Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (4): 340-349, 2007
ISSN 1816-496X

Molecular Modelling Analysis of the Metabolism of Ceftiofur

Fazlul Huq
School of Biomedical Sciences, Faculty of Health Sciences, C42, The University of Sydney, P.O. Box 170, Lidcombe, NSW 1825, Australia

Abstract: Ceftiofur sodium (CF) is a third generation broad-spectrum cephalosphorin, that is active against both Gram-positive and Gram-negative pathogenic bacteria of veterinary importance and has been approved for subcutaneous treatment of certain respiratory diseases in cattle, horses, pigs, poultry and dogs. CF is rapidly metabolized to its active metabolite desfuroylceftiofur (DFC) and furoic acid (FA) after parenteral administration. DFC is further metabolized to disulfides such as desfuroylceftiofur dimer (DFC-D), desfuroylceftiofur cysteine disulfide (DFC-CYS) and desfuroylceftiofur glutathione (DFC-GS) and desfuroylcetiofur protein conjugate that may be playing a role in the activity and efficacy of CF. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that metabolite DFC-D has the lowest LUMO-HOMO energy difference so that it would be most reactive kinetically. CF and DFC are also expected to be significantly more labile than FA and DFC-CYS. The higher kinetic lability and the presence of electron-deficient regions on the molecular surfaces of DFC-D, CF and DFC mean that the compounds would react more readily with reduced form of glutathione and nucleobases in DNA. The depletion of glutathione level will induce cellular toxicity resulting from oxidative stress and oxidation of nucleobases in DNA will cause DNA damage. In actual fact, the effects of such adverse reactions may be lower in the case of most reactive metabolite DFC-D because of its much greater ease in excretion.

Key words: Gram-positive bacteria, streptococcus zooepidemiincus, cattle, horses ceftiofur sodium, molecular modelling

INTRODUCTION

Ceftiofur sodium (CF; (6R,7R)-((2-amino-4-thiazolyl)-Z-(methoxyimino)acetyl)amino-3-((2-+++furanylcarbonyl)thio)methyl-8-oxo-5-thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid) is a third generation broad-spectrum cephalosphorin that is active against both Gram-positive and Gram-negative pathogenic bacteria of veterinary importance (Becker *et al*. 2003). It has been approved in the EU for subcutaneous treatment of certain respiratory diseases in cattle, horses, pigs, poultry and dogs. In horses, CF has been found to be effective in the treatment and control of respiratory infections caused by *Streptococcus zooepidemiincus* (Folz *et al*., 1992) and also in the treatment against septic arthritis (Mills *et al*., 2000). CF can be administered as an i.m. or s.c. injection. It is minimally bioavailable after oral administration.

The metabolism of CF has been described in rats (Jaglan *et al*., 1989) and swine (Gilbertson *et al*., 1995). CF is rapidly metabolized to the active metabolite desfuroylceftiofur (DFC) and Furoic Acid (FA) after parenteral administration. The half-life of the parent drug is less than 10 min, because of rapid cleavage of the thioseter bond. DFC which has a free thiol group is further metabolized to disulfides such as desfuroylceftiofur dimer (DFC-D), desfuroylceftiofur cysteine disulfide (DFC-CYS) and desfuroylceftiofur glutathione (DFC-GS) and desfuroylcetiofur protein conjugate (Olson *et al*., 1998; Beconi-Barker *et al*., 1995 a,b; Glilbertson *et al*., 1995;

Beconi-Barker *et al*., 1995) which may be playing a role in the activity and efficacy of CF. CF has been shown to produce chromosomal aberrations following extended treatment of Chinese hamster ovary cells in culture at high doses (Aaron *et al*., 1995). The clastogenic effect due to CF is found to be due to prolongation of cell cycle. In this study molecular modelling analyses have been carried out to obtain information on the relative toxicity of CF and its metabolites. The study was done in The School of Biomedical Sciences, The University of Sydney during February to July 2006.

