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Investigation of the Binding Affinity between Styrylquinoline Inhibitors and HIV Integrase Using Calculated Nuclear Quadrupole Coupling Constant (NQCC) Parameters (A Theoretical ab initio Study)

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In this work, the calculated nuclear quadrupole coupling constants of ^{17}O in some styrylquinoline conformers were presented. The calculations were carried out to find the relationships between the charge distribution of styrylquinolines and their pharmaceutical behavior and to explore the differences among the electronic structures of some conformers of these potent HIV IN inhibitors. Furthermore, the HIV IN inhibitory of **R1** and **R2** rotamers was compared. On the basis of our results: - Charge density on oxygen atoms of carboxyl moiety has a dominant role in the drug activity. - The **a** conformer in which a divalent hydrogen atom is a link, has more capability in antiviral drug treatment. - The **R1** conformer, as a $\mathbf{Mg}^{+2}$ chelating agent, is better than **R2** conformer and thus it is more inhibitor of HIV IN.

Key Words: AIDS, Charge density, HIV, NQR, Styrylquinoline

Introduction

The Acquired Immunodeficiency Syndrome (AIDS) is a major epidemic with more than 33 million infected people worldwide.[1] Its etiological agent has been identified as human immunodeficiency virus type 1 (HIV-1). Current approved therapies target four steps of the HIV life cycle (fusion, reverse transcription, integration and proteolytic maturation).[2,3]

Theoretically, an anti-HIV agent may exert its activity by inhibiting a variety of steps in the life cycle of the virus. However, medicinal chemists have focused their attention predominantly on the following stages: Viral binding to target cells, Virus cell fusion, Virus uncoating, Reverse transcription of genomic RNA, Viral integration, Gene expression, Cleavage event and finally Virion maturation, by hitting any of these stages, the viral replication can be terminated.[4]

Integrase (IN) is an enzyme encoded by the HIV genome and represents a well established target. It catalyses the insertion and integration of the proviral DNA into the genome of the host cell in two steps: 30-processing (endonucleolytic sequence-specific hydrolysis of the 30-ends of the viral cDNA) and strand transfer (ligation of the viral 30-OH cDNA ends to the phosphate backbone of the host DNA acceptor).[5-7]

Integration of HIV-1 DNA into the host genome ensures stable maintenance of the viral genome in the host organism and, therefore, is a key process in the virus life cycle. IN is responsible for two distinct, consecutive catalytic steps in the integration process.[8] All reactions require a metallic cofactor, Mg^{2+} or Mn^{2+}, and, except for disintegration,[9,10] all reactions require the full-length IN. There are several experimental evidences to suggest that Mg^{2+} is more physiologically relevant as a cofactor, particularly because Mg^{2+}-dependent catalysis exhibits weaker non-specific endonucleolytic cleavage and the tolerance of sequence variation at the ends of the viral DNA is much greater in the presence of Mn^{2+} than in the presence of Mg^{2+}.[11-14]

The emergence of viral strains resistant against available drugs and the dynamic nature of the HIV-1 genome support a continued effort towards the discovery and characterization of novel targets and anti-viral drugs. Due to its central role in the HIV-1 life cycle, IN represents a promising therapeutic target. In the past, *in vitro* IN assays were extensively used to find IN inhibitors.[15] Current inhibitors can be separated into two main classes, depending on their mechanisms of action: (i) Compounds that competitively prevent the DNA binding of IN to the viral DNA. These compounds are mainly directed against the 3'-processing reaction as they bind to the donor site within the catalytic site − i.e. the 'specific' DNA-binding site for the viral DNA −. This group is referred to as 'integrase DNA-binding inhibitors' (INBI) and includes styrylquinoline compounds.[16,17] (ii) The second class includes compounds that cannot bind to the DNA-free IN. They bind to the pre-formed IN – viral DNA complex.

