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Pharmacophoric Features of Nucleosidic HIV-1RT Inhibitors

Arpita Yadav* and Sanjeev Kumar Singh

Department of Chemistry, Institute of Engineering and Technology, CSJM University, Kanpur 208024, India

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Abstract—A quantum pharmacological study on nucleosidic inhibitors of HIV-1RT has been performed. The main aim of this study is to discuss the pharmacophoric features (conformational and electrostatic) of nucleosidic inhibitors and compare them with normal substrate dNTP. Present study stresses on the need to refine nucleosidic drugs, as combination therapy to date is still one of the best remedies for AIDS. The results of ab initio HF calculations indicate very little effect of 3′ substituent on ring puckering and suggest that potency regulation may be via very intricate phosphate-catalytic triad interactions. Our MESP maps also show charge complementarity between the drug and receptor.

Introduction

Acquired immuno deficiency syndrome (AIDS) is a deadly disease and hence considerable efforts have been made to find out effective drugs against this disease.[1,2] It is a viral disease that spreads rapidly by the replication of the virus. There are several stages in the replication of the virus catalyzed by various enzymes. One obvious way to stop the replication of the virus and in turn control the disease is to design inhibitors for one of these enzymes[3] referred to as the nucleosidic drugs. HIV-1Reverse Transcriptase (RT) is one such DNA enzyme involved in the transcription phase of replication of the virus. The main function of this enzyme is to produce double stranded DNA from single strand RNA so that the genetic information can be integrated into host cell chromosome and the virus can replicate. The present study will focus on inhibitors for this enzyme. Although it is realized that resistance to currently available chemotherapeutics invariably emerges, there is a high medical need to develop more potent, safe and selective antiviral agents. In the present study we shall discuss pharmacophoric features of nucleosidic drugs, which is the first step towards designing more potent drugs.

Nucleosidic drugs or inhibitors to be specific can inhibit a DNA enzyme like HIV-1RT by several mechanisms—the most common of all is the incorporation of the nucleosidic drug in the growing DNA chain. Apart from this the drug may show competitive inhibition by binding to the free enzyme; it may bind to the enzyme–DNA complex or it may even bind to the product-binding site. Examples of all these inhibitory mechanisms are known[4–8] but the commonly used drugs which are modified nucleosides act by incorporation in the growing DNA chain.[9] Although they undoubtedly show inhibition of HIV-1RT they all lead to host toxicity because they can only be partially selective at best.

To be able to develop more selective drugs, which can also overcome drug resistance problems, we must understand the drug's pharmacophoric features and its interactions with the receptor.

Some commonly clinically used nucleosidic drugs[10–16] that we have considered in this study are 2′3′dideoxy nucleosides, 2′3′didehydro dideoxy nucleosides, azidothymidine (AZT) (Fig. 1). The characteristics of these drugs will be compared against the normal substrate for HIV-1RT, that is dNTP deoxynucleotide triphosphate. (cf. Fig. 1) It is to be emphasized that although the nucleosidic drugs lead to host toxicity and drug resistance problems still, practically, one cannot avoid usage of nucleosidic drugs (combination therapy at best) as they are readily available in market, also their bioavailability is good and they are sure to work orally as well as intravenously.

Recent theoretical studies have been more towards Monte Carlo simulations of non-nucleosidic inhibitors

*Corresponding author. Tel.: +91-512-591637; fax: +91-512-590007; e-mail: arpitayadav@yahoo.co.in

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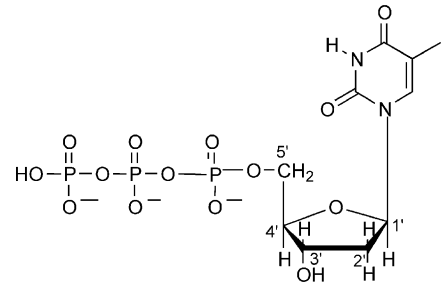
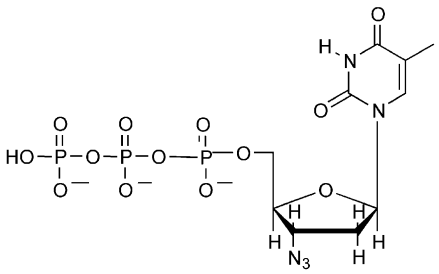
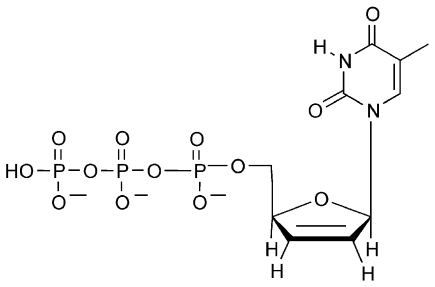