COMPUTATIONAL METHODS

The geometries of CF and its metabolites DFC, FA, DFC-D and DFC-CYS have been optimized based on molecular mechanics (Fig. 1) semi-empirical and DFT calculations, using the molecular

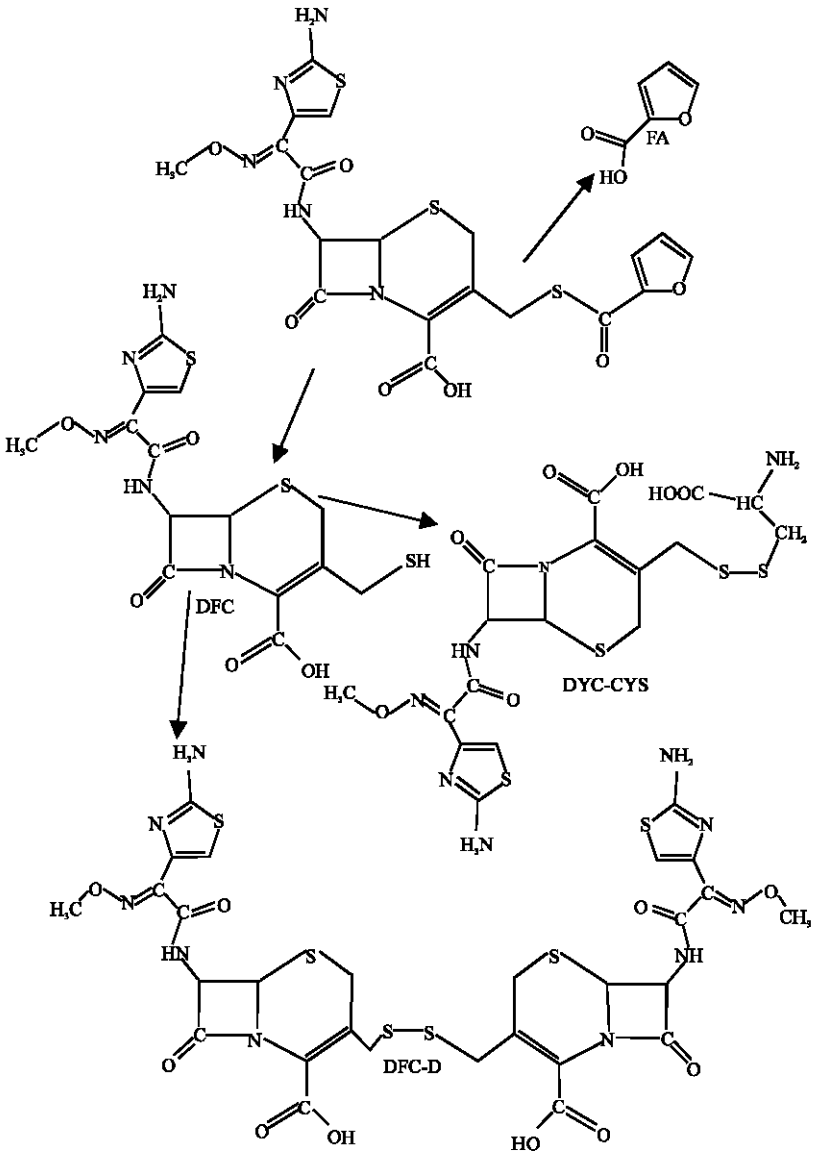


Fig. 1: Metabolic pathway for ceftiofur (Based on De Baere *et al*., 2004)

