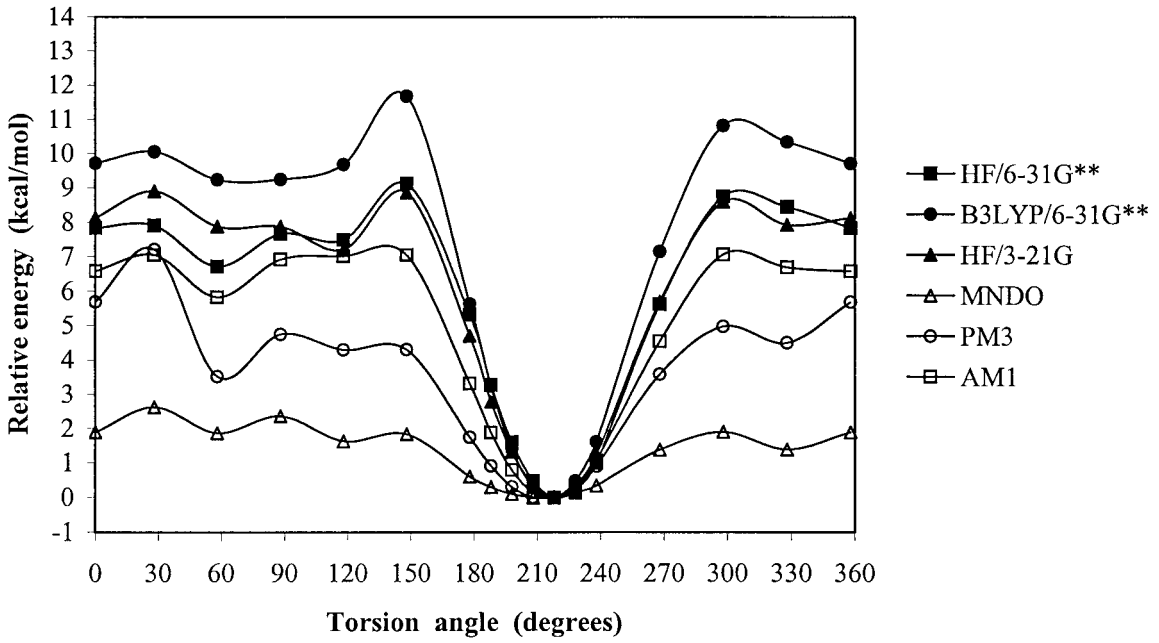


Figure 3. The rotational potential (kcal/mol) of dihedral angle, α, obtained from semiempirical (MNDO, AM1 and PM3), ab initio at the HF/3-21G and HF/6-31G** levels and density functional theory at the B3LYP/6-31G** level.

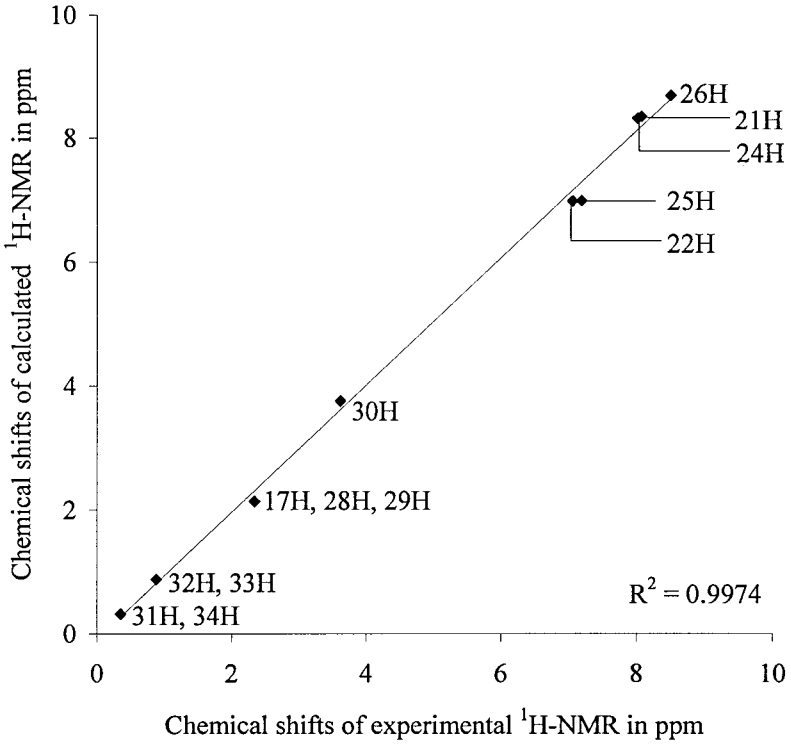


Figure 4. Plot of calculated and experimental chemical shifts of ^{1}H-NMR.

