Studying the Chemical Reactivity Properties of Cytosine and its Antiviral Analogues: Zebularine, 5-Aza-Cytosine And 5-Aza-5,6-Dihydro-Cytosine through Density Functional Theory

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Research Article

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Abstract

Zebularine, 5-aza-cytosine and 5-aza-5,6-dihydro-cytosine are structurally similar to cytosine, but their biological functions are rather different. Cytosine can be methylated which is a gene lesion that can cause human disease. On the contrary, zebularine and 5-aza-cytosine are inhibitors of DNA methylation. 5-aza-5,6-dihydro-cytosine is specifically designed to induce lethal mutagenesis in HIV for its structurally variability. Here, theoretical research into their chemical properties through density functional theory is reported. Molecular hardness and molecular electronic surface potential were analysed. Compared to cytosine, the main reason for the inability of methyl addition of zebularine is the reduced nucleophilicity of C5 atom. The lack of a hydrogen atom at N5 atom in 5-aza-cytosine is responsible for the incomplete reaction of methyl transfer. Variability of 5-aza-5,6-dihydro-cytosine is responsible for the mutagenesis treatment by paring with guanine or adenine with its different tautomers. Aspect of these chemical reactivities can be accounted for the distinctive biological functions of these molecules.

Introduction

Nucleoside analogues as agents for anticancer and antiviral therapeutics have been studied and used for fifty years[1]. The naturally occurring nucleosides represent a unique starting point for drug design due to their involvement in numerous critical biological processes as well as the fact that they serve as essential building blocks for DNA/RNA synthesis. On such a point of view, modifications of natural nucleobases can be designed or refined, based on the interactions identified in the binding site of target enzymes. Of the four nitrogenous bases of DNA, cytosine was selected for discussion since an abundance of mutagenesis data concerning this base are available in the scientific literature[2]. Modifications of cytosine would lead to disruption or termination of replication or other biological processes[3]. Here zebularine, in the absence of the amino group at C4 position, and 5-aza-cytosine analogue (termed as 5-azaC, also known as decitabine), modified at C5 position by an atom N, and its saturated form 5-aza-5,6-dihydro-2'-deoxycytosine (5-azaHC) are reported. Cytosine is introduced a methyl group via C5 position catalysed by methyltransferase enzymes[4-6], while the three analogues are the most important nucleoside analogue inhibitors of cytosine methylation[7-9]. The addition of the extra nitrogen also endowed 5-azaC with profound anticancer properties[10-13]. 5-azaHC is known to be incorporated into a viral (HIV) genome to extinguish HIV viruses by elevating its already high mutation rates above the error catastrophe limit, in which limit no viable progeny can be sustained and the viral population collapses[14-16].

These different biological functions are closely related to their specific molecular structures. Amino group is an important biologically substituent. The absence of the amino group (zebularine) or nitrogen substitution to the structure of cytosine (5-azaC and 5-azaHC) can have profound effects. The motivation for the present study was to quantify the chemical reactivity parameters of the geometrically similar molecules: zebularine, Cytosine, 5-azaC and 5-azaHC. The properties of these molecules are discussed here emphasizing their different aspects dependent of chemical hardness, and surface electrostatic potential.

Theoretical Background

Theoretical background:

1. **Reactivity parameter**

In density functional theory, the ground-state properties of a many-electron system are uniquely determined by its electron density ρ(r) as expressed below[17]

$$E[\rho] = F[\rho] + \int \upsilon(r)\rho(r)dr$$

Where υ(r) is the external potential that includes the nuclear potential, and $F[\rho]$ is the universal Hohenberg-Kohn functional composed of the electronic kinetic energy and the electron-electron interaction energy. The global hardness η is defined as the second partial derivative of E[r] with respect to the number of electrons N at constant external potential, and an approximation to η is used by a finite difference method[18, 19].

$$\eta = \left(\frac{\partial^2 E}{\partial N^2}\right)_{\upsilon(r)} \; ; \; \frac{1}{2}(I - EA)$$

Where E, IP and EA are respectively the total energy, ionization potential and electronic affinity of an N-electron system.

