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# Molecular Modelling Analysis of the Metabolism of Entecavir

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**Abstract:** In this study, molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations have been carried out to investigate the relative stability of ETV and its metabolites with the aim of providing a better understanding of their relative toxicity. The results of the analyses show that both ETV and its major metabolites have LUMO-HOMO energy differences so that they would be kinetically inert. The molecular surface of ETV is found to posses neutral, electron-rich and electron-deficient regions so that the compounds may be subjected to lyophilic, electrophilic and nucelephilic attacks. Nucleophilic attacks can be due to cellular nucleophiles such as glutathione and nucleobases in DNA. However, because of the kinetic inertness of the molecules the rates of such adverse reactions are expected to be low so that ETV and its metabolites may not cause high toxicity.

**Key words:** Entecavir, hepatitis B, molecular modelling

## INTRODUCTION

Entecavir (ETV) is a cyclopentyl guanosine analogue launched in 2005 for the once-daily treatment of chronic hepatitis B virus (HBV) infection that affects two billion people worldwide (Lai *et al*., 2003; Matthews, 2006) and remains a clinical challenge. HBV infection is the tenth leading cause of death worldwide, resulting in approximately one million deaths per year due to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (Lavanchy, 2004). ETV is approved in the US, the EU, China, Japan, Korea, Taiwan and Latin America for the treatment of chronic HBV infection in adults with active viral infection (Robinson *et al*., 2006; Matthews, 2006; Rivkin and Marie, 2005).

In mammalian cells, ETV is phosphorylated to the active triphosphorylated form which inhibits all the three activities of the HBV polymerase: (1) base pairing, (2) reverse transcription of the negative strand of the pregenomic messenger RNA and (3) synthesis of the positive strand of the HBV DNA (Hegde and Schmidt, 2006). Entecavir triphosphate (ETV-TP) is a potent inhibitor of HBV DNA polymerases α, β, γ and δ and mitochondrial DNA polymerase γ. Following oral administration, peak plasma levels of ETV are reached between 0.5 to 1.5 h. The drug is minimally metabolized, primarily to glucuronide and sulfate conjugates. The drug is eliminated primarily in the urine via glomerular filtration and tubular secretion (Matthews, 2006; Yamanaka *et al*., 1999). The most common adverse side effects of ETV therapy include headache, upper respiratory tract infection, cough, abdominal pain, diarrhoea, fatigue, dizziness and nausea (Matthews, 2006). ETV has a low protein-binding capacity (13), 100% oral bioavailability and an apparent volume distribution in excess of total body water. It is predominantly eliminated in the urine via glomerular filtration and tubular secretion, with urinary recovery of unchanged drug to range from 62 to 73% of the administered dose.

In this study, molecular modelling analyses have been carried out using the program Spartan '04 (Spartan, 2004) to investigate the relative stability of ETV and its metabolites with the aim of providing a better understanding on their relative toxicity. The study was carried out in the Discipline of Biomedical Science, Faculty of Medicine, The University of Sydney during January to March 2007.

## COMPUTATIONAL METHODS

The geometries of ETV and its metabolites ETV-MP, ETV-DP and ETV-TP (Fig. 1) have been optimised based on molecular mechanics, semi-empirical and DFT (Density Functional Theroy) calculations, using the molecular modelling program Spartan '04. Previous studies have shown that Spartan'04 are able to attain optimized geometry quickly (Huq, 2006). Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried at B3LYP/6-31G* level. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimised structures, single point calculations were carried out to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO (Highest Occupied Moleculer Orbital) and LUMO (Lowest Occupied Moleculer Orbital). The order of calculations: Molecular mechanics followed by semi-empirical followed by DFT ensured that the structure was not embedded in a local minimum. To further check whether the global minimum was reached, some calculations were carried

