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► To cite this version:

Clifford W Fong. Efavirenz free radicals, oxidative stress and CNS toxicity in HIV infections and anti-cancer therapies: quantum mechanical study. [Research Report] Eigenenergy Adelaide, South Australia, Australia. 2022. <hal-03744488>

HAL Id: hal-03744488

<https://hal.science/hal-03744488v1>

Submitted on 3 Aug 2022

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Efavirenz free radicals, oxidative stress and CNS toxicity in HIV infections and anti-cancer therapies: quantum mechanical study

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Keywords: Efavirenz, oxidative stress, HIV, anticancer, free radicals, dissociative electron attachment, quantum mechanics

Abbreviations

Efavirenz EFV, Dolutegravir DTG, Reactive oxygen species ROS, Dissociative electron attachment DEA, Lipid peroxidation LPO, Transition state TS

Summary

Evidence is presented that EFV is quite labile in aqueous solution, forming free radical species by DEA, as well as undergoing photolysis. DTG can also form radical species similarly to EFV, but with more ease, but does not undergo dissociation. The DTG• radical is more stable than the EFV• radical. It is likely that these radical species are involved in oxidative stress in HIV infections and adverse neuropsychiatric side effects.

The free radical properties of EFV has implications for the use of EFV as an anticancer drug, particularly as a radiosensitizing effect could make it a promising drug in combination with radiotherapy.

Introduction

The effects of oxidative stress may contribute to several neurodegenerative conditions, such as Alzheimer's disease and Parkinson's disease. The brain is particularly vulnerable to oxidative stress because brain cells consume about 20 percent of the total amount of oxygen the body needs. Brain cells generate free radicals required for brain cell growth, neuroplasticity, and cognitive functioning. During oxidative stress, excess free radicals can damage structures inside brain cells and even cause cell death, which may increase the risk of Parkinson's disease. Oxidative stress may modify amyloid-beta peptides and contribute to the accumulation of amyloid plaques in the brain, a key marker of Alzheimer's disease. [1,2]

There is increased oxidative stress in HIV-infected patients, as measured by LPO and GSH levels in plasma of HIV-infected individuals. Lipid peroxidation (LPO) is a means of determining oxidative stress. There is also a deficiency of glutathione in HIV infection. Persistent oxidative load leads to an accelerated rate of consumption of glutathione (GSH). [3]

HIV infection leads to a pronounced oxidative stress, and triggers ROS production. The impact of HIV on antioxidant defense systems, and HIV-driven oxidative stress on the associated pathology has been analysed showing that reactive oxygen species underlie a wide spectrum of events in infected cells and tissues. [4]

Efavirenz (EFV) is an approved antiretroviral medication used to treat and prevent HIV/AIDS. It is generally recommended for use with other antiretrovirals. It is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and works by blocking the function of reverse transcriptase. The adverse effects of EFV include neuropsychiatric effects, disturbed sleep, cognitive impairment and depression. The neurotoxicity of EFV appears to be caused by interfering with the mitochondrial function, which may be caused by inhibiting creatine kinase, by disrupting mitochondrial membranes or by interfering with nitric oxide signalling. The neuropsychiatric adverse effects are dose-dependent. [5]]

Pharmacologically relevant concentrations of EFV can impair cell viability of endothelial cells and that these effects may be aggravated by either elevated concentrations of EFV or by a combined use of EFV with other oxidative stress-inducing medications. [6] EFV is associated with increases in apoptosis, increased mitochondrial mass, and oxidative stress in hepatic cells. EFV causes a decrease in ATP concentration and increases ROS production, which leads to an elevation in the lipid content of hepatic cells. [7] EFV has been shown to affect mitochondrial function in a reversible manner, inducing a decrease in mitochondrial membrane potential and an increase in mitochondrial superoxide production, followed by a reduction in cellular glutathione content. The toxic effect of EFV was partially reversed by antioxidant pretreatment, suggesting ROS generation is involved in this effect. [8]

It has been shown that EFV has a radiosensitizing effect making it a promising drug for combination with radiotherapy. The efficacy of EFV combined with irradiation was assessed in BxPC-3 pancreatic cancer cells. The antitumor potential of EFV and radiotherapy are additive. Oxidative stress and mitochondrial membrane depolarization appeared in the cancer cells immediately after EFV radiotherapy treatment. [9]