deoxy ribonucleoside-5'-triphosphate (dNTP)
(normal substrate for HIV-1RT)



3'azido 2'3'dideoxy thymidine (AZT)



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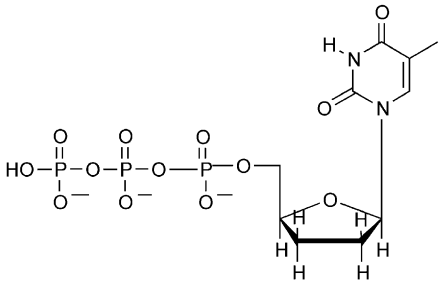
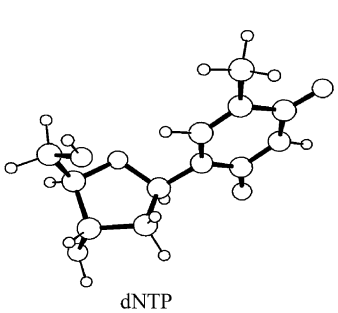
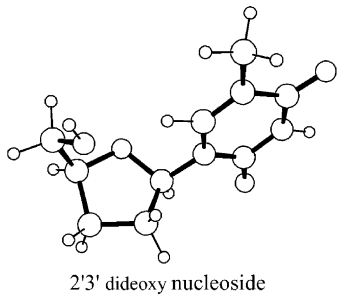


2'3' dideoxy thymidine

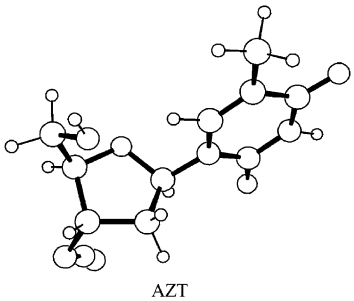
Clinically used broad spectrum antiviral Nucleosidic drugs



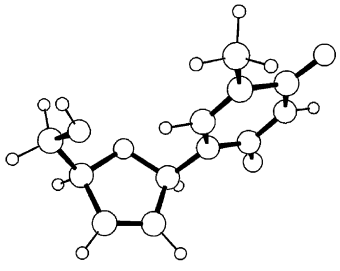
dNTP



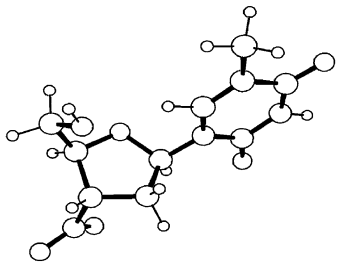
2'3' dideoxy nucleoside



AZT



2'3' didehydro dideoxy nucleoside



3' nitro 2'3' dideoxy nucleoside

Optimized bioactive conformations of nucleosidic drugs

Figure 1. Clinically used nucleosidic drugs and their optimized bioactive conformations.

(NNI's) docked in the NNI active site of HIV-1RT. The drug–receptor interaction energy is calculated through simulation.[17,18] In this study we stress more on trying to refine nucleosidic drugs and also understanding in detail the pharmacophoric features and interactions with the receptor.

