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Molecular Modelling Analysis of the Metabolism of Letrozole

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Abstract: Letrozole (LET) is a new orally active, potent and selective non-steroidal aromatase inhibitor that is currently in use for the treatment of hormone-sensitive advanced breast cancer in postmenopausal women. LET therapy is associated with minimal side effects except its adverse effects on bone metabolism of uncertain clinical significance. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that LET and its primary metabolite M1 have large LUMO-HOMO energy differences so that they would be kinetically inert. However, another likely metabolite M2 has a much smaller LUMO-HOMO energy difference - of 3.2 eV for from DFT calculations as compared to 5.3 eV for LET and 5.2 eV. This means that M2 would be highly reactive. The molecular surface of M2 is found to abound in electron-deficient regions so that it may be subject to significant nucleophilic attack by glutathione and nucleobases in DNA. Depletion of glutathione would induce cellular toxicity by compromising the antioxidant status of the cell whereas oxidation of nucleobases in DNA would cause DNA damage. Since M2 may not be forming in any significant, in actual fact the consequences of such adverse reactions may remain low.

Key words: Breast cancer, postmenopausal women, letrozole, aromatase inhibitor, molecular modelling

Introduction

Letrozole [1-(bis-(4-cyanophenyl)methyl)-1,2,4-triazole, Femar, LET, CGS 20 267] is a new orally active, potent and selective non-steroidal aromatase inhibitor, currently in use for the treatment of hormone-sensitive advanced breast cancer in postmenopausal women (Pfister *et al.*, 2001; Harper-Wynne *et al.*, 2002). For patients with hormone-receptor-postmenopausal breast cancer, the risk of relapse remains significant even after the successful completion of 5 years of adjuvant tamoxifen therapy (Goss, 2006).

Biotransformation of LET is the main mechanism of its elimination. Its primary metabolite is bis-4-cyanophenylmethanol (M1; CGP 44 645) which is a secondary alcohol produced from the replacement of triazole ring with the hydroxyl group. The glucuronide of M1 is the predominant species found in the urine (Sioufi *et al.*, 1997). M1 can be further oxidized to form the corresponding ketone (M2) but so far this has not been detected.

LET therapy is associated with minimal side effects except its adverse effects on bone metabolism of uncertain clinical significance (Goss, 2006). In this study molecular modelling analyses have been carried out of LET and its primary metabolite M1 and suspected metabolite M2 in order to obtain a better understanding of their toxicity. The study was carried out in the School of Biomedical Sciences, The University of Sydney during February to June 2006.

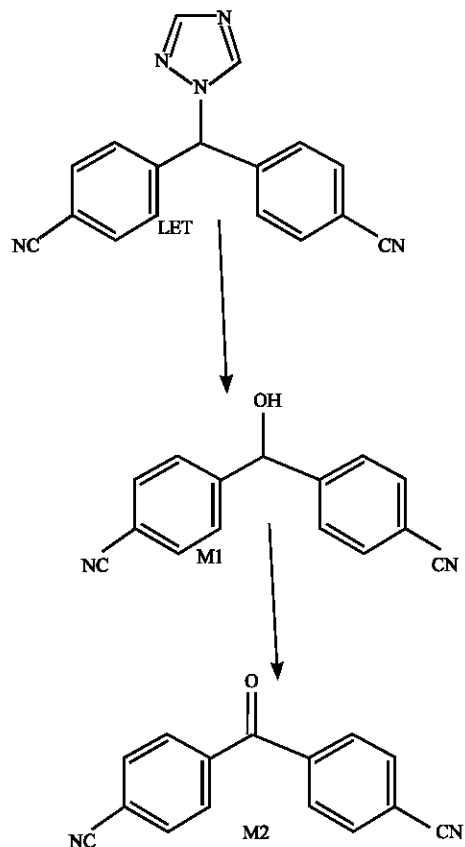


Fig. 1: Metabolic pathway of letrozole (Based on Marfil and Pineau *et al.*, 1996)

Computational Methods

The geometries of LET and its metabolites have been optimised based on molecular mechanics (Fig. 1), semi-empirical and DFT calculations, using the molecular modelling program Spartan '02. Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried using the program Spartan '02 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 to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO and LUMO. 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 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, energies of HOMO and LUMO as per both PM3 and