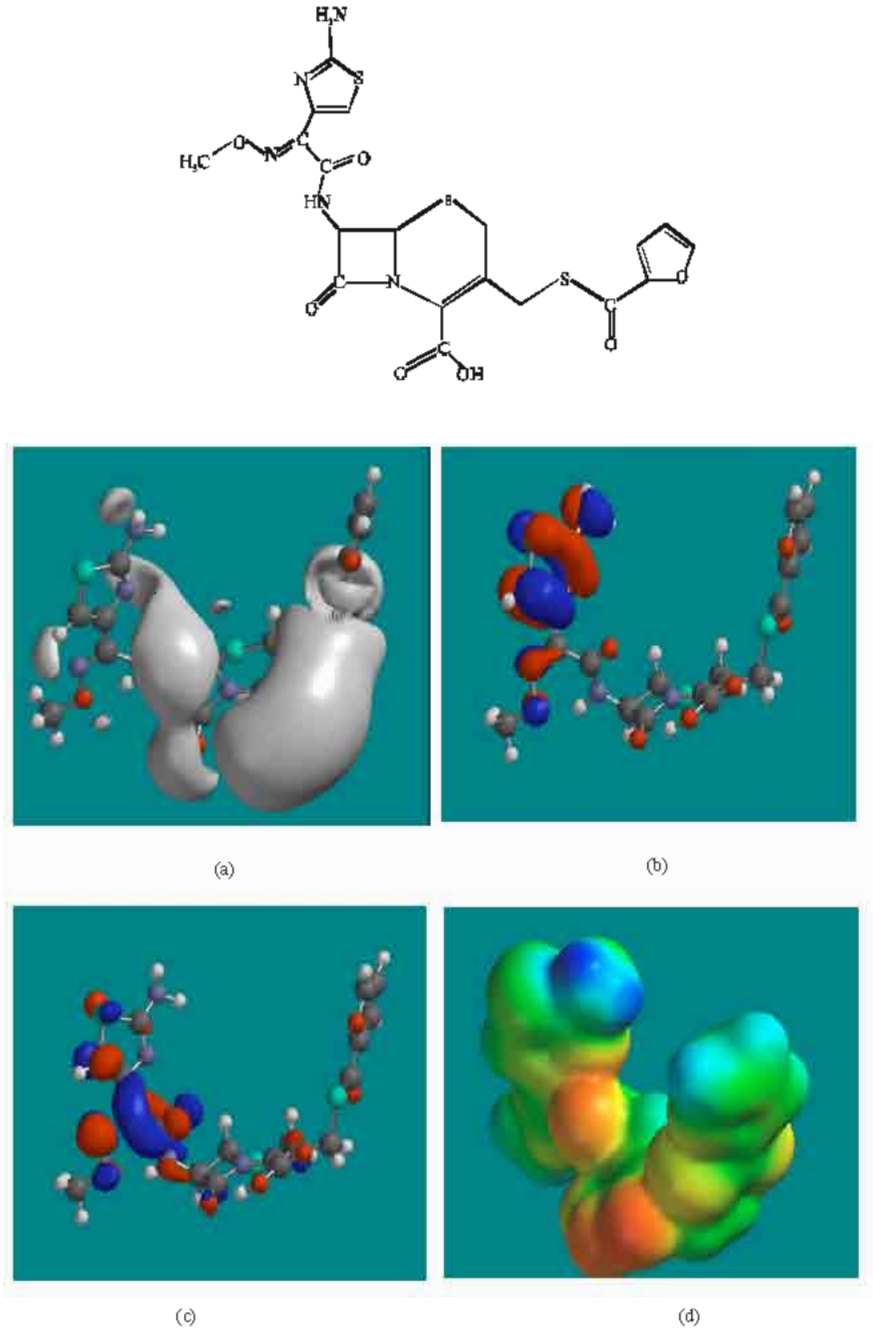


Fig 2 Structure of CF giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative,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

