Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 2 (1): 50-57, 2007
ISSN 1815-9923

Molecular Modelling Analysis of the Metabolism of Fenbufen

Fazlul Huq
School of Biomedical Sciences, Faculty of Health Sciences, C42,
The University of Sydney, Lidcombe, NSW, Australia

Abstract: Fenbufen (FN) is an orally and parenterally effective NSAID, used in the treatment of rheumatoid arthritis and characterized by low water solubility, particularly at low pH. It is actually a pro-drug of [1,1'-biphenyl]-4-acetic acid (BPAA) formed in its metabolism. Like other NSAIDs it shows activity in humans as wells a wide variety of animals including mice, rats, guinea pigs and dogs. However, FN is also known to produce a high incidence of skin rash especially in women. In aerated solution FN was found to sensitize the formation of singlet oxygen indicating FN is a strong photodynamic agent. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that FN and its metabolites differ in their LUMO-HOMO energy differences indicating that they would differ in their kinetic ability. The molecular surface of the metabolite BPEN is found to abound most in electron-deficient regions so that BPEN is more likely to react with cellular antioxidant glutathione and nucleobases in DNA resulting in glutathione depletion and DNA damage respectively. Depletion of reduced form of glutathione will induce cellular toxicity by compromising the antioxidant status of the cell.

Key words: Fenbufen, NSAID, rheumatoid arthritis, toxicity, glutathione, molecular modelling

Introduction

Fenbufen [FN; γ-oxo-(1,1'-biphenyl)-4-butanoic acid] is an orally and parenterally effective non-steroidal anti-inflammatory drug (NSAID) that is used in the treatment of rheumatoid arthritis but is characterized by low water solubility, particularly at low pH (Di Martino *et al*., 1999. FN is actually a pro-drug of [1,1'-biphenyl]-4-acetic acid (BPAA) formed in its metabolism. Like other NSAIDs it shows activity in humans as wells a wide variety of animals including mice, rats, guinea pigs and dogs. However, FN is also known to produce a high incidence of skin rash especially in women (Navaratnam and Jones, 2000. As with other NSAIDs, the primary mode of action of FN (more exactly that of its active metabolite BPAA) is associated with inhibition of cylcooxygenase and lipoxygenase pathways (Vane, 1971). COX exists in at least two different isoforms COX-1 and COX-2. COX-1 is present in most cells under physiological conditions whereas COX-2 is induced mainly in response to inflammatory stimuli (Levoin *et al*., 2004).

Besides BPAA, other metabolites of FN are: [1,1'-biphenyl]-4-(γ-hydroxybutanoic acid) (BPHBA), [1,1'-biphenyl]-4-butanoic acid (BPBA), [1,1'-biphenyl]-_(β,γ-dihydroxy-(R*,S*)-butanoic acid) (BPDBA), 4'-hydroxy([1,1'-biphenyl]-4-acetic acid (4'HBPAA) and (1-[1,1'-biphenyl]-4-yl-ethanone (BPEN) (Chiccareli *et al*., 1980; Siluveru and Stewart, 1996). FN and its metabolites BPAA, BPHBA, BPDBA and 4'HBPAA can undergo conjugation with glucuronic acid to form the corresponding acyl glucuronides FN-GLU, BPAA-GLU, BPDBA-GLU and 4'HBPAA-GLU (Fig. 1).

Corresponding Author: Dr. Fazlul Huq, School of Biomedical Sciences, Faculty of Health Sciences, University of Sydney, P.O. Box 170 East Street Lidcombe NSW 1825, Australia
Tel: 061 2 9351 9522 Fax: 061 2 9351 9520