Table 1: Calculated thermodynamic and other parameters of LET 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 (cal mol ⁻¹ K ⁻¹)	Solvation energy (kcal mol ⁻¹ K ⁻¹)
LET	PM3	168.94	180.04	164.54	138.18	-11.10
	DFT	-928.15		166.73	137.11	-13.45
M1	PM3	67.55	76.24	142.37	124.08	-8.70
	DFT	-762.30		144.04	123.15	-10.37
M2	PM3	169.80	175.68	126.30	115.27	-5.88
	DFT	-760.97		127.89	114.18	-7.39

Molecule	Calculation type	Free energy (kcal mol ⁻¹)	Area (Å ²)	Volume (Å ³)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
LET	PM3	123.34	310.82	294.62	3.0	-10.32	-1.20	9.12
	DFT	125.87	312.26	294.40	3.9	-7.32	-1.98	5.34
M1	PM3	105.37	265.64	247.48	4.0	-10.03	-0.98	9.05
	DFT	107.34	265.64	247.48	5.1	-7.13	-1.93	5.20
M2	PM3	91.93	257.57	242.06	2.7	-10.14	-1.69	8.45
	DFT	93.86	257.57	242.06	3.7	-6.74	-3.55	3.19

* In atomic units from DFT calculations

DFT calculations for LET and its metabolites M1 and M2. Figure 2-4 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 the optimised structures of LET and its metabolites M1 and M2.

The calculated solvation energies of LET, M1 and M2 from PM3 calculations in kcal mol⁻¹ are, respectively -11.10, -8.70 and -5.88 and their dipole moments from DFT calculations are 3.9, 5.1 and 3.7, respectively. Relatively low values for solvation energy suggest that LET and its metabolites will have low solubility in water and higher solubility in lipids. This means that the compounds would have a low clearance rate. The hypothetical metabolite M2 has the lowest solvation energy indicating it would have the lowest solubility in water and highest solubility in lipid.

The calculated LUMO-HOMO energy difference is found to be high for LET and its metabolite M1 (5.34 and 5.20 eV for LET and M1, respectively from DFT calculations) and quite low for the M2 (3.19 eV from DFT calculations). This means that whereas LET and M1 would be kinetically inert, M2 would be highly labile.

The high lability of M2 and the presence of electron-deficient regions on its molecular surface (Fig. 4d) mean that M2 would react readily with glutathione and nucleobases in DNA. Reaction of M2 with glutathione would induce cellular toxicity by compromising the anti-oxidant status of the cell whereas the oxidation of nucleobases in DNA would cause DNA damage. As stated earlier although M2 is a likely metabolite of LET, it has not been detected so far. It is possible that M2 is formed only in small amounts that quickly disappear because of its high reactivity. However, if M2 is formed in any significant amount, the low rate of clearance and rapid reaction with glutathione and nucleobases in DNA, would make M2 a highly toxic metabolite. It may be noted the molecular surface of LET also abounds in electron-deficient reactions. However, the kinetic inertness of the molecule would provide protection against such adverse reactions.

In the case of LET, the electrostatic potential is found to be negative around nitrogen centres, indicating that the positions may be subject to electrophilic attack. In the case of M1 and M2, the electrostatic potential is found to be negative around nitrogen and oxygen centres, once again indicating that the positions may be subject to electrophilic attack.

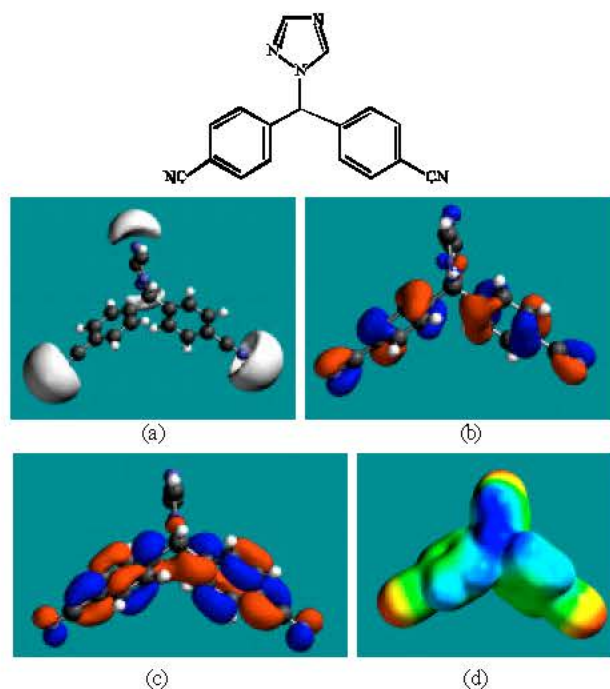


Fig. 2: Structure of LET giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative,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