The World Health Organization 2019 recommends dolutegravir (DTG) as the first- and second-line treatment for all persons with HIV. DTG and EFV side effects involve mitochondrial dysfunction impairing both mitochondrial ATP generation and REDOX activity.[10] DTG toxicity was associated with reproductive deficits and oxidative stress induction in *D. melanogaster* resulting in reduced fly fecundity, mean lifespan, AChE activity, antioxidant parameters, and elevated MDA level. It was inferred that there were concerns for long term use of DTG by HIV patients. [11]

DTG is superior to EFV for HIV antiretroviral therapy, but may be associated with an increased risk of neural tube defects in newborns if used by women at conception. Neuropsychiatric adverse effects with EFV are by far the most common reasons for discontinuation of treatment. Persistence of central nervous system toxicity, which may impact quality of life negatively on a long-term basis is the main reason for discontinuation of EFV. [12,13]

DTG is an integrase strand transfer inhibitor (INSTI). These inhibitors halt the HIV integrase enzyme from forming covalent bonds between host-cell and viral DNA, which prevents effective incorporation of the viral DNA into the host chromosome. These drugs have been associated with less peripheral toxicities than other classes, and less neuroglial toxicity.

EFV has also been shown to be an effective repurposed therapeutic against prostate, pancreatic and triple negative breast cancer (TNBC). The fatty acid metabolism pathway is a key regulator in EFV-induced TNBC anticancer processes. [14] DTG has been shown to be active against many cancer cell lines, and its antiproliferative potency was positively correlated with the expression levels of the human endogenous retrovirus type K. [15]

Study objectives

- (a) Examine the free radical properties of EFV and its role in oxidative stress that may be related to how this drug may cause neuropsychiatric side effects, or have anticancer properties
- (b) Compare the properties of free radicals formed by EFV and DTG and how these properties may be involved in oxidative stress

Results

The formation of free radicals from EFV and DTG is examined in water. Electrons resulting from oxidative stress via mitochondrial and lipid sources can attach to EFV and DTG. Figure 1 shows EFV and DTG. Figure 2 shows two configurations of EFV, named EFV the most stable configuration and EFV_{alt} a less stable alternate configuration (by ca. ΔG -42.3 kcal/mol). The EFV configuration has C-O 1.46Å and \angle O-C-CF₃ 105deg compared to the less stable EFV_{alt} configuration which has C-O 1.57Å, \angle O-C-CF₃ 151deg. To test the accuracy of the structures and computational algorithms, the UV spectra of the EFV configuration in CHCl₃, CH₃CN and CH₃OH solution were shown to closely replicate experimental data.[16] (see Materials and methods) The UV spectra of the EFV_{alt} configuration did not match the experimental spectra.

It is also known that UV photolysis (ca 95 and 112 kcal/mol UV sources), of EFV in water or human plasma leads to decarbonylation [16] or decarboxylation [17] respectively. Also thermolysis of EFV in water is known to lead to first order decarbonylation. [19] Figure 3 shows the UV photolysis as measured by adiabatic TD DFT of the EFV and EFV_{alt} configurations and the TS for the EFV photolysis, ΔG^a 33.2 kcal/mol (excluding UV hv energy input) from the ground state with C--O 1.46Å to the TS C--O 2.20Å. These computed data are clearly in accord with experimental results since the energy of the UV photolysis sources exceed ΔG^a . Both images clearly show elongation of the C--O bond as expected for decarbonylation or decarboxylation to occur. The bond elongation is clearly more facile in the less stable EFV_{alt} configuration compared to the stable EFV configuration as expected.

Figure 4 shows the vertical addition of a hydrated electron (ΔG -34.6 kcal/mol, [19]) to the stable EFV configuration, EFV• with $\Delta\Delta G$ -39.69 - -34.6 = -5.1kcal/mol, and C-O bond length 1.46Å. Figure 5 then shows the adiabatic optimization of EFV• via the dissociated electron attachment or transfer (DEA or DET) mechanism [20-25] resulting in a C-O elongation to 2.81Å from 1.46Å, with the TS ΔG^* -44.5 kcal/mol. This indicates that EFV• species is reactive and dissociates easily to radical precursors leading to decarbonylation or decarboxylation, similarly to that observed in photolysis of EFV.

Figures 6 and 7 show the same process (as Figs 4 and 5) of addition of a hydrated electron to the less stable EFV_{alt} configuration followed by DEA with a starting C-O bond length of 1.57Å then

elongating to 1.78Å after DEA. As expected both processes are more facile since the starting configuration EFV^{alt} is less stable than EFV.