1. **Molecular Electrostatic Surface Potential (MESP)**

Electrostatic potential is an important property that plays a key role in the interaction of molecules and is well established as an effective tool for interpreting and predicting molecular reactive behaviour[20-22]. The electronic potential of a molecule is an expression of Coulomb's law:

$$V_{tot}(\mathrm{r}) = V_{nu}(\mathrm{r}) + V_{ele}(\mathrm{r}) = \sum_A \frac{Z_A}{|\mathrm{r} - R_A|} - \int \frac{\rho(\mathrm{r}')}{|\mathrm{r}-\mathrm{r}'|} d\mathrm{r}' \qquad (1)$$

where Z_A is the charge on nucleus A, located at R_A. is the electronic density of the molecule and r' is a dummy integration variable. Depending on whether the effect of the nuclei or the electrons is dominant in any given region, may be either positive or negative. The most negative-valued points in the MESP topography, usually indicated with the notation V_{min} is widely used to gauge the electron donating properties of a molecule[23]. Similarly, V_{max} is used as a parameter to measure the electron drawing abilities of a molecule[24, 25].

Computational Methods

Hybrid meta-GGA functional M06-2X has been performed to study the different reactivity descriptors concerning IP, electronic affinity, hardness. This method has been proved to give the best correlation with experimental data[26, 27]. The split-valence 6-311++G(2d, 2p) has been employed in this study. To reduce computational complexity, N1 was substituted by a methyl group instead a nucleoside for these molecules. The geometries of all the molecules were optimized to find the neutral ground electron states. The spin multiplicity corresponding to singlet and doublet has been considered for the ground, cationic and anionic forms of the molecules, respectively. All calculations were performed with Gaussian 09 software[28]. The wave function generated in the computations is used for the calculation of the MESP.

Results And Discussion

The calculated chemical reactivity parameters for the interested molecules are listed in Table 1. They are ionization potential (IP), defined as the first ionization potential which is used to measure the extraction energy of the outermost electron of an atom of the system and thus indirectly reveal how tightly an electron is bound within the nuclear attractive field; electron affinity (EA), which measures the ability of a molecule to accept an electron and form anions; chemical hardness (η)[29], a descriptor shows the resistance of the chemical potential of a molecule to a change in its electron distributions. All these global reactivity descriptors are required to understand the biological activity of a molecule.

The calculated EA of cytosine is close to the experimental value which extrapolated the electron affinity of the valence anion of bare cytosine to be 0.13 ± 0.12 eV[30] and the IP is 8.49 eV, very close to the experimental value: 8.68 eV[31], indicating that the computational method is feasible. The IP values are similar for zebularine, cytosine and 5-azaHC, except 5-azaC which is less than half of any of the three mentioned. This indicates that 5-azaC is rather easy to lose an electron. The EA values are rather different and their singly occupied molecular orbitals (SOMO) are shown in Figure 1. The SOMO of zebularine is a π orbital with excess electron locating on C5, corresponding to its reduction potential; more negative than cytosine and 5-azaHC by a few tenths of an eV. The SOMOs of cytosine and 5-azaC are the typical characteristics of the □* antibond orbitals. The SOMO of 5-azaHC, the highest in energy, belongs to lone pair electron localized on N5. The negative EA of zebularine and 5-azaC indicate that the anions are electronically unbound at the equilibrium geometries of the corresponding neutral, and they are unstable.

Bisecting the sum of IP and EA values produces the chemical hardness η. The largest negative EA of zebularine induces the largest η. The least IP with the EA close to zero of 5-azaC results in the smallest η. The more hardness of molecules, the more stable they are, as stated, “at equilibrium, chemical systems are as hard as possible” [32]. The η value of 5-azaC, half of that of cytosine, is accounting for the fast decomposition in aqueous solution[33, 34]. The largest η value of zebularine indicates that it is least reactive of the four molecules, whereas the respective η values of cytosine and 5-azaHC suggest that they are relatively active.