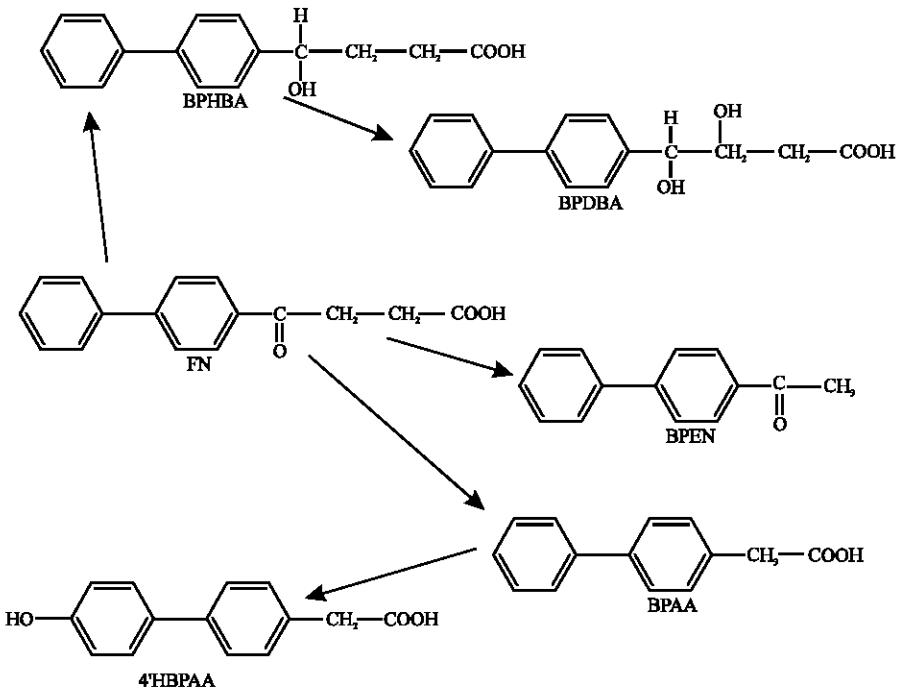

Fig. 1: Metabolic pathways for ketoprofen (Based on Chiccareli *et al*., 1980; Siluveru and Stewart, 1996)

A serious problem with a number of NSAIDs is the hepatotoxicity induced by reaction of metabolites with proteins, enzymes, cellular antioxidants such as glutathione and DNA (Sildenius *et al*., 2004). Because of idiosynchratic adverse reactions, nine NSAIDs were in fact withdrawn during the period 1974 and 1993. Acyl glucuronides formed from NSAIDs containing a carboxylic acid moiety are known to bind covalently with glutathione and proteins (Boelsterli *et al*., 1995). However, thus far, no direct correlation between acyl glucuronide covalent binding and acute toxicity due to NSAIDs has emerged. On the contrary, there is evidence suggesting that the inhibition of glucuronidation results into increased NSAID cytotoxicity (Siraki *et al*., 2005). Also, there is evidence to suggest that other metabolites of NSAIDs may be making significant contributions to their toxicity. A recent study has shown that for diclofenac (another NSAID), the toxicity is more likely to be due ketonic metabolites obtained from oxidation rather glucuronides (Huq and Alshehri, 2006). FN, BPAA, BPHBA and BPDBA may also form CoA thioesters that are believed to be more reactive than acylglucuronides (Sidenius *et al*., 2004). In aerated solution FN was found to sensitize the formation of singlet oxygen with a quantum yield close to unity, indicating FN is a strong photodynamic agent (Navaratnam and Jones, 2000).

In this study, molecular modelling analyses have been carried out using the program Spartan '02 (Spartan, 2002) to investigate the relative stability of FN and its metabolites with the aim of providing a better understanding of their relative toxicity. No calculations were done for FN-GLU, BPAA-GLU, BPDBA-GLU and 4'HBPAA-GLU.

Computational Methods

The geometries of FN and its metabolites have been optimized based on molecular mechanics, 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 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 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. The study was carried out in the School of Biomedical Sciences, The University of Sydney during February to June 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, energies of HOMO and LUMO as per both PM3 and DFT calculations for FN and its metabolites BPAA, BPHBA, BPDBA, 4'HBPAA and BPEN. 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 density of electrostatic potential (where red indicates negative, blue indicates positive and green indicates neutral) in (d), as applied to the optimized structures of FN and its metabolites BPAA, BPHBA, BPDBA, 4'HBPAA and BPEN.