Thus among the numerous classes of emerged HIV IN inhibitors, only a very few of them exhibit a substantial level of antiviral activity at nontoxic concentrations. Styrylquinolines have been recognized as potent HIV IN inhibitors that block the replication of HIV in cell-based assays.[18,19] Styrylquinolines fall within the general category of inhibitors characterized by two aryl groups linked through a central spacer.

It seems that characterization of electronic structures and detailed analysis of charge density on oxygen atoms in Styrylquinolines to be useful to determine the active sites of these drugs.

Nuclear Quadrupolar Resonance (NQR) spectroscopy[20] is a very sensitive technique for determination of electronic charge distribution around quadrupolar nuclei ($I \geq 1$). This method can be used as a probe to obtain information about the environment of a given quadrupolar nuclei and consequently to determine the electronic structure of molecules and complexes. Quantum mechanical approach has been shown to be a very effective method in determination of the charge distributions in molecules.[21]

Despite the remarkable properties of styrylquinolines, the binding mode of these inhibitors remains to be elucidated. A more accurate knowledge of the role of charge distribution in the molecular interactions between these compounds and their presumed target was needed to further improve their pharmacological properties. Determination of the most active configuration of the drug (tautomeric forms) is a good approach which should be supported by computations. In the present paper, calculated nuclear quadrupole coupling constants (NQCCs) of quadrupolar nuclei in styrylquinolines were used to explore the electronic structure and steric factors controlling inhibitory of these drugs.

Computational Details

Quantum mechanical calculations were performed using the Gaussian 98 program.[22] All structures were fully optimized without any restriction. From the calculated vibrational frequencies, the optimized structures were characterized as minima (NIMAG = 0). Like in many previous studies on quinoline derivatives,[23-25] the B3lyp functional was used, which combines Becke's three-parameter[26] exchange functional with the correlation functional of Lee, Yang and Parr.[27,28]

In addition, since there is no experimental data on NQCCs of styrylquinoline, a rather small basis set such as 6-31G* is able to lead us to the qualitative results using calculated NQCCs, which seems to be reasonable, because a qualitative prediction may be obtained faster.

In this work, ab initio calculations were performed at the B3LYP/6-31G* level to compute the components of the electric field gradient (EFG) tensor in the principal axes system. The selected level and basis sets give the rather acceptable qualitative NQCCs of considered quadrupolar atoms.

Evaluations of NQCCs. The formulation employed in the evaluation of NQR parameters can be found elsewhere.[29] Briefly, the EFG is a traceless, symmetric second-rank tensor that principal axes are chosen so that its components satisfy $|q_{ZZ}| \geq |q_{YY}| \geq |q_{XX}|$, $(eq_{ij} = \frac{\partial^2 V}{\partial i \partial j})$ where $i, j = X, Y, Z$, e is electron charge and V is the external electronic potential.[30] The expression $\chi = \frac{e^2 Q\, q_{ZZ}}{h}$ is termed as nuclear quadrupole coupling constant and has the unit of frequency (Hz). h is the Planck's constant, Q is nuclear electric quadrupole moment and q_{ZZ} is the Z component of EFG tensor in principal axes system.

Similar to the many previous studies,[31,32] here we assumed that the nuclear electric quadrupole moments act as a simple constant or scaling parameter, and we do not parameterize it. Among the wide range of published standard values of quadrupole moments, we selected the recent value of $Q(^{17}O) = 25.58$ *mb* reported by Pyykko.[33]

Results and Discussion

In this work ab initio NQCC computations on some styrylquinolines have been performed so that a possible relationship between their electronic structure and biological activity could be investigated. The computed interaction energies are not related in a simple way to experimental values because entropy and solvent effects have not been fully taken into account in our computations. Since the obtained values do not correspond to experimental dissociation constants therefore calculated NQCCs of quadrupolar nuclei seems to be, in this case, a valid tool. In other words, the relation between the electronic structure of styrylquinoline and its IN inhibitory ability can be considered using NQR parameters, because these parameters are highly sensitive to local charge distribution.