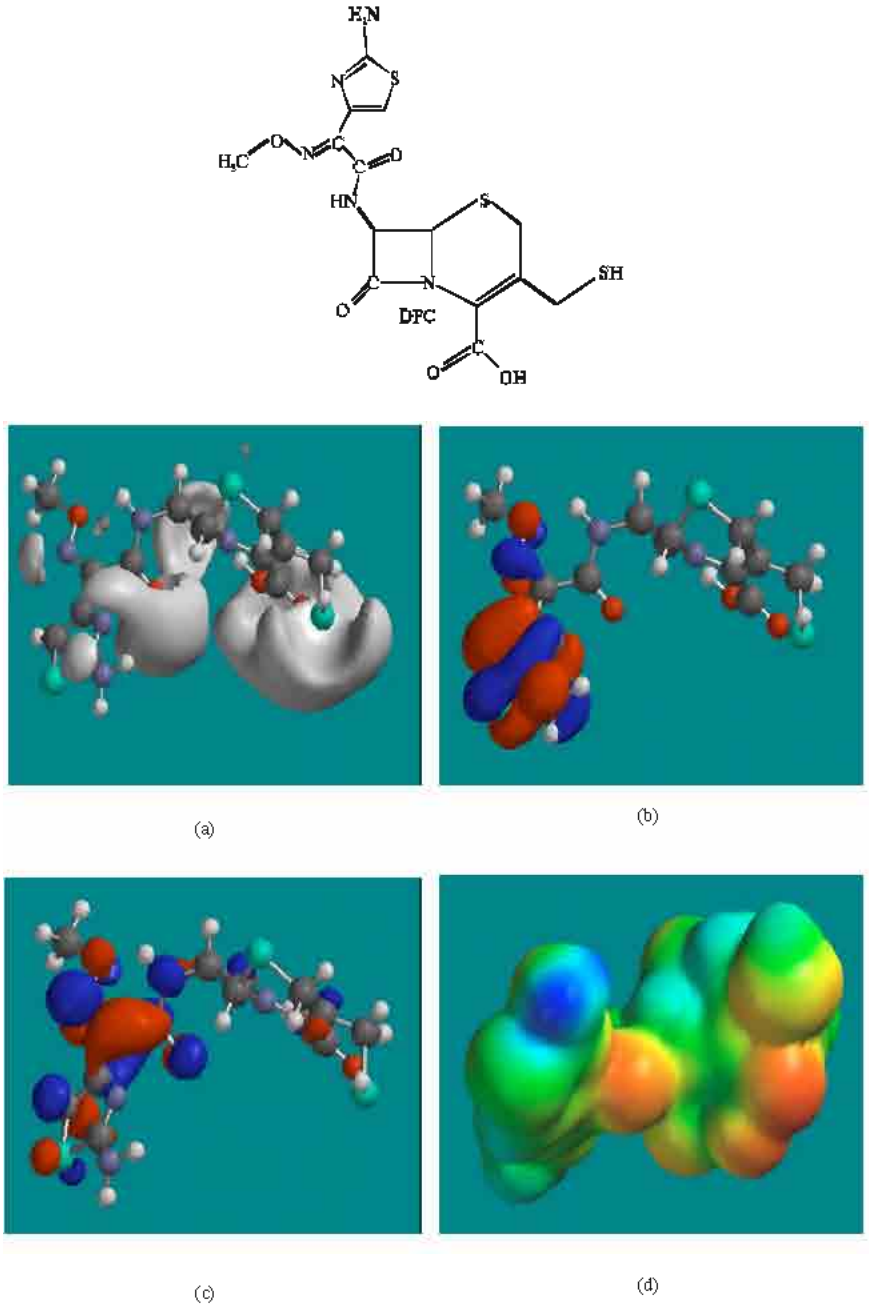


Fig 3 Structure of DFC giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates