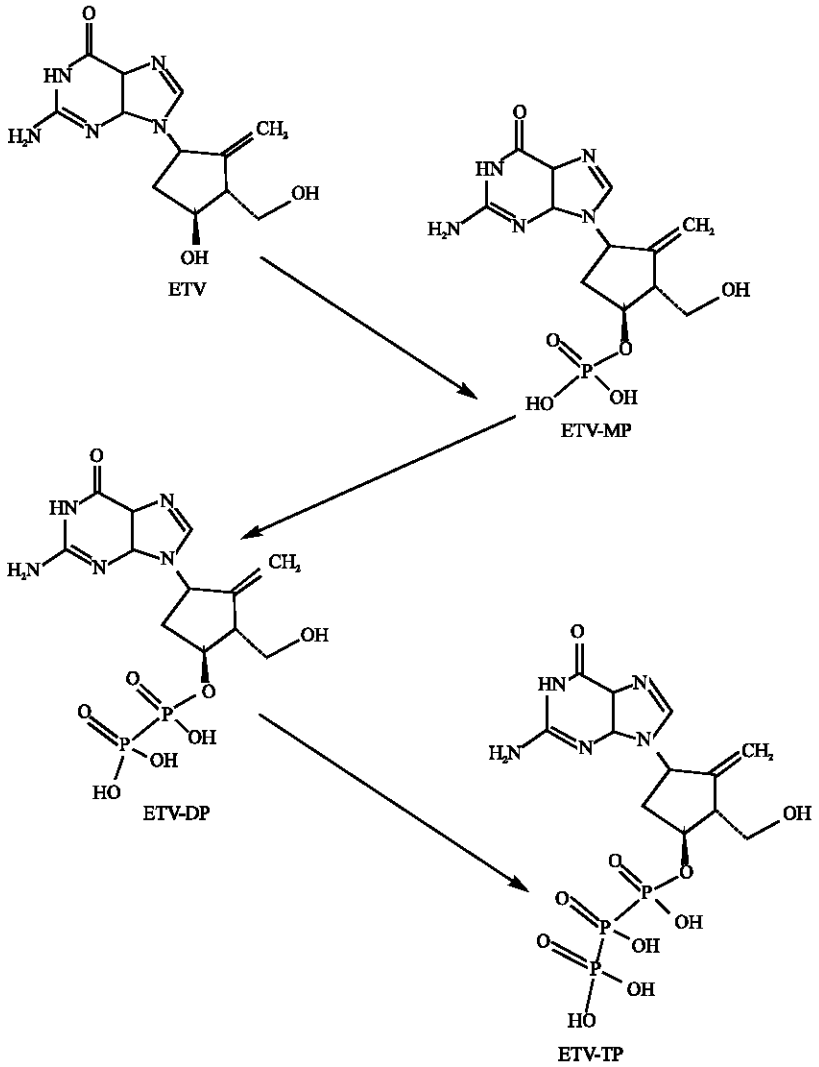



Fig. 1 Metabolic pathway for ETV

out with improvable structures. It was found that when the stated order was followed, structure corresponding to the global minimum or close to that could ultimately be reached in all cases. Although RMS gradient of 0.001 may not be sufficiently low for vibrational analysis, it is believed to be sufficient for calculations associated with electronic energy levels.

## RESULTS AND DISCUSSION

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, surface area, volume, dipole moment and energies of HOMO and LUMO as per both PM3 and DFT calculations for ETV and its metabolites ETV-MP, ETV-DP and ETV-TP. Fig. 2-5 give the regions of negative electrostatic potential (greyish-white envelopes) in (a), HOMOs (where red indicates HOMOs with high electron density) in (b), LUMOs in (c) and density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to optimised structures of ETV, ETV-MP, ETV-DP and ETV-TP.

The LUMO-HOMO energy differences for ETV and its metabolites ETV-MP, ETV-DP and ETV-TP from DFT calculations are respectively 5.35, 4.92, 4.78 and 4.72 eV, indicating that ETV is most kinetically inert and ETV-TP would be least inert.

In the case of ETV, ETV-MP, ETV-DP and ETV-TP, the electrostatic potential is found to be more negative around the oxygen centres, indicating that the positions may be subject to electrophilic attack.

In the case of ETV, ETV-MP, ETV-DP and ETV-TP, the HOMOs with high electron density are found to be close to the non-hydrogen atoms of the fused rings whereas the LUMOs are found to be close to the non-hydrogen atoms of the five-membered carbon ring.

The overlap of HOMO with high electron density and region of negative electrostatic potential at some positions, gives further support to the idea that the positions may be subject to electrophilic attack.