Figure 8 shows the thermolysis (at 298K) of EFV^{alt} in water, which is a quite facile process for the less stable configuration. Thermolysis does not occur easily for the more stable EFV configuration.

Figure 9 shows the addition of a hydrated electron to DTG with $\Delta\Delta G$ -15.0kcal/mol. Compared to Figure 4 which shows ΔG -5.1kcal/mol for the addition of an electron to EFV, indicating that DTG more easily forms DTG• than EFV• in water, probably due to the greater delocalization of the electron in the molecular ring structure of DTG• compared to EFV•.

Discussion

It is shown that EFV is quite reactive to UV photolysis in water solution, but is particularly reactive to the addition of hydrated electrons. The results for EFV show that it is quite labile to decarbonylation or decarboxylation caused by facile elongation of the C-O bond upon electron addition followed by DEA. EFV can exist in less stable minor configurations in solution which are more reactive to photolysis and DEA. Figure 10 shows the photolysis and DEA for EFV which can finally result in the formation of CO₂ or CO and further radical species after bond elongation of the tertiary C---O bond.

Since HIV infection is known to lead to pronounced oxidative stress which triggers ROS production, the facile ease of free radicals formed from EFV is highly likely to be an important contributing factor. The neurotoxicity of EFV is also thought to be caused by interfering with the mitochondrial function, implicating free radical forms of EFV in the neurotoxicity. It is known that the toxic effect of EFV was partially reversed by antioxidant pretreatment, suggesting ROS generation is involved in this effect.

EFV is known to have a radiosensitizing effect, which is consistent with the ease it forms free radical species. The antitumor potential of EFV and radiotherapy are additive. Oxidative stress and mitochondrial membrane depolarization appear in the cancer cells immediately after EFV radiotherapy treatment. These data show that the free radical properties of EFV make it a promising anticancer drug for combination with radiotherapy.

DTG is known to be superior to EFV for HIV antiretroviral therapy, mainly because of neuropsychiatric adverse side effects with EFV. DTG and EFV side effects involve mitochondrial dysfunction impairing both mitochondrial ATP generation and REDOX activity, but DTG is less toxic. This study shows that DTG more easily forms DTG• than EFV• in water, but DTG• does not form further radical species as EFV• ie DTG• is more stable than EFV•. DTG is also more lipophilic than EFV, aiding cell membrane permeability.

Conclusions

Evidence is presented that EFV is quite labile in aqueous solution, forming free radical species by DEA, as well as undergoing photolysis. DTG can also form radical species similarly to EFV,

but with more ease, but does not undergo dissociation. The DTG• radical is more stable than the EFV• radical. It is likely that these radical species are involved in oxidative stress in HIV infections and adverse neuropsychiatric side effects.

The free radical properties of EFV has implications for the use of EFV as an anticancer drug, particularly as a radiosensitizing effect could make it a promising drug in combination with radiotherapy.

Materials and methods

All calculations were carried out using the Gaussian 09 package. Energy optimizations were at the DFT/B3LYP/6-31G(d,p) (6d, 7f) level of theory for all atoms in water, with the empirical dispersion correction GD3BJ. Selected optimizations at the DFT/B3LYP/6-31+G(d,p) (6d, 7f) with the empirical dispersion correction GD3BJ level of theory gave very similar results to those at the lower level. DFT, TD DFT and thermochemical energy calculations and molecular electrostatic potential surface maps were conducted at the B3LYP/6-31+G(d) GD3BJ using the IEFPCM/SMD solvent model in water. TD DFT calculations for EFV UV and photolysis studies also showed UV spectral calculations in CHCl₃ (4.98eV, 249nm, osc 0.28), CH₃CN (4.99eV, 249nm, osc 0.25) and methanol (4.98eV, 249nm, osc 0.26) of the EFV configuration were very close to the experimental values (247, 246, 248 nm). The UV spectra in water showed 4.97eV, 249nm, osc 0.254. The free energy of the hydrated solvated electron is -34.6 kcal/mol. [19] C-O bond elongation studies were not all transition states but were demonstrations of the ease of bond elongation during adiabatic optimizations under certain conditions eg vertical addition of an electron in water to EFV followed by adiabatic optimization to reveal elongation of the C-O bond, or photolysis of EFV as measured by adiabatic TD DFT UV. TSs had one negative vibration frequency. EFV UV photolysis in water conducted at 315-280 nm (ca 95 kcal/mol) [16] and in human plasma at 254 nm (112 kcal/mol) [17]. Lipophilicity measurements: EFV CDS n-octanol 1.03 kcal/mol compares with DTG CDS n-octanol 2.34 kcal/mol