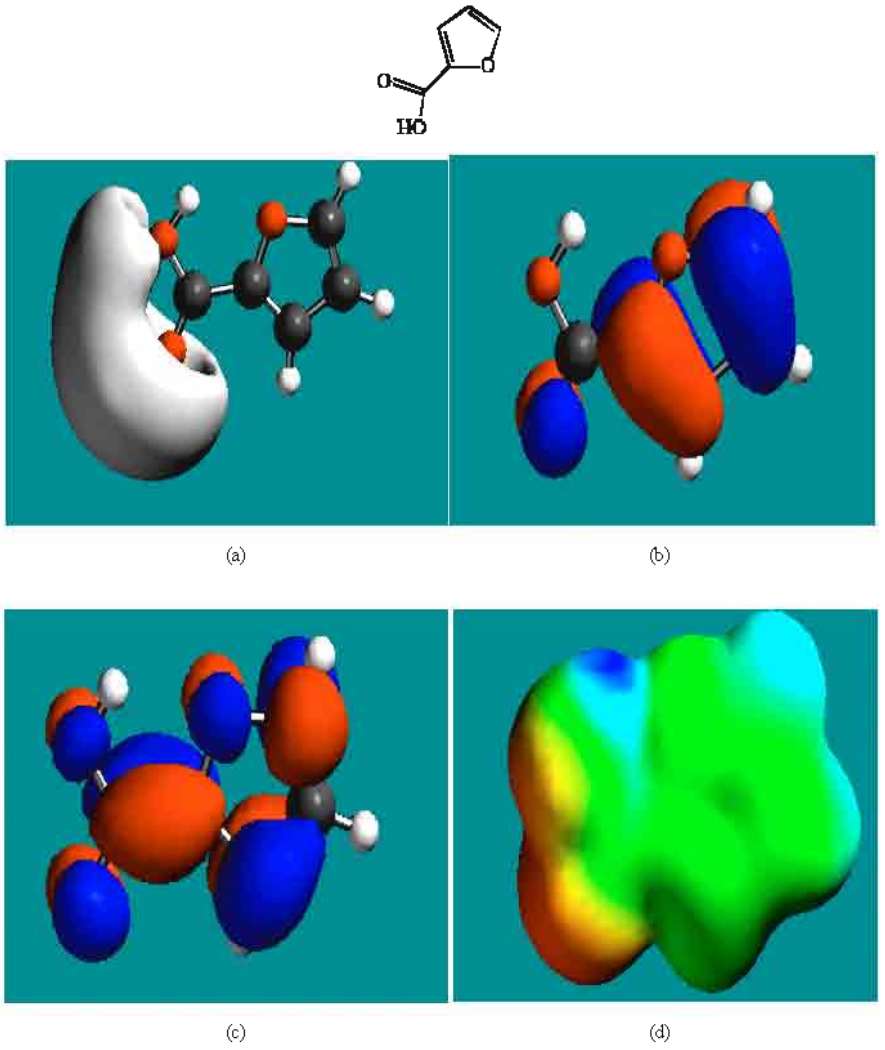
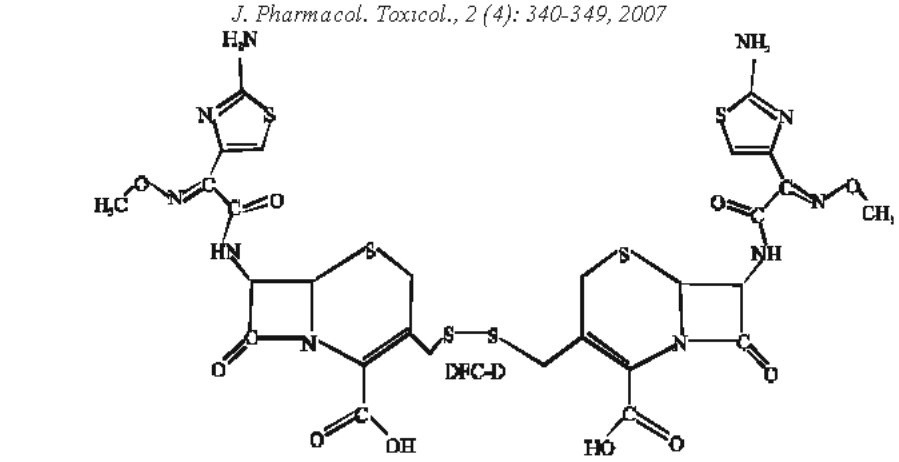
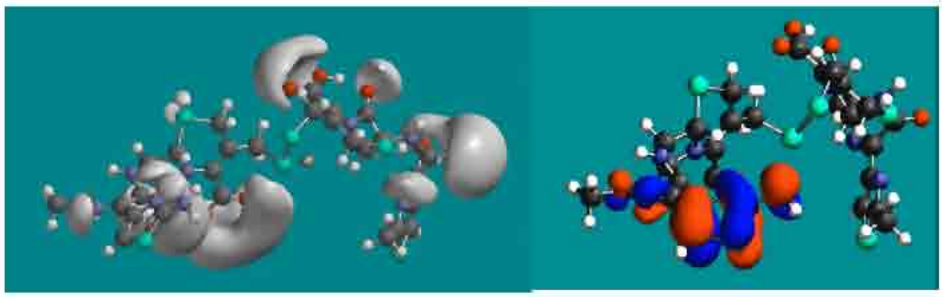


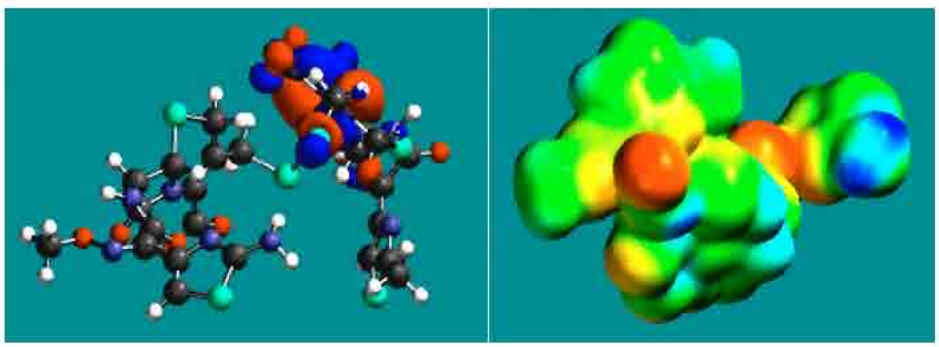
Fig. 4: Structure of FA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative,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

modelling program Spartan 02 (Spartan 2002). No calculations were done for DFC-GS. 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 optimized structures, single point calculations were carried out 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