Table I. Reactivity parameters concerning ionization potential (*IP*), electron affinity (EA), and chemical hardness (η) of cytosine, 5-azaC and 5-azaHC calculated at the M062X/6-311++G(2d,2p) level of theory. Units are in eV.

molecules	IP	EA	η
cytosine	8.49	0.11	4.19
zebularine	8.85	-0.33	4.59
5-azaC	4.10	-0.08	2.09
5-azaHC	8.40	0.21	4.10

The instability of 5-azaC is closely related with N5 atom. With the electron-lone pair and more electronegativity, more electronic charge accumulates on N5. This makes the angle C4N5C6 a little sharp. It is 113.7°, smaller than the corresponding angle of cytosine (115.9°) and 5-azaHC (115.0°). The angle of C2N3C4 is 118.7° for 5-azaC, the identically moiety in cytosine is 120.4° and that of 5-azaHC is 119.0°. The reduced angles increase the triazine tension, causing 5-azaC unstable. In alkaline and water solutions 5-aza-deoxycytidine (Scheme 1) undergoes a rapid and reversible opening of the 5-azacytosine ring, followed by irreversible decomposition[35, 36].

The MESP is a well-established approach for the determination of reactive behaviour of a molecule. The electrostatic potential on individual atoms and the values of three maximum electrostatic potential (V_{max}, in orange) and two minimum electrostatic potential (V_{min}, in cyan) of the systems (except zebularine) are presented in Figure 2. The blue surface surrounding some nitrogen and oxygen atoms represents the region of maximum electro negativity and the red colour surface accumulated on some hydrogen atoms shows their electro positivity. The V_{min} values vary from -48.6 to -54.5 kcal/mol and large or small V_{max} values scatter around N-H or C-H hydrogens. If we compare the MESP of zebularine and cytosine, we can observe that both the three V_{max} and one V_{min} are slightly smaller for zebularine (37.8, 27.5, 20.7 and -49.8 kcal/mol, respectively) than for cytosine (48.4, 36.8, 33.8 and -53.5 kcal/mol, respectively). The lesser basicity of N3 atom makes zebularine unfavourable to be protonated and unsusceptible to enzyme-mediated reaction[37, 38]. Second, the electrostatic potential around C5 in cytosine is -5.0 kcal/mol, while it is -0.03 kcal/mol for zebularine, the less nucleophilicity impedes DNA methyltransferases enzymes to methylate the C5 position when zebularine acts as a substrate. Lastly, the absence of the hydrogen bond interactions formed by the exocyclic amino group makes zebularine difficult to locate in a right position for the nucleophilic addition[8]. Compared to zebularine, the amino N atom enhances π electron delocalization around cytosine, producing more positive V_{max} and more negative V_{min} and the consequent favourable substrate for methylation. The amino hydrogen atoms are

likely to transfer to N3 atom for its strong proton affinity, forming tautomers of E/Z conformations (Cyt-E/Cyt-Z, see SI). Tautomerization of cytosine is less abundant, since the amino hydrogens possess relatively low V_{max} (compared to those of 5-azaHC). The Boltzmann population of the Cyt-E and Cyt-Z tautomers is 5.9 %, close to the experiment that traces of the imino population (~ 12 %) exist in 1-methyl-cytosine vapor[33, 34]. The Cyt-Z conformation is relatively more populated than Cyt-E, for the easier abstraction of the amino hydrogen with a more positive MESP potential of 48.4 kcal/mol around.