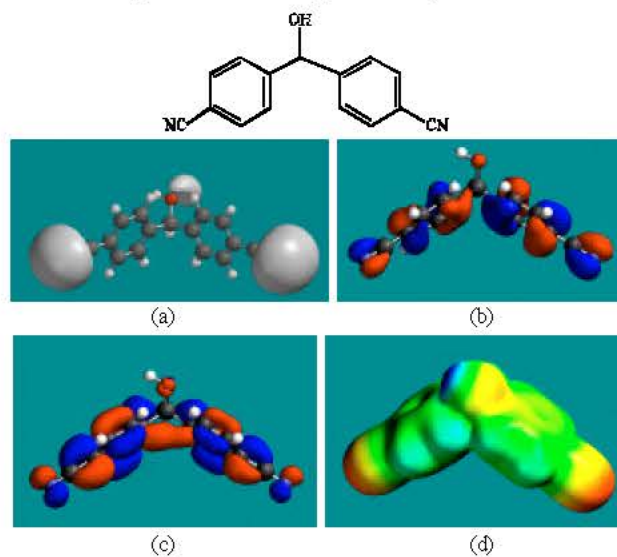


Fig. 3: Structure of M1 giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

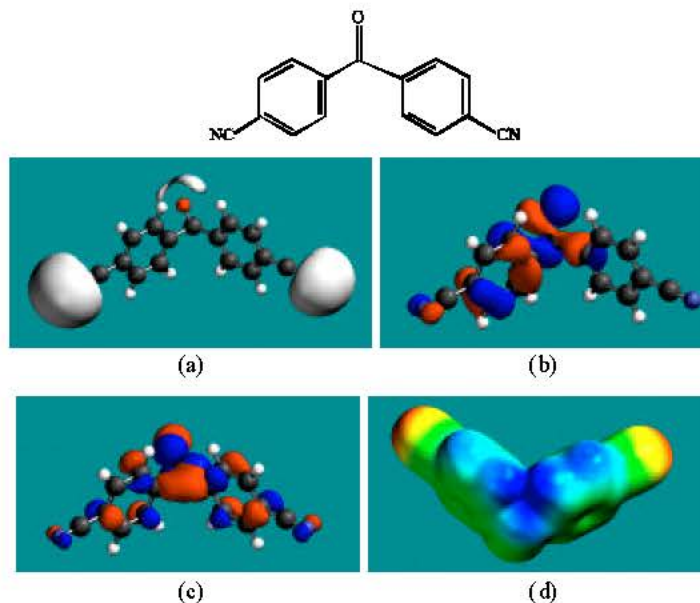


Fig. 4: Structure of M2 giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

When the surface area and volume of LET, M1 and M2 are compared, it is found that LET has distinctly different values from those of M1 and M2 so that M1 and M2 may not act as substrates for the receptors to which LET binds.

In the case of LET and M1, both the HOMOs with high electron density and LUMOs are found close to the almost all the non-hydrogen atoms. In the case of M2, the HOMOs with high electron density are found close some of the non-hydrogen atoms whereas LUMOs are found close to almost all the non-hydrogen atoms. Convergence or close proximity of positions of HUMOs with high electron density and positions of high electrostatic at some points give further support to the idea that the positions may be subject to electrophilic attack.

Conclusions

Molecular modelling analyses based on semi-empirical and LET calculations show that LET and its primary metabolite M1 have large LUMO-HOMO energy differences so that they would be kinetically inert. This means the rates of any possible reaction of LET and M1 with glutathione and nucleobases in DNA would be low. The suspected metabolite M2 would be much more reactive and would react readily with glutathione and nucleobases in DNA. However, as M2 has not been detected it may be concluded that it is not formed in any significant amount and therefore the consequences of such adverse reactions would be low.

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