Table 1: Calculated thermodynamic and other parameters of fenbufen 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})
FN	PM3	-99.98	-83.57	175.34	132.58	-6.41
	DFT	-843.83		176.67	131.89	-8.23
BPAA	PM3	-58.22	-46.97	148.76	117.10	-11.25
	DFT	-691.18		149.88	116.45	-13.56
BPHBA	PM3	-113.34	-96.51	189.35	134.88	-13.49
	DFT	-845.01		190.23	133.97	-15.72
BPDBA	PM3	-181.60	-162.42	182.47	143.47	-19.18
	DFT	-994.26		183.55	142.67	-22.06
4'HBPAA	PM3	-108.03	-92.14	152.99	122.56	-15.90
	DFT	-766.40		154.02	121.53	-19.36
BPEN	PM3	1.34	6.43	144.77	112.83	-5.08
	DFT	-615.95		145.89	112.14	-6.97

Molecule	Calculation type	Free energy (kcal mol^{-1})	Area ($Å^2$)	Volume ($Å^3$)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
FN	PM3	135.81	281.20	266.27	6.0	-9.36	-1.06	8.30
	DFT	137.40	287.10	268.23	3.3	-6.41	-1.81	4.60
BPAA	PM3	113.85	243.92	228.19	4.8	-9.45	-0.62	8.83
	DFT	115.18	244.12	228.76	5.3	-6.37	-1.18	5.19
BPHBA	PM3	149.13	288.49	271.66	3.8	-9.39	-0.50	8.89
	DFT	150.31	291.09	273.18	3.7	-6.21	-0.99	5.22
BPDBA	PM3	139.69	303.80	282.61	3.0	-9.54	-0.96	8.58
	DFT	141.03	294.45	280.80	5.1	-6.58	-2.30	4.28
4'HBPAA	PM3	116.45	252.79	235.34	5.5	-9.11	-0.59	8.52
	DFT	117.80	253.03	235.86	6.3	-5.95	-1.04	4.91
BPEN	PM3	111.13	237.38	221.54	2.9	-9.33	-0.47	8.86
	DFT	112.47	236.11	221.89	3.3	-6.31	-1.65	4.66