Methodology

Ab initio Hartree fock (HF) molecular orbital (MO) calculations have been performed on nucleosidic drug molecules using 6-31G basis set.[19,20] Complete geometry optimizations have been performed for all using an optimally conditioned method of Davidon and Nazareth.[21,22] Optimized conformations have been plotted with the ORTEP package. Charge environment has been studied using complete molecular electrostatic potential contours and the same have been illustrated with the help of the graphics package MOLDEN.[23] To understand the differences in the conformation of the drug and the normal substrate dNTP, drugs have been mapped one by one onto dNTP. This is referred to as conformational mapping. The idea of conformational mapping is to understand the variations in the conformation of the drug with respect to a normal substrate (which binds perfectly) or with respect to a clinically tested potent drug (which can again be assumed to bind perfectly). The above calculations comprise the first two steps of a quantum pharmacological study. The first step in a quantum pharmacological study is to find out common conformational features (in terms of internuclear distances, specific angles or torsions etc.) responsible for drugs activity, that is defining the pharmacophore. The next step is the receptor mapping. In this study, we will deal with the above described steps of quantum pharmacology.

Result and Discussion

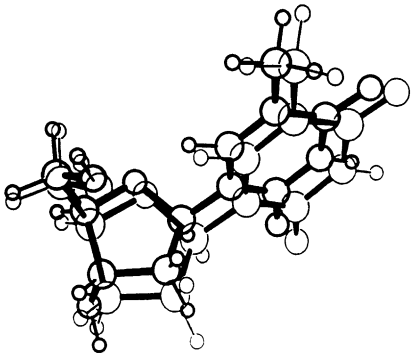
The ORTEP plots of completely optimized geometries of nucleosidic inhibitors of HIV-1RT at the HF level with the 6-31G basis set are shown in Figure 1. These are the bioactive β-D-ribose conformations and we have taken the thymidine base throughout for the sake of comparison. The next figure shows conformational mapping. Each drug has been superimposed one by one onto the normal substrate dNTP.

The conformational mapping indicates that ring puckering is not affected by change of the substituent at the 3′ position. The thymidine base disposition may vary a little. This conformational mapping also suggests that potency may be regulated by very intricate interactions with the catalytic triad. It is not directly regulated through the substituent at the 3′ position.

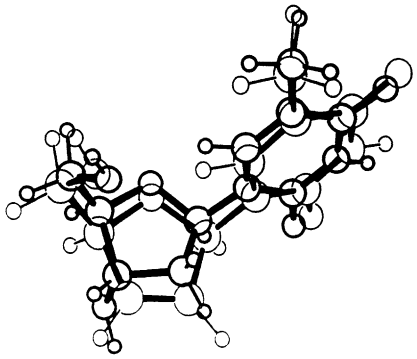
To understand the effect of these minor conformational changes on the drug–receptor interactions and monitoring the potency we have simulated the active site for the nucleosidic HIV-1RT inhibitors. Figure 2 also shows the normal substrate dNTP with the active site catalytic triad. The three aspartics 110, 185 and 186 constituting the catalytic triad have been docked in with dNTP. The aspartics have been placed according to C_α coordinates for protein reported by Jacobo Molina et al.[24] and also a 3-D molecular model reported in 1994.[25] The recent ternary complex crystal structure reported by Steve Harrison and Greg Verdine contains the positions of active site residues in the non-nucleosidic inhibitor binding pocket of HIV-1RT.[26] Figure 3 shows the various nucleosidic drugs mapped onto the normal substrate in the presence of catalytic triad. The mapping indicates the importance of catalytic triad in interacting with the phosphate. Figure 3 highlights the simple fact that the nucleosidic inhibitor binding to the HIV-1RT active site is controlled mainly by the attractive interactions between the CH_2OH (taken in place of triphosphate) and the aspartics. The interactions between the phosphate and catalytic triad are not only important for the binding of these drugs but may also play an indirect role in controlling their potency. Our results are in agreement with a previous molecular model of binding of dNTP suggested by Kaushik et al.[27] wherein all three aspartics participate in binding dNTP, Mg^{2+} and subsequently asp186 and asp110 stabilize the transition state.

Previous studies have concentrated on the binding of nucleosidic drugs not on their potency regulation. Our conformational mapping indicates that only minor differences in interactions of the phosphate with the catalytic triad induced by the 3′-substituent could be responsible for differences in potencies of these drugs as otherwise the conformations of various inhibitors are very similar.