From expresion $\chi = \frac{e^2 Q\, q_{ZZ}}{h}$, it is obvious that NQCC of nuclei is directly proportional to q_{ZZ}. There are two factors controlling the value of q_{ZZ} in a nucleus; charge density on the nucleus and symmetry of EFG around the quadrupolar nucleus. It is evident that an increase of the charge density causes the q_{ZZ}, and consequently χ, to increase. If charge distribution were such that the symmetry of EFG increased, then q_{ZZ} and consequently χ will decrease.

Since the contribution of nonbonding electrons (lone pairs p and d) in the nonspherical charge distribution is greater than the bonding electrons and charges on neighboring ions, therefore in atoms with nonbonding electron pairs (such as oxygen), the EFG is more asymmetric than that of the others and increasing the charge density in these atoms causes the effect of the nonbonding electron pairs to become modest and the symmetry of EFG around the nucleus to be increased. Therefore, the values of q_{ZZ} and χ for oxygen atoms decrease when its charge density increases.

Some previous researches[34] indicate that the presence of a 7-carboxyl-8-hydroxyl patterns in the quinoline half and an aromatic or heteroaromatic ancillary subunit is required for the biological activity of styrylquinoline (Figure 1).

It is manifest that the presence of divalent cations is essential for the catalytic activity of HIV integrase.[36-38] The styrylquinoline compounds lie in a nearly coordination to magnesium cation. As shown in docking results, the oxygen atoms of carboxyl group and hydroxyl group may coordinate with Mg^{+2} ion within the enzyme catalytic site.[39] This docking mode was the same as the experiment report given by zouhiri *et al.*[34] Thus, based on previous studies the oxygen atoms of carboxyl group and hydroxyl group in styrylquinolines have dominant role in drug activity regardless of determining the active sites.

In this study in order to determine the active sites of these drugs we considered some experimental examined compounds in ref. 34 (Figure 2).

From ref. 34, Styrylquinolne in which the carboxyl group at

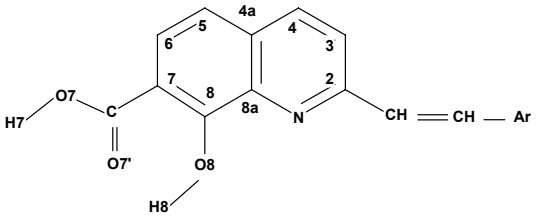


Figure 1. The numbering of atoms and their substituent of quinoline ring.

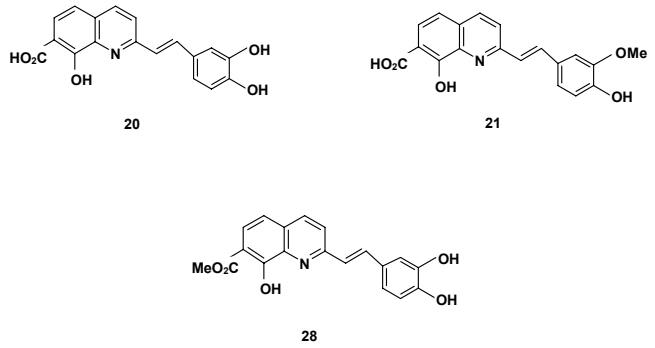


Figure 2. Some active (20 and 21) and inactive (28) styrylquinoline derivatives which their HIV-1 IN inhibitory experimentally determined in ref. 34.

Table 1. Comparison of calculated NQCCs of oxygen atoms in some active and inactive styrylquinolines

Compound	$\chi_{O7}^{calc}(MHz)$	$\chi_{O8}^{calc}(MHz)$	$\chi_{O7'}^{calc}(MHz)$
20	8.89	8.45	7.67
21	8.89	8.45	7.67
28	9.52	8.45	7.91

C-7 in quinolne half is replaced by a carbomethoxy group (Fig. 2, compound 28) is accompanied by a complete loss of inhibitory potency ($IC_{50} > 100$ μM).