The molecular surfaces of ETV, ETV-MP, ETV-DP and ETV-TP are found to possess neutral (green) negative (yellow and red) and some electron-deficient (blue) regions so that they may

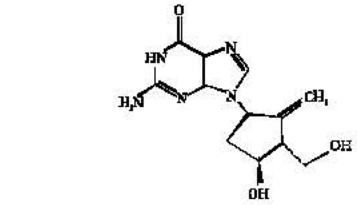
Table 1: Calculated thermodynamic and other parameters of ETV and its metabolites

| Molecule | Calculation type | Total energy (kcal $mol^{-1}$/ atomic unit*) | Heat of formation (kcal $mol^{-1}$) | Enthalpy (kcal $mol^{-1}$ $K^{-1}$) | Entropy (kcal $mol^{-1}$ $K^{-1}$) | Free energy (kcal $mol^{-1}$) |
|---|---|---|---|---|---|---|
| ETV | PM3 | | -68.07 | 698.38 | 565.23 | 533.94 |
| | DFT | -926.42 | | 701.33 | 564.13 | 533.29 |
| ETV-MP | PM3 | | -264.01 | 849.71 | 642.98 | 658.01 |
| | DFT | -1495.15 | | 850.94 | 641.68 | 659.72 |
| ETV-DP | PM3 | | -484.21 | 847.49 | 764.53 | 619.55 |
| | DFT | -2061.87 | | 848.91 | 763.67 | 621.34 |
| ETV-TP | PM3 | | -683.64 | 925.31 | 838.47 | 675.31 |
| | DFT | -2629.58 | | 926.78 | 837.34 | 677.25 |

Table 1: Continued

| Molecule | Calculation type | Area ($Å^2$) | Volume (Å3) | Dipole moment (debye) | HOMO (eV) | LUMO (eV) | HOMO-LUMO (eV) |
|---|---|---|---|---|---|---|---|
| ETV | PM3 | 263.61 | 242.03 | 3.0 | -9.02 | -0.73 | 8.29 |
| | DFT | 260.12 | 239.95 | 3.8 | -5.97 | -0.62 | 5.35 |
| ETV-MP | PM3 | 325.68 | 301.38 | 4.4 | -8.90 | -0.60 | 8.30 |
| | DFT | 306.60 | 283.85 | 2.9 | -5.94 | -1.02 | 4.92 |
| ETV-DP | PM3 | 376.56 | 333.90 | 3.8 | -8.98 | -0.69 | 8.29 |
| | DFT | 351.01 | 327.11 | 5.4 | -6.33 | -1.55 | 4.78 |
| ETV-TP | PM3 | 418.50 | 376.47 | 2.1 | -9.08 | -0.78 | 8.30 |
| | DFT | 407.16 | 371.97 | 5.9 | -6.33 | -1.61 | 4.72 |

* In atomic units from DFT calculations



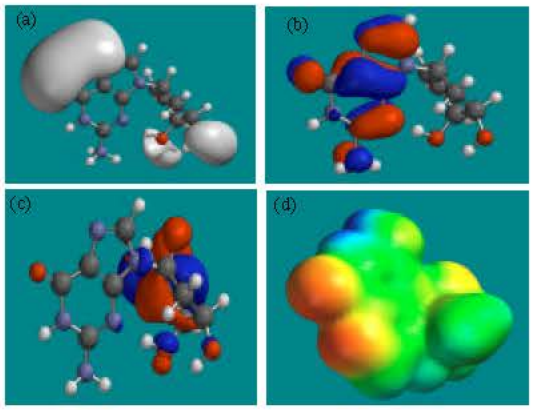
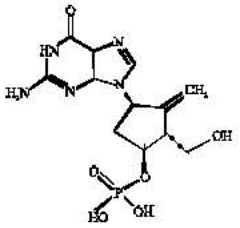



Fig. 2: Structure of ETV giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)