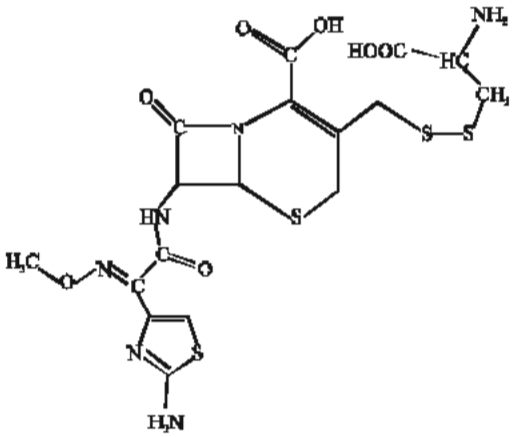
(a) (b)



(c) (d)

Fig. 5: Structure of DFC-D 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)

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.



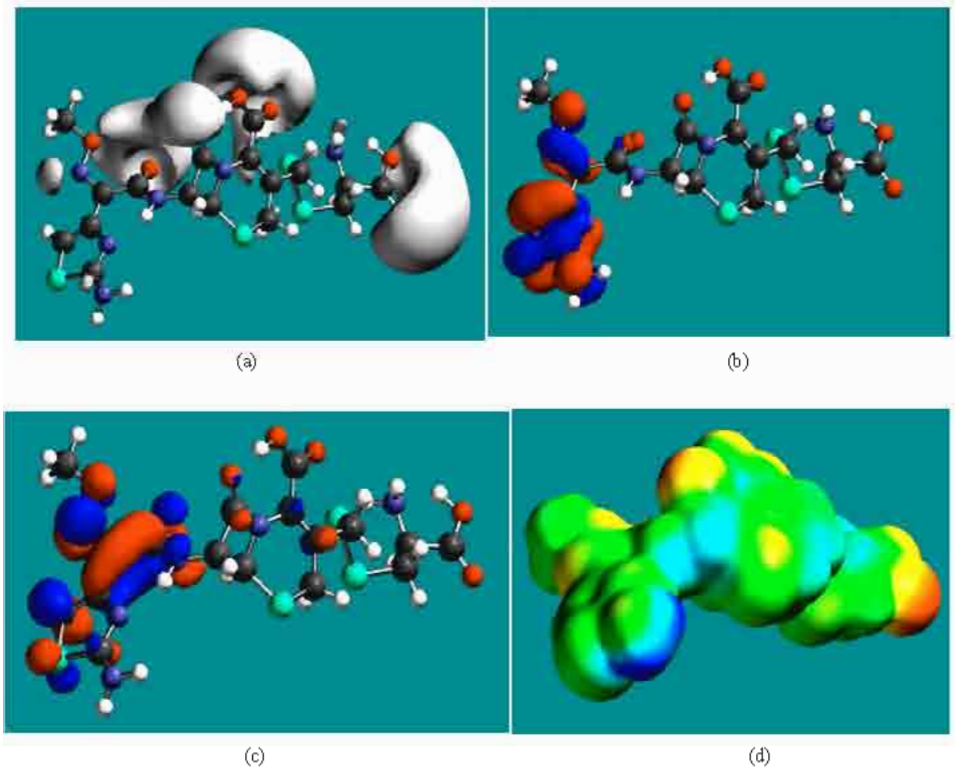


Fig. 6: Structure of DFC-CYS giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

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 CF 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})	Free energy (kcal mol^{-1})
CF	PM3	-101.11	-70.76	244.86	221.39	-30.34	178.86
	DFT	-2729.05		246.31	220.27	-26.12	-180.67
DFC	PM3	-72.61	-44.91	200.83	188.69	-27.71	144.57
	DFT	-2386.88		202.15	187.09	-25.89	146.40
FA	PM3	-100.22	-90.44	57.82	82.55	-9.79	33.21
	DFT	-418.60		57.45	80.61	-6.40	33.42
DFC-D	PM3	-136.46	-92.32	401.65	331.58	-44.14	302.78
	DFT	-4756.53		403.79	330.13	-40.67	305.41
DFC-CYS	PM3	-163.94	-126.59	264.33	236.99	-36.69	193.67
	DFT	-3107.61		266.11	235.71	-34.57	195.87