In contrast, 3',4'-dihydroxy and 3'-methoxy-4'-hydroxy derivatives (20 and 21 compounds respectively) show good anti −HIV potencies (IC_{50} between 1 and 3 μM). In other words the compounds 20 and 21 can be roughly classified as active and 28 as inactive derivatives of styrylquinoline.[34] The calculated NQCCs of oxygen atoms of hydroxyl group and carboxyl group of these compounds were listed in Table 1.

The results show that NQCCs of O7 and O7' in active compounds substantially differ from that of inactive compound; the major difference among oxygen atoms of hydroxyl group and carboxyl group in active and inactive compounds is the charge density on O7 and O7' atoms. This indicates that the charge distribution on carboxyl moiety may play an important role in determining of pharmaceutical behavior of styrylquinolines. In 20 and 21 compounds, O7 and O7' have less NQCC (630 KHz and 240 KHz, respectively) and consequently more charge density than that of 28 compound. These calculations were performed for all other compounds in ref. 34 with IC_{50} between 1 and 3 μM and this point observed in all cases. This may be evidence that NQCCs may correlate with activity well and the oxygen atoms of carboxyl moiety are the active sites of these drugs.

In continue, two required structural subunits of this drug (quinoline half and ancillary phenyl ring) were considered separately.

Comparison of Calculated ^{17}O-NQCCs of Some Conformations of Quinoline Half

In styrylquinoline derivatives, the carboxyl and hydroxyl substituents lie at adjacent positions of C_7 and C_8 (Figure 1),

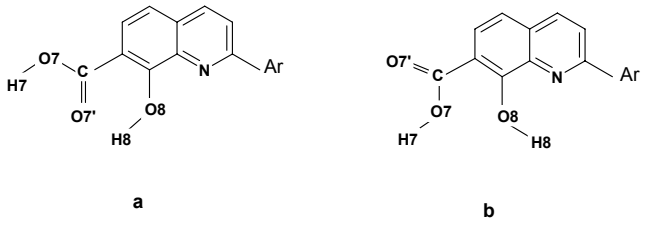


Figure 3. Two selected conformations of styrylquinoline derivatives in this work.

Table 2. Comparison of calculated NQCCs of various atoms in two conformations **a** and **b**

Conformations	$\chi_{O7}^{calc}(MHz)$	$\chi_{O8}^{calc}(MHz)$	$\chi_{O7'}^{calc}(MHz)$	
a	8.90	8.44	7.65	
b	8.70	8.68	8.88	
Complex	$\chi_{O7}^{calc}(MHz)$	$\chi_{O8}^{calc}(MHz)$	$\chi_{O7'}^{calc}(MHz)$	$E(Hartree)$
a-Mg^{+2}	8.62	8.31	7.63	−0.33
b-Mg^{+2}	8.37	8.43	8.53	−0.30

exactly as in salicylic acid.

In salicylic acid, the neighborhood of hydroxyl and carboxyl substituents favors the formation of a chelate ring, in which a divalent hydrogen atom is a link.[35] It is interesting to consider this model in styrylquinoline compound. Here we calculate the ^{17}O-NQCCs of two selected conformations of ortho-hydroxy acid group in styrylquinoline (Figure 3; **a**, **b**). To achieve this, the electric field gradient at the site of oxygen atoms was calculated.

As mentioned above, styrylquinoline may exist as two conformers **a** and **b**; Figure 3.

The calculated NQCCs of oxygen atoms were shown in Table 2.

Table 2 shows NQCC and therefore charge density of these atoms in two conformers (**a** and **b**) are different from each other. The O7'-NQCC of **a** conformer is obviously different from that of **b** conformer and O7'-NQCC in **a** conformer is about 1 MHz less than that of **b** conformer.

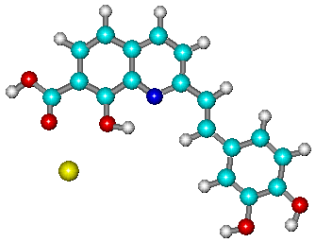
Based on these results the mentioned oxygen atom (O7') in **a** conformer has greater charge density than that of in **b** conformer.