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Fig 1. Efavirenz (EFV) (L) and Dolutegravir (DTG) (R)

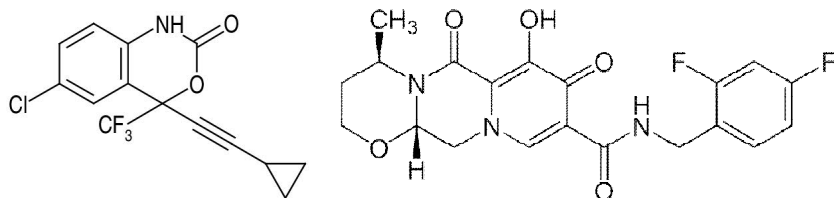
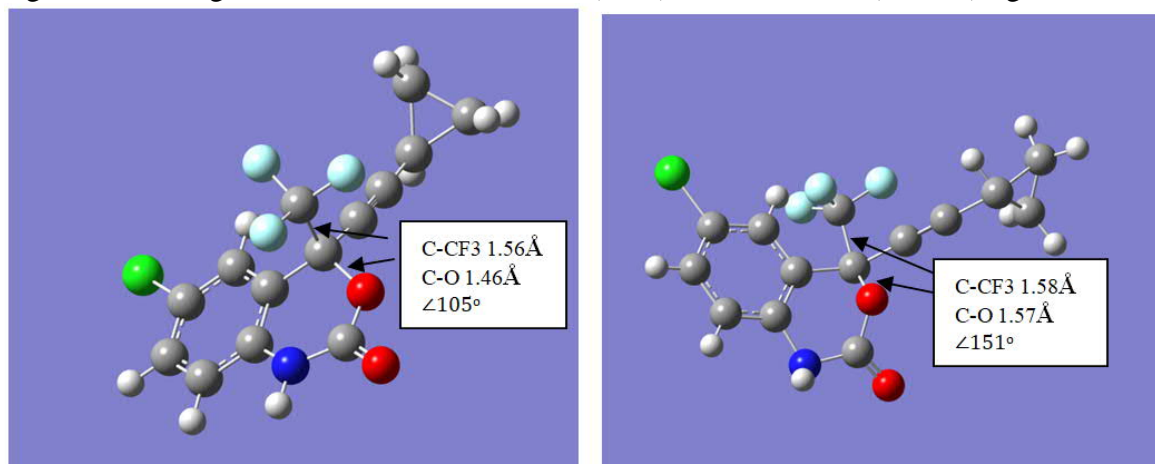


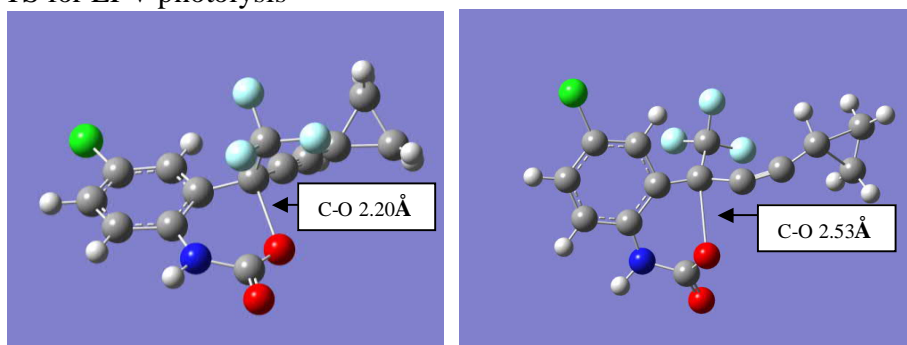
Fig 2. Two configurations of EFV, most stable (EFV) left, less stable (EFValt) right, in water