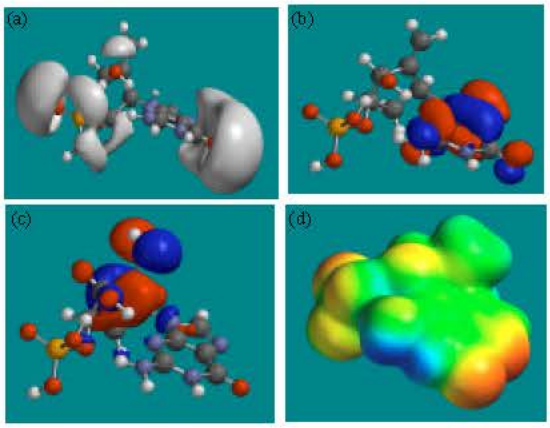



Fig. 3: Structure of ETV-MP giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

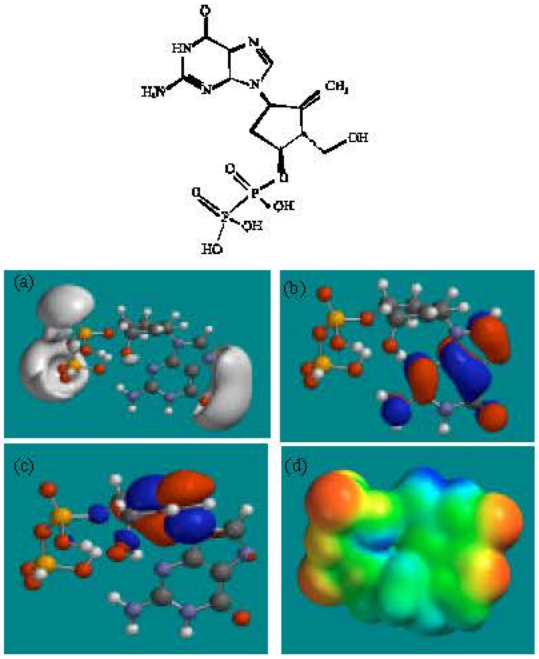



Fig. 4: Structure of ETV-DP giving in: (a) the electrostatic potential greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

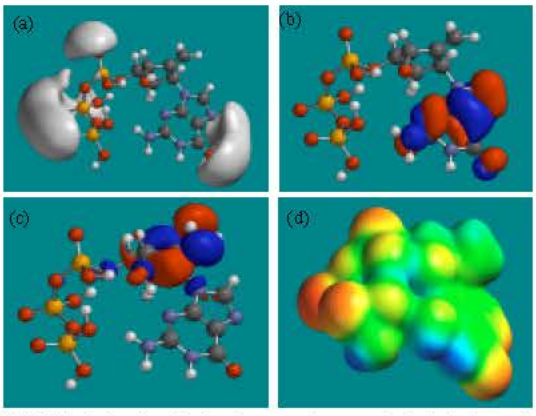



Fig. 5: Structure of ETV-TP giving in: (a) the electrostatic potential greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

be subject to lyophilic, electrophilic and nucleophilic attacks. Nucleophilic attacks can be due to glutathione and nucleobases in DNA. Reaction with glutathione can induce cellular toxicity by compromising the antioxidant status of the cell whereas that with nucleobases in DNA can cause DNA damage. However, as stated earlier, since ETV and its metabolites are expected to be kinetically inert, the rate of such adverse reactions may be low unless speeded up enzymatically.

## CONCLUSION

Entecavir (ETV) is a cyclopentyl guanosine analogue launched in 2005 for the once-daily treatment of chronic hepatitis B virus (HBV) infection that affects two billion people worldwide. The most common adverse side effects of ETV therapy include headache, upper respiratory tract infection, cough, abdominal pain, diarrhoea, fatigue, dizziness and nausea. In this study, molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations have been carried out to investigate the relative stability of ETV and its metabolites with the aim of providing a better understanding of their relative toxicity. The results of the analyses show that molecular surfaces of ETV and its metabolites have some electron-deficient regions so that they can react with glutathione and nucleobases in DNA. Reaction with glutathione can induce cellular toxicity associated with oxidative stress whereas oxidation of nucleobases can cause DNA damage. However, ETV and its metabolites are found to have large LUMO-HOMO energy differences so that they would be kinetically inert so that the rates of such adverse reactions may be low, unless speeded up enzymatically.

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