This point is based on this evidence that increasing charge density on oxygen atoms causes that effect of the nonbonding electron pairs of these atoms to become modest and therefore the values of q_{ZZ} and consequently the NQCC values of these atoms to decrease.

In this part using calculated NQCCs, we try to predict which conformer is better chelating of Mg^{+2}. It is obvious that Mg^{+2} cation has electron deficiency, in order to form a binding, the electrical charge must be transferred from styrylquinoline molecule to Mg^{+2} cation. For a strong binding the mentioned charge transfer must be significant and more complete.

Based on the calculated NQCCs (Table 2), charge density of O7' atom in two conformers (**a** and **b**) is different from each other.

The results show that **a** conformer has more contribution in the activity of styrylquinoline derivatives, because the active



Bond	Mg-O7' = 1.79
	Mg-O8 = 2.38
Angle	O7'-Mg-O8 = 80.09

Figure 4. A proposed binding mode of the drug on Mg^{2+} in the vicinity of the active site according to the calculated NQCCs.

site of this drug i.e. O7' in this conformer has less NQCC and therefore greater charge density. Since the Mg^{2+} ion is a cofactor required for the enzymatic activity of HIV IN, therefore chelating a cation such as Mg^{2+} by **a** conformer is better than the **b** conformer. It is noticeable that the difference between binding energy in two considered complexes is very small (about 0.03 hartree) and in this case comparison of binding energies could not help us for determination of preferred complex.

Accordingly, a presumed binding mode of the drug on Mg^{2+} in the vicinity of the active site may be derived (Figure 4).

Comparison of the Calculated NQCCs of ^{17}O of Two Rotamers of Styrylquinoline; R1 and R2

Styrylquinoline-like molecules exist as different conformational isomers involving rotation around the quasi-single bond between the styryl and quinoline moieties.[40-42] Denisov *et al.* showed that the enol form prevails in solution.[43] Since compound **1** (Scheme 1) is a trance-2-styrylquinoline-like, therefore different conformational geometries (tautomers and rotamers) of the drug should exist in solution.[40-42]

It has been shown that compound **1** is present in the solution as a mixture of two stable rotamers with different photophysical and photochemical properties (Scheme 1, **R1** and **R2**).

The equilibrium between the conformers is strongly PH and solvent dependent. In acidic medium, only the **R2** rotamer is present while in aprotic solvents (dioxane) only the **R1** rotamer exists.[44] It is important to know which conformer of the drug has more ability to chelate the divalent cations (Mg^{+2}).

Theoretical calculations, in particular calculation of NQCCs of nuclei, seem to be a proper tool for gaining better understanding of the electronic structure of these conformers. Table 3 shows the calculated ^{17}O-NQCCs of the two conformations of compound **1** (Scheme 1).

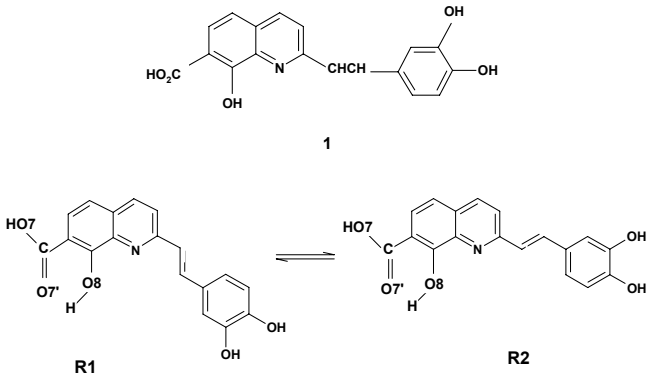
From our previous results in this work, we proposed a binding mode of the drug on Mg^{+2} in the vicinity of the active site (Figure 4).

Based on this hypothesis, charge density of O7' has a dominant role in the biological activity of styrylquinolines. Therefore it is expected that for a better metal-chelating of quinoline part, the charge densities on O7' atom should be increased and thus any status that increase the charge distribution on O7' atom may cause the biological activity of styrylquinoline to be increased.