Molecule	Calculation type	Area ($Å^2$)	Volume ($Å^2$))	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
CF	PM3	485.70	447.98	6.0	-9.25	-1.01	8.24
	DFT	473.38	443.38	6.8	-5.92	-1.74	4.18
DFC	PM3	393.22	360.08	7.8	-9.29	-1.06	8.23
	DFT	389.11	358.08	7.7	-5.93	-1.79	4.14
FA	PM3	128.26	106.34	4.7	-10.04	-0.58	9.46
	DFT	126.63	105.61	5.1	-6.92	-1.36	5.56
DFC-D	PM3	770.54	718.65	3.4	-9.23	-2.35	6.88
	DFT	713.23	755.16	5.3	-5.91	-1.94	3.97
DFC-CYS	PM3	505.51	457.51	7.1	-9.29	-2.59	6.70
	DFT	496.65	454.64	11.6	-6.18	-1.80	4.38

* in atomic units from DFT calculations

DFT calculations for CF and its metabolites DFC, FA, DFC-D and DFC-CYS. Figure 2-6 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 surface charges (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimized structures of CF and its metabolites DFC, FA, DFC-D and DFC-CYS.

The calculated solvation energies of CF and its metabolites DFC, FA, DFC-CYS and DFC-D from PM3 calculations in kcal mol^{-1} are -30.34, -27.71, -9.79, -44.14 and -36.69 and their dipole moments from DFT calculations are 6.8, 7.7, 5.1, 5.3 and 11.6 , respectively. The values suggest that CF and its metabolites would vary in their solubility in water with the terminal metabolites DFC-D and DFC-CYS having much greater solubility.

CF and its metabolites have LUMO-HOMO energy differences ranging from 4.0 to 5.6 eV from DFT calculations, indicating that the compounds would differ in their kinetic lability. DFC-D, CF and DFC are expected to be more labile as they have smaller LUMO-HOMO energy differences, the most labile one being DFC-D.

In the case of CF, DFC, DFC-D and DFC-CYS, electrostatic potential is found to be more negative around the various oxygen centers so that they may be more likely subject to electrophilic attack. In the case of DFC, electrostatic potential is also found to be more negative around one of the sulfur centers so that it may also be subject to electrophilic attack. In the case of FA, electrostatic potential is found to be more negative around the two oxygen centers of the carboxyl group so that they may be more likely subject to electrophilic attack. In the case of DFC-CYS, electrostatic potential is found to be more negative around the two oxygen centers of the carboxyl group so that they may be more likely subject to electrophilic attack.

In the case of CF and DFC, HOMOs with high electron density are found close to the most of the non-hydrogen atoms of the thiazolyl ring whereas LUMOs are found close to the non-hydrogen atoms of the linking chain between thiazolyl ring and the four-membered and six-membered fused rings. In the case of DFC-D, the positions of HOMOs with high electron density and LUMOs are found to be quite different but not in the case of DFC-CYS. In the case FA, both HOMOs with high electron density and LUMOs are found close to the most of the non-hydrogen atoms.

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

When the surface areas and volumes are considered, it is found that the active metabolite DFC has distinctly different values from those of CF and other metabolites, suggesting that CF and its other metabolites may not be able to act substrates for the enzyme(s) to which the active metabolite DFC associates.

It can be seen that the molecular surfaces of CF and all its metabolites have some electron-deficient regions (blue) so that they may be subject to nucleophilic attacks such as those by glutathione and nucleobases in DNA. This means that DFC-D, CF and DFC can react more readily with cellular glutathione and nucleobases in DNA resulting into glutathione depletion and oxidation of nucleobases , respectively. Oxidative stress induces cellular toxicity whereas oxidation of nucleobases causes DNA damage.