For 5-azaC, the replacement of the C-H group by a nitrogen atom provides an additional basic center (-20.0 kcal/mol) which in turn affects the basicity of the other nitrogen atoms of the ring (Figure 1c). The MESP distribution around 5-azaC, whether V_{max} or V_{min}, are both lower than those in cytosine. The second V_{min} of cytosine lies on the amino N atom, whereas 5-azaC provides the second V_{min} near N5 atom. At this site, methylation can be formed by a covalent bond; however, β-elimination of DNMT1 (DNA (cytosine-5)-methyltransferase 1) cannot occur due to the lack of a hydrogen atom at this position[39, 40]. Consequently, methylation of DNMT1 is unable to continue. In the case of 5-azaHC, the MESP is redistributed upon the saturation of N5 and C6. π electrons are more localized within N3 and the oxygen atom, leading to gained V_{max} and V_{min} values (Figure 1d). The sites as well as their V_{max} values (53.9, 52.1 kcal/mol) of the amide and amino hydrogens are very close. This intramolecular proton-proton repulsion causes increased proton kinetic energy, which would enhance isomerization rates. Seven tautomers were observed in the experiment[41], and their respective structures are listed in the SI.

Conclusion

In summary, C5 is a docking site for methylation for cytosine. Nitrogen substitution at this site makes zebularine and 5-azaC an inhibitor for methyl addition, while 5-azaHC has multiple tautomers for the active hydrogen atom on N5 and this molecule acts as a mutagenesis treatment. Herein, is can be found that the effects of chemical modifications of cytosine impinge great influences on biological activities. Chemical modification of natural nucleobases opens a new field in the search for effective antiviral and antitumor therapy. In this work, MESP and molecular hardness are valuable tools for description of molecular activities and can give reasonable information for the experiments.

Declarations

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Code availability: (No)

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Figures

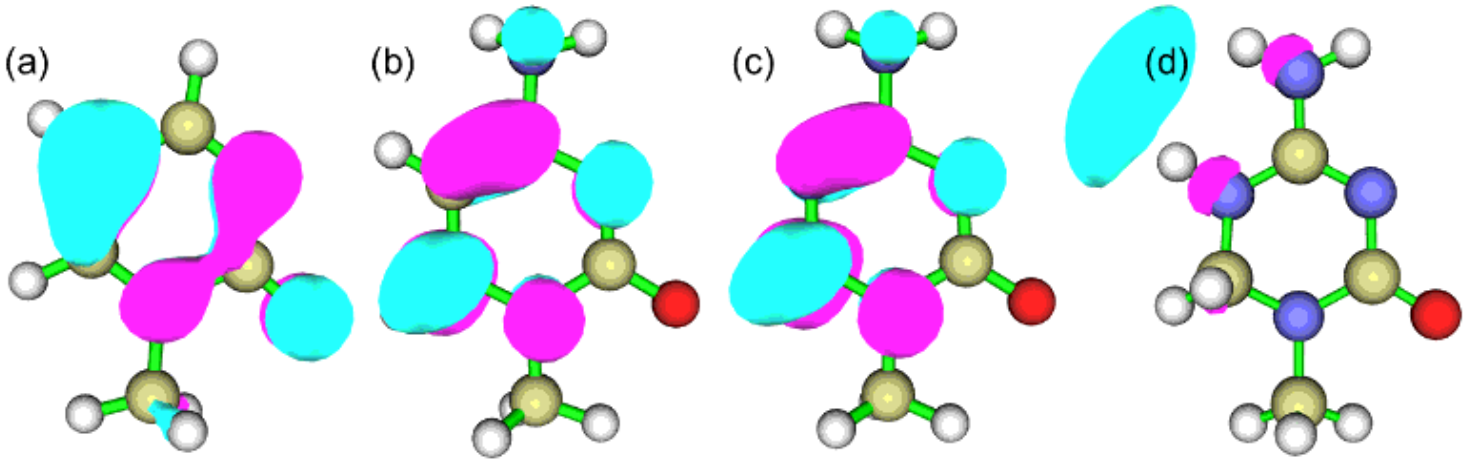

Figure 1

The singly occupied molecular orbital (SOMO) of (a) zebularine, (b) cytosine, (c) 5-azaC and (d) 5-azaHC. The isovalue is 0.05 Bohr for (a), (b) and (c). It is 0.04 Bohr for (d).