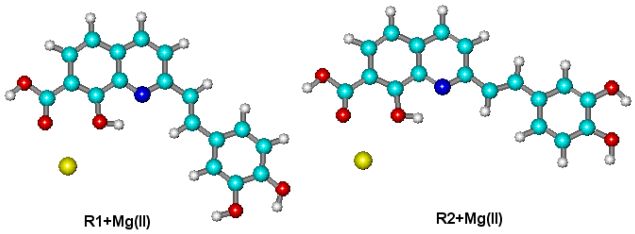
Using calculated ^{17}O-NQCCs (Table 3), we can compare the HIV IN inhibitory of these conformers; **R1** and **R2**. The results showed that in **R1** conformation, O7' has more charge density (less NQCC; about 20 kHz) than those in **R2** conformation. Therefore it is expected that compound **1** in **R1** conformer is better chelater of Mg^{+2} and thus better inhibitor of HIV IN. The calculated NQCCs of complexes of these rotamers with Mg^{+2} (Figure 5) confirm this point. The results suggest that the change of charge density on O7' is greater than that of O8. In other words, O7' has a dominant role in the interaction with Mg^{+2} and its charge density is more affected after complexation. Such as previous section, the difference between binding energies of

Table 3. The calculated NQCCs of O7' and O8 in R1 and R2 conformers of styrylquinoline

Conformations	$\chi_{O7}^{calc}(MHz)$	$\chi_{O8}^{calc}(MHz)$	$\chi_{O7'}^{calc}(MHz)$	
R1	8.90	8.44	7.65	
R2	8.90	8.46	7.67	
Complexes	$\chi_{O7}^{calc}(MHz)$	$\chi_{O8}^{calc}(MHz)$	$\chi_{O7'}^{calc}(MHz)$	$E(Hartree)$
R1-Mg^{+2}	8.84	8.44	8.68	−0.33
R2-Mg^{+2}	8.85	8.49	8.72	−0.30



Scheme 1. The structure of compound **1** and rotamer equilibrium between the two rotamers; **R1** and **R2**



Bond	Mg-O7' = 1.79	**Bond**	Mg-O7' = 1.81
	Mg-O8 = 2.38		Mg-O8 = 2.383
Angle	O7'-Mg-O8 = 80.09	**Angle**	O7'-Mg-O8 = 84.26

Figure 5. The optimized structure of complexes Mg^{+2} with **R1** and **R2** conformers.

R1-Mg^{+2} and R2-Mg^{+2} complexes are very small (about 0.03 hartree) and using calculated binding energies, we cannot compare the HIV IN inhibitory of these conformers.

Since, only the **R1** rotamer exists in aprotic solvents,[38] use of this drug in the aprotic solvents such as dioxane may enhance its biological activity.

Conclusion

Styrylquinolines have been recognized as potent HIV IN inhibitors that block HIV replication in cell-based assay. The present study indicated that the calculated NQCC parameters could give us helpful hints for interpretation of the binding affinity between these inhibitors and integrase. According to the data obtained from charge distributions, it is concluded that:

− The oxygen atoms of carboxyl moiety are active sites of styrylquinolines and their charge density is more affected after complexation.

− Conformer **a** in which a divalent hydrogen atom is a link, has more capability in antiviral drug treatment.

− Based on the calculated NQCCs, we can compare the HIV IN inhibitories of **R1** and **R2** conformers. The results showed that in **R1** conformation, O7' has more charge density (less NQCC; about 20 kHz) than that of **R2** conformation. Therefore it is expected that styrylquinoline in **R1** conformer to be better chelater of Mg^{+2} and thus better inhibitor of HIV IN.

− The results showed that although the binding energies differ between conformers, the difference is small enough that there can be no definite answer on the question of what will be preferred structure of a complex. Therefore the NQR parameters are more sensitive and precise than the binding energies of Mg^{+2} cation with various conformers.

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