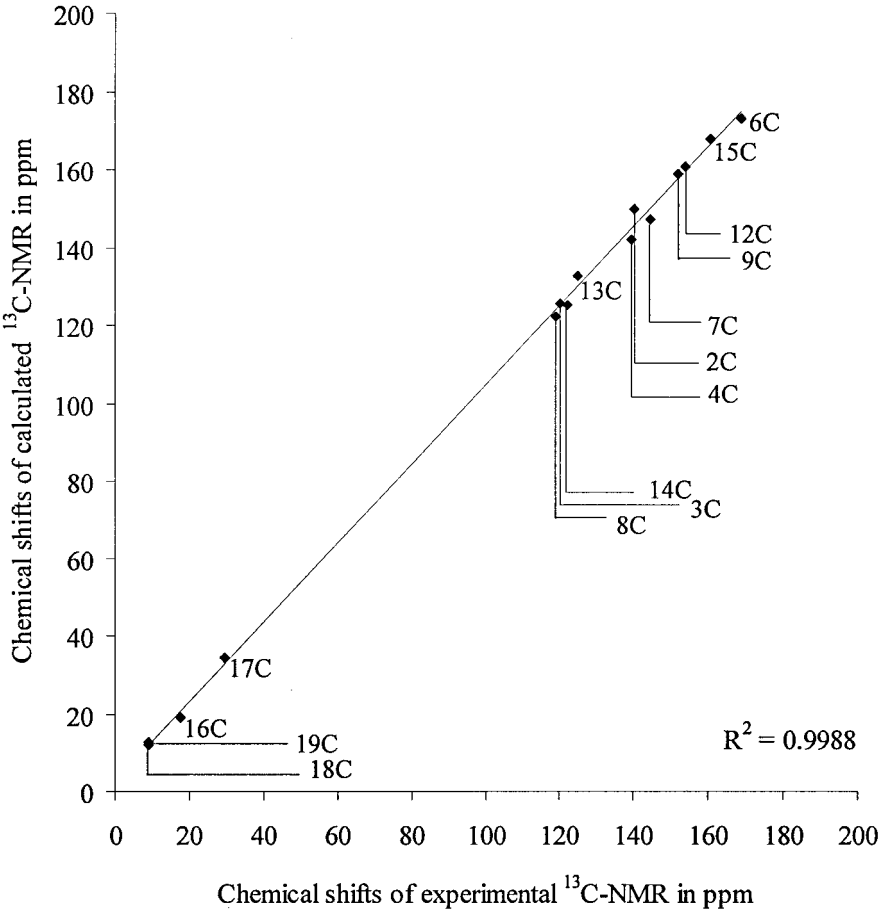


Figure 5. Plot of calculated and experimental of chemical shifts of ^{13}C-NMR.

Table 2. The comparison of experimental and calculated ^{1}H-NMR chemical shifts, δ (ppm)

Position of proton	Expt. δ	Cal. δ[a]	Residual
21H	8.08	8.35	−0.27
22H	7.07	6.98	0.09
24H	8.02	8.32	−0.30
25H	7.20	6.99	0.03
26H	8.51	8.69	0.18
27H, 28H, 29H	2.34	2.14	0.20
30H	3.62	3.76	0.14
31H, 34H	0.35	0.32	0.03
32H, 33H	0.88	0.88	0.00
SD[b]		0.20	

[a]At the B3LYP/6-311++G** level, using the B3LYP/6-31G** optimized structure.
[b]Standard deviation
$(SD) = [\Sigma(x_{Cal.} - x_{Expt.})^2/n - 1]^{1/2}$.

Based on our previous investigation on the conformational analysis of HEPT and TIBO [29–31], it was found that there are two energy minima of each structure. The main reason is due to the number of rotatable single bonds and the flexibility of the inhibitor structures. In both compounds, the comparison of calculated ^{1}H-NMR spectra with experiment indicates that the preferable minimum of each compound corresponds to the geometry of the molecules in the association complex. However, it was found that, for TIBO, the nitrogen inversion effect plays an important role on conformational change and gives the butterfly-like shape of TIBO similar to the complex structure with HIV-1 RT. Taken into account, it can be concluded that, in solution, nevirapine and some other NNRTIs exist in one conformation which corresponds to the similar structure of molecule existing in the complex.

Table 3. The comparison of experimental and calculated ^{13}C-NMR chemical shifts, δ (ppm)

Position of proton	Expt. δ	Cal. δ[a]	Residual
2C	140.36	149.91	9.55
3C	120.35	125.57	5.22
4C	139.52	142.12	2.60
6C	169.06	173.05	3.99
7C	144.47	147.18	2.71
8C	118.99	122.10	3.11
9C	152.15	158.83	6.68
12C	154.17	160.74	6.57
13C	124.97	132.83	7.86
14C	122.13	125.23	3.10
15C	160.73	167.75	7.02
16C	17.86	18.88	1.02
17C	29.65	34.35	4.70
18C	8.88	12.65	3.77
19C	9.15	12.13	2.98
SD[b]		5.43	

[a]At the B3LYP/6-311++G** level, using the B3LYP/6-31G** optimized structure.
[b]Standard deviation
$(SD) = [\Sigma(x_{Cal.} - x_{Expt.})^2/n - 1]^{1/2}$.

Conclusions

The performance of the conformational analysis of nevirapine was based on quantum chemical calculations which were investigated by semiempirical, *ab initio* and density functional theory methods. The pronounced energetic structure concerns the dihedral angle α. The conformational minima appear for a dihedral angle α equals to 218.47 deg. Based on density functional theory at the B3LYP/6-31G** level, the local minima structure, shows an almost identical structure as the geometry of the molecule in the complex with HIV-1 RT. The calculated ^{1}H-NMR and ^{13}C-NMR spectra for the energy minimum geometry agree well with the experimental result which indicated that the geometry of nevirapine in solution is very similar to that of the molecule in the inhibition complex.

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