Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (8): 748-752, 2007
ISSN 1816-496X

Molecular Modelling Analysis of the Metabolism of Clotrimazole

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Abstract: Clotrimazole (CTZ; bispheny l (2-chlorophenyl)-methan)) is an N-substituted imidazole drug that is used therapeutically as a topical antifungal agent. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that both CTZ and its major metabolite 2-chlorophenybiphenylmethanol (2CLBPM) have LUMO-HOMO energy differences so that they would be kinetically inert. The molecular surface CTZ is found to abound in electron-deficient regions so that it can react with cellular nucleophiles such as glutathione and nucleobases in DNA thus causing depletion of glutathione and oxidation of nucleobases. The former would induce cellular toxicity due to oxidative stress and the latter would cause DNA damage. However, because of kinetic inertness of CTZ, the rates of such adverse reaction are expected to be low unless speeded up enzymatically.

Key words: Clotrimazole, fungicide, CYP3A, molecular modelling

INTRODUCTION

Clotrimazole (CTZ) is an N-substituted imidazole drug that is used therapeutically as a topical antifungal agent. It impairs integrity of fungal cell membranes by inhibiting biosynthesis of membrane lipids, through inhibition of a fungal cytochrome P450 involved in ergosterol biosynthesis (Pappas and Franklin, 1993; Turan *et al*., 2001; Shah *et al*., 2001). At low concentrations, CTZ acts a fungistatic agent by inhibiting *de novo* synthesis of sterols required for the formation of cell membrane in actively growing organisms (Beam, 1992). At high concentrations (which are not achievable in clinical medicine), it plays a fungicidal role by causing direct damage and increased permeability of the fungal cell membranes. The drug has low solubility in water and is poorly absorbed after oral administration.

CTZ is a potent inhibitor of its own hepatic microsomal enzymes, resulting in progressively less drug being available during a course of therapy. CTZ has been found to be a potent inhibitor of 6 β-hydroxylation of testosterone in rat that is catalysed by CYP3A (Turan *et al*., 2001). It is also a potent *in vitro* inhibitor of mammalian cytochrome P450s including the ones responsible for the metabolism of xenobiotics (Sheets *et al*., 1986; Heuser and Franklin, 1985; Suzuki *et al*., 2000).

The main *in vivo* metabolite of CTZ is 2-chlorophenybiphenylmethanol (2CLBPM) produced from deamination reaction which is catalysed by CYP3A. CTZ binds to iron (III) cytochrome P450 to produce a Type II spectrum (Sheets *et al*., 1986) that is characteristic of many nitrogenous compounds. Figure 1 depicts the metabolic pathway for CTZ.

In this study, molecular modelling analyses have been carried out using the program Spartan '02 (2002) to investigate the relative stability of CTZ and its metabolites with the aim of providing a better understanding on their relative toxicity. Previous studies have shown that xenobiotics or their metabolites which are kinetically labile and abound in electron-deficient regions on the molecular surface tend to induce cellular toxicity due to glutathione depletion and cause DNA damage due to oxidation of nucleobases in DNA (Huq, 2006a, b).

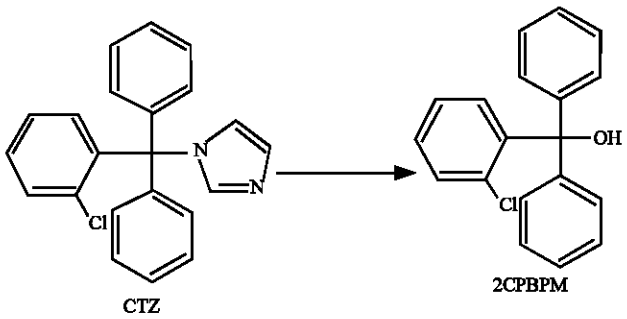

Fig. 1: Metabolic pathway for CTZ

MATERIALS AND METHODS

This being an entirely theoretical study, only molecular modelling calculations were carried out.

Computational Methods

The geometries of CTZ and its metabolite 2CPBPM have been optimised based on molecular mechanics, semi-empirical and DFT (density functional theory) calculations, using the molecular modelling program Spartan '04. Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT (Density functional theory) 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 Molecular Orbital) and LUMO (Lowest Unoccupied Molecular 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 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 (Huq and Alsheri, 2006).

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 CTZ and its metabolite 2CPBPM. Figure 2 and 3 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 CTZ and its metabolite 2CPBPM.

The LUMO-HOMO energy differences for CTZ and its metabolite 2CPBPM from DFT calculations are respectively 5.05 and 5.88 eV, indicating that both the compounds would be kinetically inert with 2CPBPM being the more inert one.