EFV C-O 1.46Å C-CF₃ 1.56Å ∠O-C-CF₃ 105deg

EFValt C-O 1.57Å C-CF₃ 1.58Å, ∠O-C-CF₃ 151deg

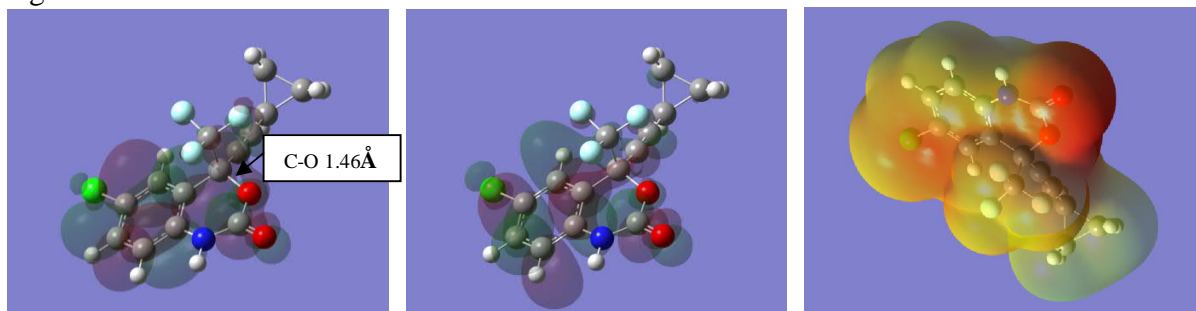
Fig 3. Photolysis EFV and EFValt configurations in water showing C-O bond elongations and TS for EFV photolysis



EFV+hν C-O 1.46→2.20Å, ΔG[‡] 33.2kcal/mol

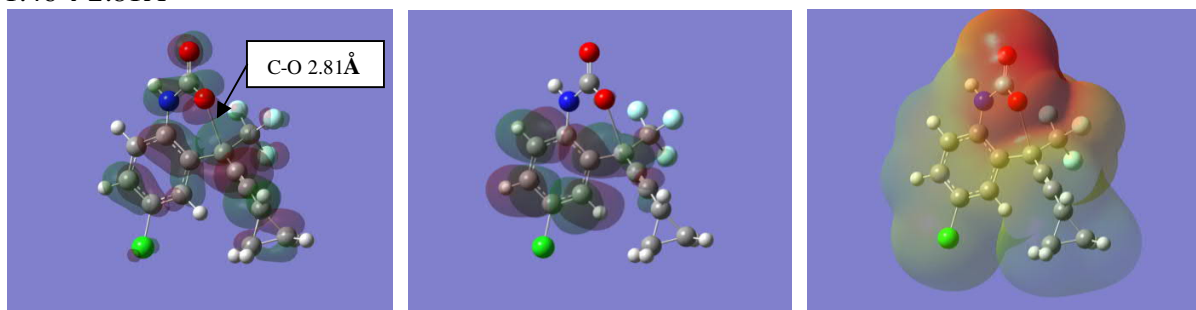
EFValt+hν C-O 1.44→2.53 Å, ΔΔG -217.5kcal/mol

Fig 4. Electron addition to EFV in water



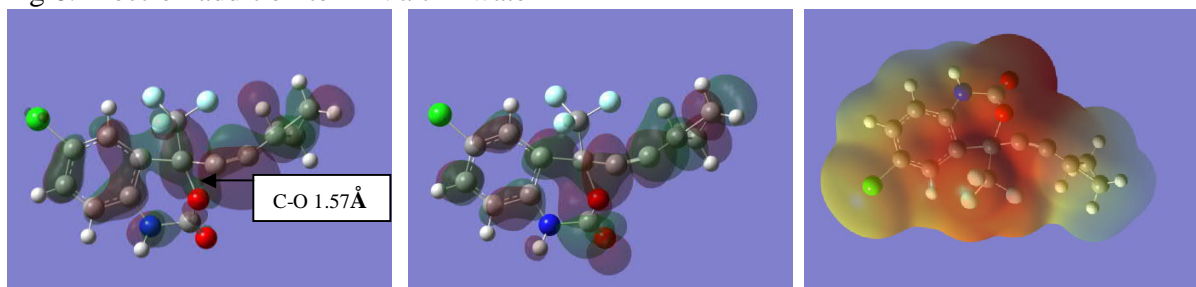
EFV + e^- C-O 1.46 Å, HOMO (L), LUMO(C), ESP(R) +/-0.183e0, $\Delta\Delta G$ -39.69 -- 34.6 = -5.1 kcal/mol

Fig 5. Dissociated electron attachment EFV in water, showing TS C-O bond elongation 1.46→2.81 Å