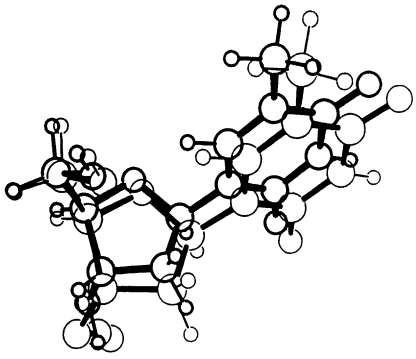
As long as the substituents at the 2′3′ positions do not disturb the ring puckering to a great extent and can have attractive interactions with the catalytic triad, the molecule could be expected to be an acceptable nucleosidic inhibitor. For example we have taken nitro group at the 3′ positions. Figure 3 indicates that with the nitro substituent the inhibitor would have identical disposition with respect to the catalytic triad as in the normal substrate dNTP. So, from a conformational point of view it appears that nitro substituent could be considered as an option for a nucleosidic inhibitor. Also, the nitrogen atom seemingly helps to enhance lipophilicity (as in AZT). Indeed, a compound with a nitro substituent at the 3′ position has been tested for anti-HIV activity in conformity with our predictions.[28] Let us now consider detailed MESP contours to understand the detailed charge environment of these drugs. The active site set up would obviously be complementary to this charge environment. The detailed MESP contours are shown in Figure 4. Detailed MESP contours indicate a slightly negative charge environment near the 2′3′ substituents becoming more negative towards the thymidine base end. Hence, one would expect almost neutral or a very slightly positively charged complementary environment on the receptor. Positive potential areas have indeed been observed in the active site of HIV-1RT where DNA binding occurs.[25] It clearly shows the complementarity of a charge environment essential for the binding of the drug to the receptor.



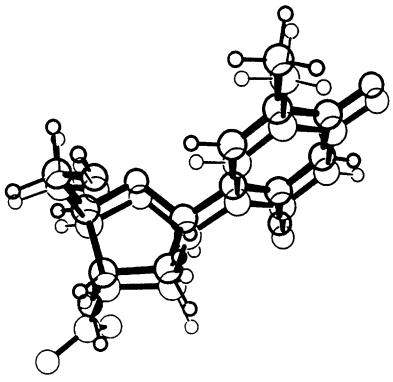
dNTP and dideoxy nucleoside



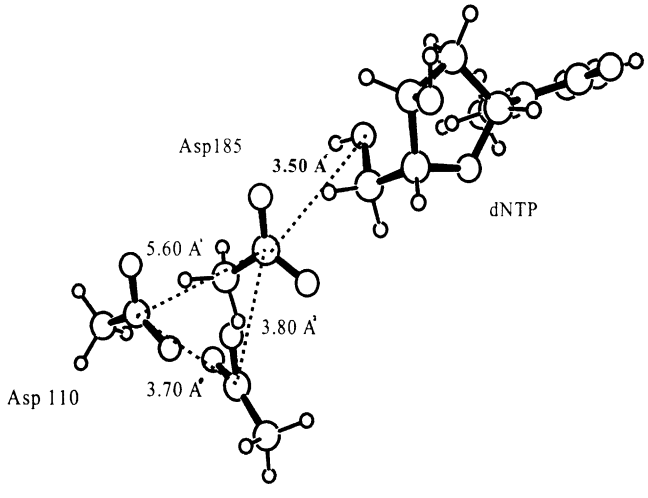
dNTP and didehydro dideoxy nucleoside



dNTP and AZT



dNTP and 3' nitro dideoxy nucleoside



dNTP with catalytic triad

Figure 2. Conformational mapping of nucleosidic drugs and dNTP with catalytic triad.