In the case of CTZ, the electrostatic potential is found to be more negative around nitrogen and chlorine atoms, indicating that the positions may be subject to electrophilic attack. In the case of 2CPBPM, the electrostatic potential is found to be more negative around chlorine and oxygen atoms and above and below the phenyl rings, indicating that the positions may be subject to electrophilic attack.

Table 1: Calculated thermodynamic and other parameters of CTZ and its metabolite 2CPBPM

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})	Free energy (kcal mol^{-1})
CTZ	PM3	116.96	125.05	218.67	146.52	174.98
	DFT	-1418.25		219.90	145.23	176.62
2CPBPM	PM3	31.78	35.48	188.96	134.14	148.96
	DFT	-1268.46		190.32	133.08	150.57

Molecule	Calculation type	Solvation energy (kcal mol^{-1})	Area (Å^2)	Volume (Å^3)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
CTZ	PM3	-8.09	341.05	352.96	5.0	-9.24	-0.36	8.88
	DFT	-8.79	344.06	354.41	5.3	-5.85	-0.80	5.05
2CPBPM	PM3	-3.70	303.00	302.11	1.5	-9.49	-0.07	9.42
	DFT	-4.45	306.26	304.21	2.0	-6.43	-0.55	5.88

*in atomic units from DFT calculations

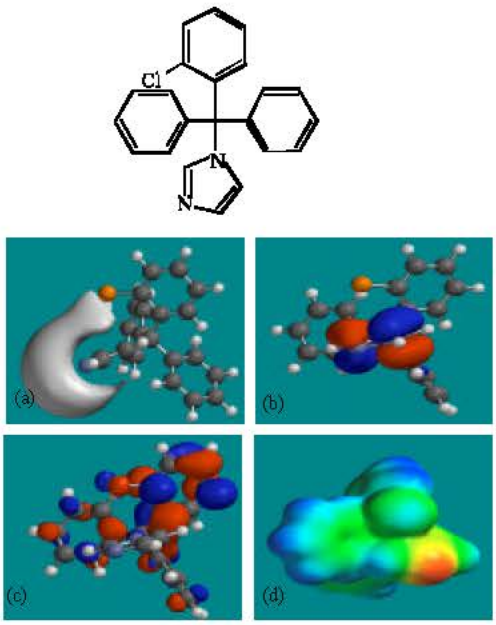

Fig. 2: Structure of CTZ giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density), (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

In the case of CTZ, the HOMOs with high electron density are found to be more localised than the LUMOs whereas in the case of 2CPBPM, they appear to have similar spreads.

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 surface of CTZ and 2CPBPM are found to possess negative (yellow and red), neutral (green) and electron-deficient (blue) regions so that they may be subject to electrophilic, lyophilic 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,

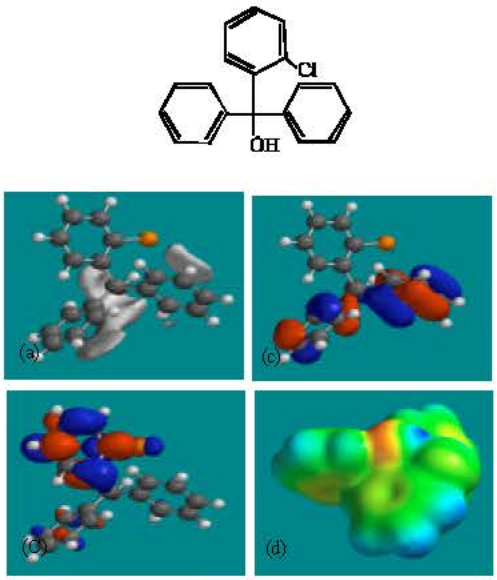

Fig. 3: Structure of 2CPBPM giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density), (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

since CTZ and 2CPBPM are expected to be kinetically inert, the rate of such adverse reactions may be low unless speeded up enzymatically. The results of the analyses thus show CTZ and its major metabolite may not induce much cellular toxicity or cause DNA damage.

CONCLUSION

CTZ is an N-subtituted imidazole drug that is used therapeutically as a topical antifungal agent. Molecular modelling analyses based on semi-empirical and DFT calculations show that both CTZ and its metabolite 2CPBPM have large LUMO-HOMO energy differences so that they would be kinetically inert. This means that although molecular surfaces of both the compounds have electron-deficient regions so that they can react with glutathione and nucleobases in DNA, the rates of such adverse reactions may be low, unless speeded up enzymatically. Slightly greater reactivity of CTZ and a greater presence of electron-deficient region on its molecular surface, may make the parent drug more toxic than its metabolite.

ACKNOWLEDGMENT

Fazlul Huq is grateful to the Discipline of Biomedical Science, School of Medical Sciences, The University of Sydney for the time release from teaching.

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