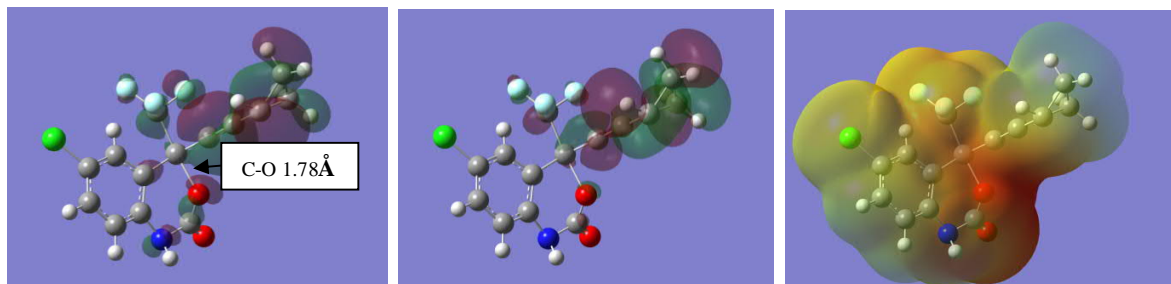
EFV + e^- DEA C-O 1.46→2.81 Å, HOMO (L), LUMO(C), ESP(R) +/-0.241e0, ΔG^* -44.5 kcal/mol

Fig 6. Electron addition to EFValt in water



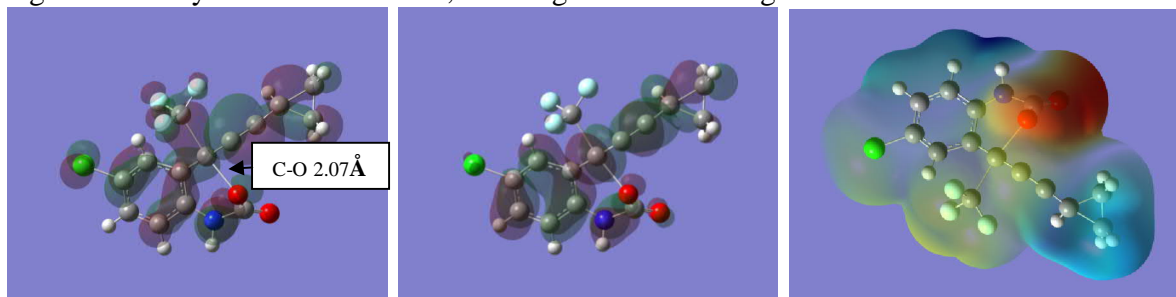
EFValt + e^- C-O 1.57 Å, HOMO (L), LUMO(C), ESP(R) +/-0.178e0, $\Delta\Delta G$ -82.0 --34.6 = -47.4 kcal/mol

Fig 7. Dissociated electron attachment EFValt in water, showing C-O bond elongation 1.57→1.78 Å



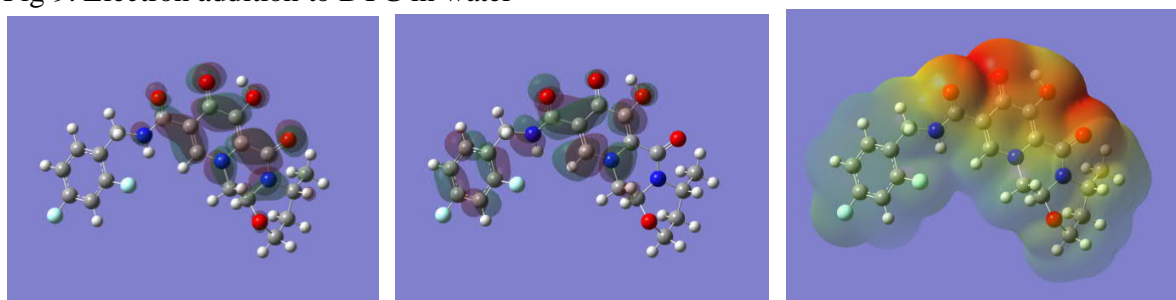
EFValt + e^- DEA C-O 1.57→1.78 Å, HOMO (L), LUMO(C), ESP(R) +/-0.213e0, $\Delta\Delta G$ -176.05 kcal/mol

Fig 8. Thermolysis EFValt in water, showing C-O bond elongation $1.57 \rightarrow 2.07 \text{ \AA}$



EFValt Thermal C-O $1.57 \rightarrow 2.07 \text{ \AA}$, HOMO (L), LUMO(C), ESP(R) $\pm 8.36 \times 10^{-2}$, $\Delta\Delta G -178.74 \text{ kcal/mol}$

Fig 9. Electron addition to DTG in water



DTG + e^- HOMO (L), LUMO(C), ESP(R) $\pm 0.267 \text{ eV}$, $\Delta\Delta G -15.00 \text{ kcal/mol}$

Fig 10. DEA and photolysis mechanism of EFV