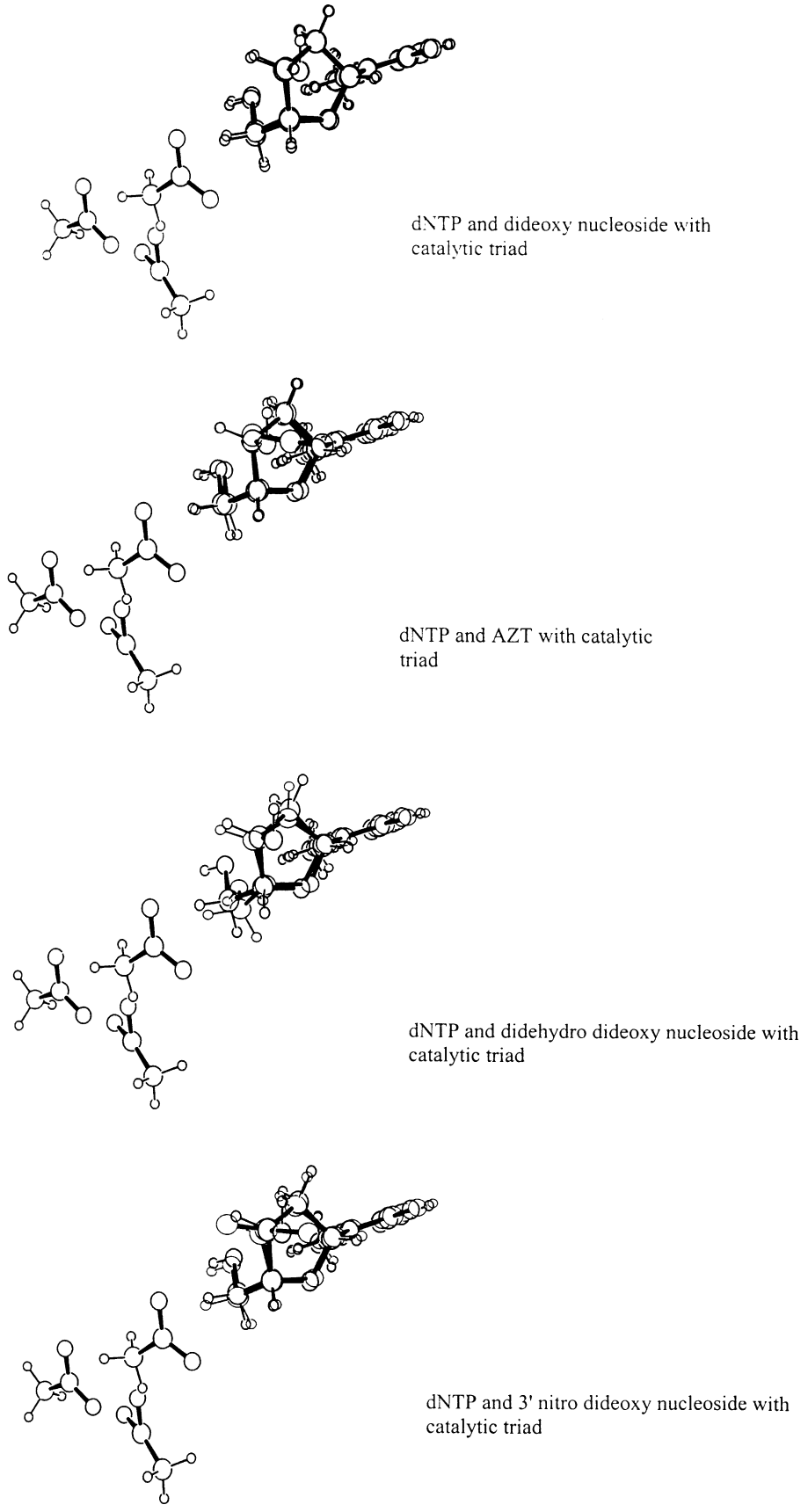


Figure 3. Conformational mapping of nucleosidic drugs in active site.