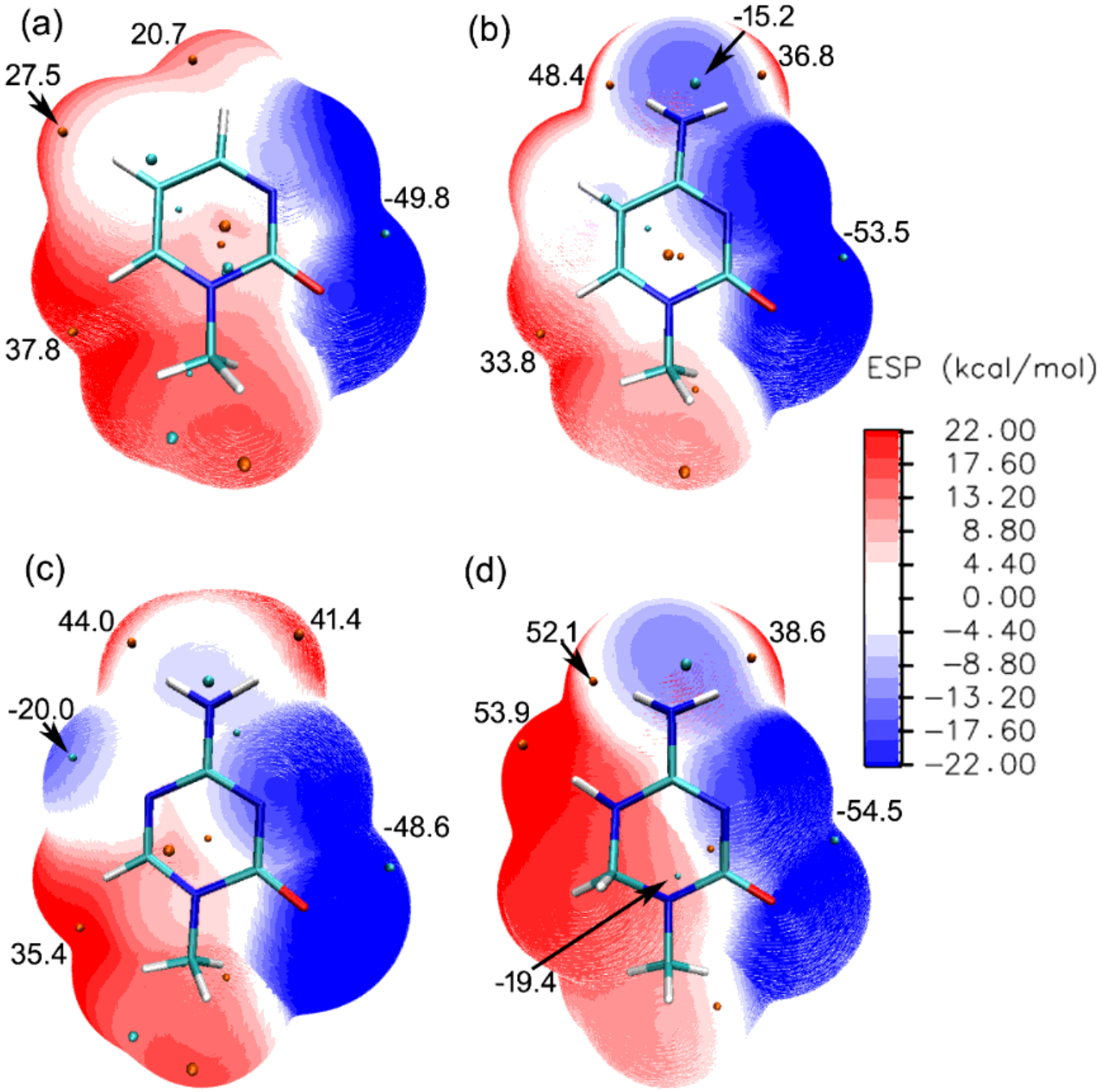

Figure 2

Molecular electrostatic potential isosurfaces for (a) zebularine, (b) cytosine, (c) 5-aza-C, and 5-aza-2HC. The electrostatic potential dots are coloured orange (positive) and cyan (negative), and the corresponding Vmax and Vmin values are given in kcal mol-1.

Supplementary Files

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- Scheme1.png