CONCLUSIONS

Molecular modelling analyses based on semi-empirical and DFT calculations show that CF and its metabolites would differ in their LUMO-HOMO energy differences such that DFC-D, CF and DFC would be more kinetically labile. The high kinetic lability and the presence of electron-deficient regions on the molecular surface mean that DFC-D, CF and DFC may react more readily with glutathione and nucleobases in DNA resulting into glutathione depletion (thus causing oxidative stress) and DNA damage, respectively. The effects of such adverse reactions may however be more significant in the case of CF and DFC than the more reactive metabolite DFC-D as DFC-D as it is likely to be more readily excreted because of much greater solubility in water.

Abbreviations

CF: Eftiofur; (6R,7R)-((2-amino-4-thiazolyl)-Z-(methoxyimino)acetyl) amino-3-((2-+++furanylcarbonyl) thio) methyl-8-oxo-5-thia-1-azabicyclo (4.2.0)oct-2-ene-2-carboxylic acid

DFC-CYS: Desfuroylceftiofur cysteine disulfide

LUMO: Lowest unoccupied molecular orbital

HOMO: Highest occupied molecular orbital

ACKNOWLEDGMENTS

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

REFERENCES

Aaron, C.S., R.L. Yu, P.S. Jaglan, R.D. Roof, C. Hamilton, R. Sorg, R. Gudi and A. Thilagar, 1995. Comparative mutagenicity testing of ceftiofur sodium: III. Ceftiofur sodium is not an *in vivo* clastogen. Mut. Res., 345: 49-56.

Becker, M., E. Zattlau and M. Petz, 2003. Quantitative determination of cetiofur-related residues in bovine raw milk by LC-MS/MS with electrospray ionization. Eur. Food Res. Technol., 217: 449-456.

Beconi-Barker, M.G., K.L. Davision, R.E. Hornish, T.S. Arnold, A.L. Craigmill, T.J. Gilbertson, E.B. Smith, T.J. Vidmar, G.A. Hoffmann and C.L. Gatchell, 1995a. (14C)ceftiofur sodium absorption, distribution, metabolism and excretion in sheep following intramuscular injections. J. Agric. Food Chem., 43:1589-1597.

Beconi-Barker, M.G., R.D. Roof, L. Lillerioux, F.M. Kausche, T.J. Vidmar, E.B. Smith, J.K. Callahan, V.L. Hubbard, G.A. Smith and T.J. Gilbertson, 1995b. Determination of ceftiofur and its desfuroylceftiofur-related metabolites in swine tissues by high-performance liquid chromatography. J. Chromatogr. B, 673: 231-244.

De Baere, S., F. Pille, S. Croubels, L. Ceelen and P. De Backer, 2004. High-performance liquid chromatographic-UV detection analysis of ceftiofur and its active metabolite desfuroylceftiofur in horse plasma and synovial fluid after regional intravenous perfusion and systematic intravenous injection of ceftiofur sodium. Analyt. Chim. Acta, 512: 75-84.

Folz, S.D., B.J. Hanson and A.K. Griffin, 1992.Treatment of respiratory infections in horses with ceftiofur sodium. Equine Vet. J., 24l: 300-304.

Gilbertson, T.J., R.D. Roof, J.L. Nappier, M.J. Zaya, R.H. Robbins, D.J. Stuart, L.F. Krezeminski and P.S. Jaglan, 1995.Disposition of Ceftiofur Sodium in Swine following Intramuscular Treatment. J.Agric. Food Chem., 43: 229-234.

Jaglan, P.S., M.F. Kubicek, T.S. Arnold, B.L. Cox, R.H. Robins, D.B. Johnson and T.J. Gilbertson, 1989. Metabolism of ceftiofur. Nature of urinary and plasma metabolites in rats and cattle. J. Agric. Food Chem., 37: 1112-1118.

Mills, C.F., B.R. Rush, G. St. Jean, E.M. Gaughan, D. Mosier, E. Gibson and L. Freeman, 2000. Determination of synovial fluid and serum concentrations and morphologic effects of intraarticular ceftiofur sodium in horses. Vet. Surg., 29: 398-406.

Olson, S.C., M.G. Beconi-Barker, R.D. Roof, L. Millerioux, F.M. Kausche, T.J. Vidmar and L.D. Adams, 1998. *In vitro* metabolism of ceftiofur in bovine tissues. J. Vet. Pharmacol. Therap., 21: 112-120.

Spartan 02, Wavefunction, Inc. Irvine, CA, USA, 2002.