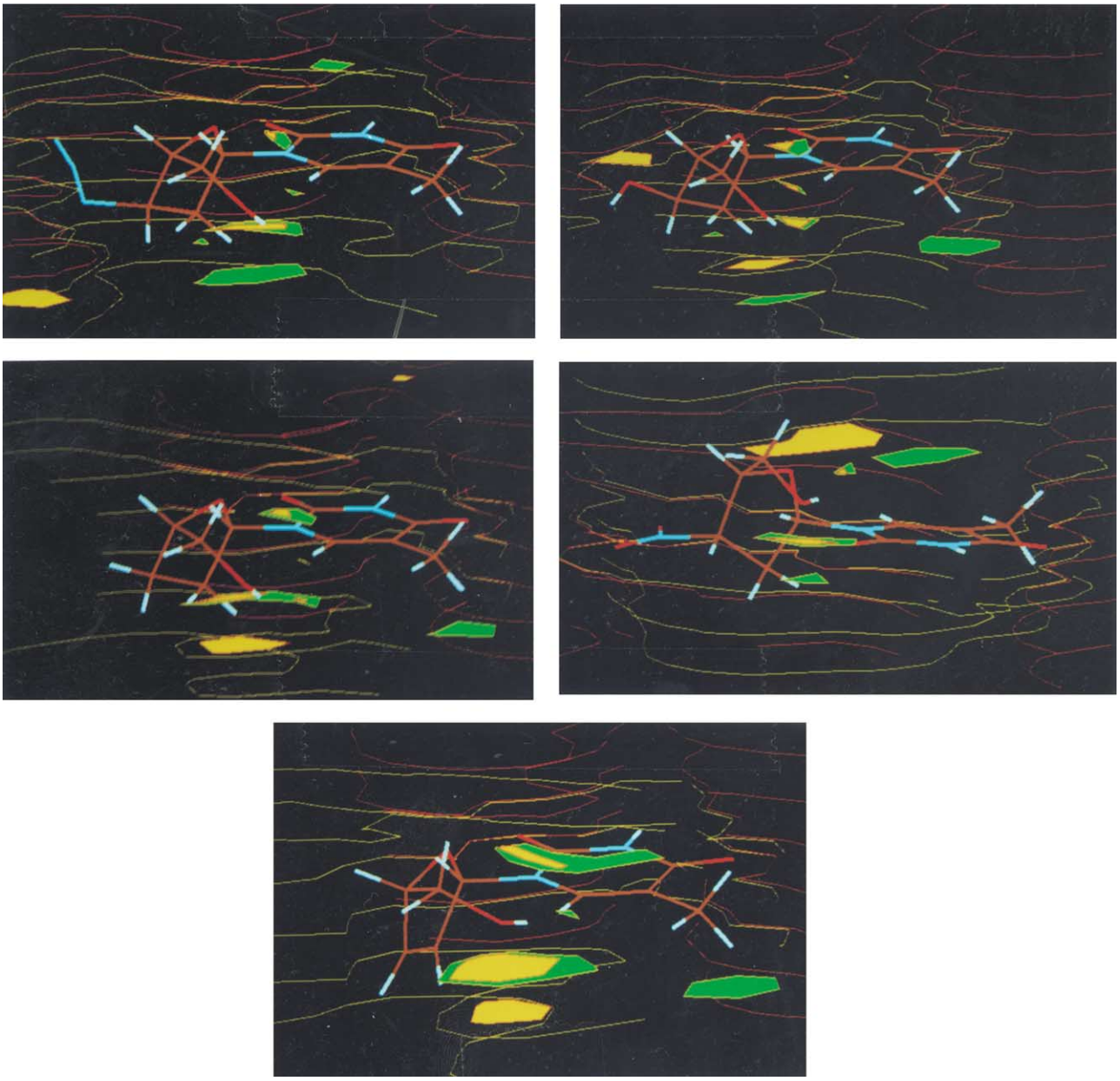


Figure 4. MESP contours for nucleosidic drugs. Red coloured contours indicate a value of −0.1 for electrostatic potential and yellow contours indicate a value of −0.05.

The next step, obviously, is to incorporate the primer, metal ions and calculate detailed drug receptor interaction energies at the microscopic level. Work along these lines is in progress and will be reported subsequently.

Conclusions

The main aspects of this study may be summarized as follows:

1. Various nucleosidic drugs with different substituents at the 3′ position show similar sugar ring puckering and only slight differences in nucleosidic base disposition and interactions with the catalytic triad.
2. Conformational mapping indicates the importance of interactions between the phosphate and the catalytic triad not only in binding the drug but also in controlling the potency.
3. Drug–receptor complementarity of the charge environment is clearly indicated by our MESP maps. Earlier studies have mostly concentrated on receptor charge environment.

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