* in atomic units from DFT calculations

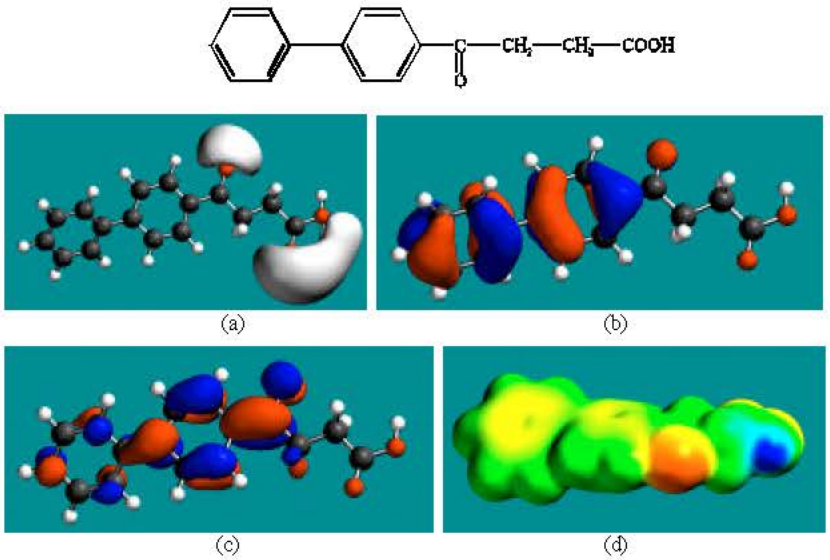

Fig. 2: Structure of FN giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

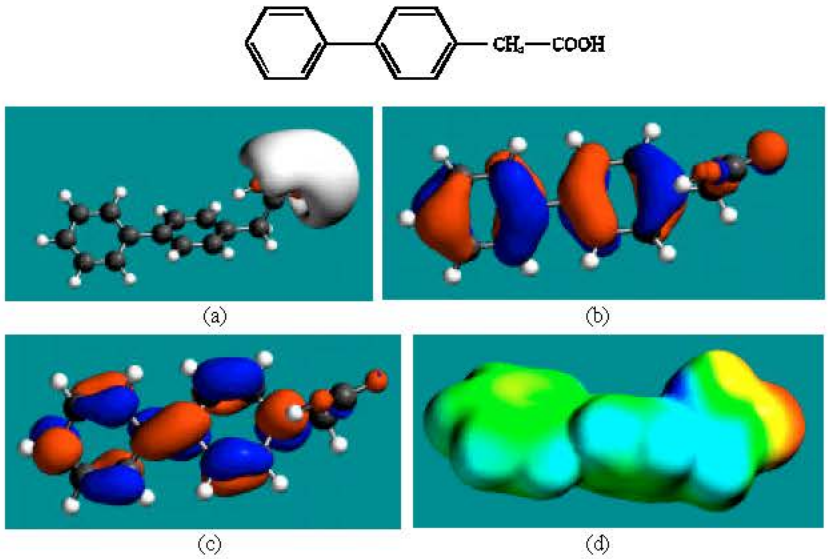

Fig. 3: Structure of BPAA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

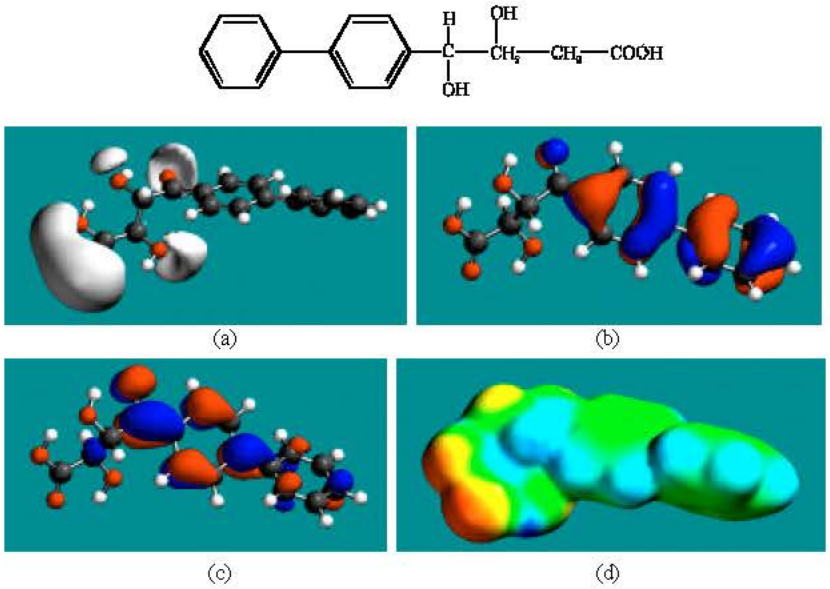

Fig. 4: Structure of BPDBA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

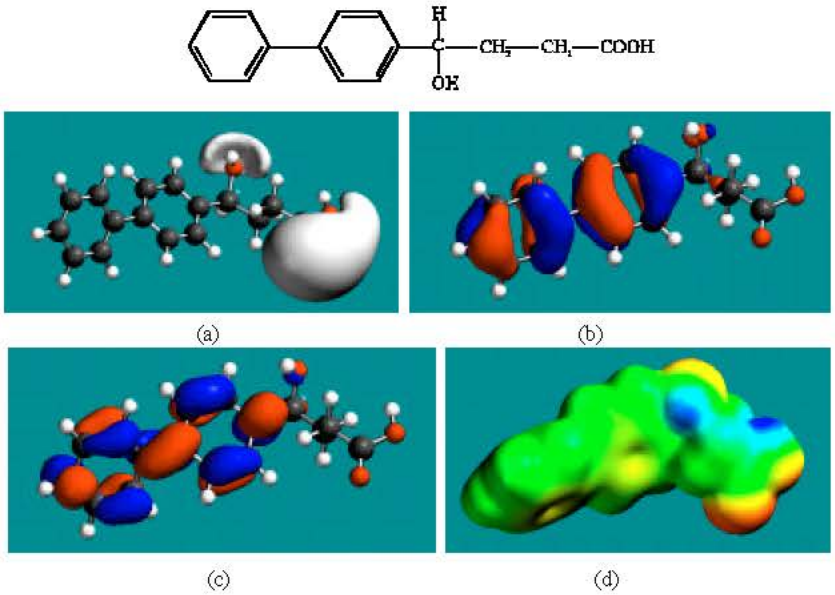

Fig. 5: Structure of BPHBA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

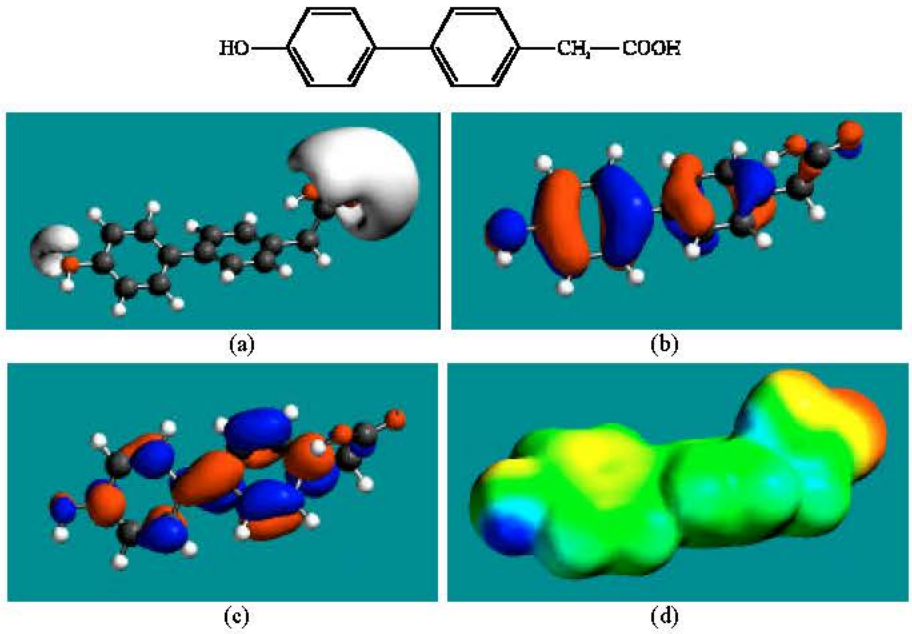

Fig. 6: Structure of 4'HBPAA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

The calculated solvation energies of FN and its metabolites BPAA, BPHBA, BPDBA, 4'HBPAA and BPEN from PM3 calculations in kcal mol^{-1} are, respectively -6.41, -11.25, -13.49, -19.18, -15.90 and -5.08 and their dipole moments from DFT calculations are 3.3, 5.3, 3.7, 5.1, 6.3 and 3.3, respectively. The values suggest FN and its metabolites would have low solubility in water except BPDBA which would have a much higher solubility in agreement with the presence of more polar groups in the molecule.

FN and its metabolites are found to differ in their LUMO-HOMO energy differences (ranging from 4.28 to 5.19 eV from DFT calculations), indicating that the compounds would differ in their kinetic lability. BPDBA has the smallest LUMO-HOMO energy difference, indicating that it would be most labile.

In the case of FN and all its metabolites, the electrostatic potential is found to be more negative around the various oxygen centers such as carbonyl, carboxyl and hydroxyl oxygen atoms, indicating that the positions may be subject to electrophilic attacks.

In the case of FN and all its metabolites, both HOMOs with high electron density and LUMOs are found to be centered mostly on the non-hydrogen atoms of the two phenyl rings. The overlap or close proximity of positions of HOMOs with high electron density and those of negative electrostatic potential give further support to the idea that the positions may be subject to electrophilic attacks.

FN and all its metabolites have harge negative heat of formation values except BPEN which has value of 6.43 kcal mol^{-1} from PM3 calculations. This may make BPEN least stable thermodynamically.

When the densities of electrostatic potential on moecular surfaces (Fig. 2d to 7d) are considered, it can be seen that the surfaces of all the compounds have both electron-rich (red) and electron-deficient (blue) regions so that they all may be subject to both electrophilic and nucleophilic attacks. BPEN is

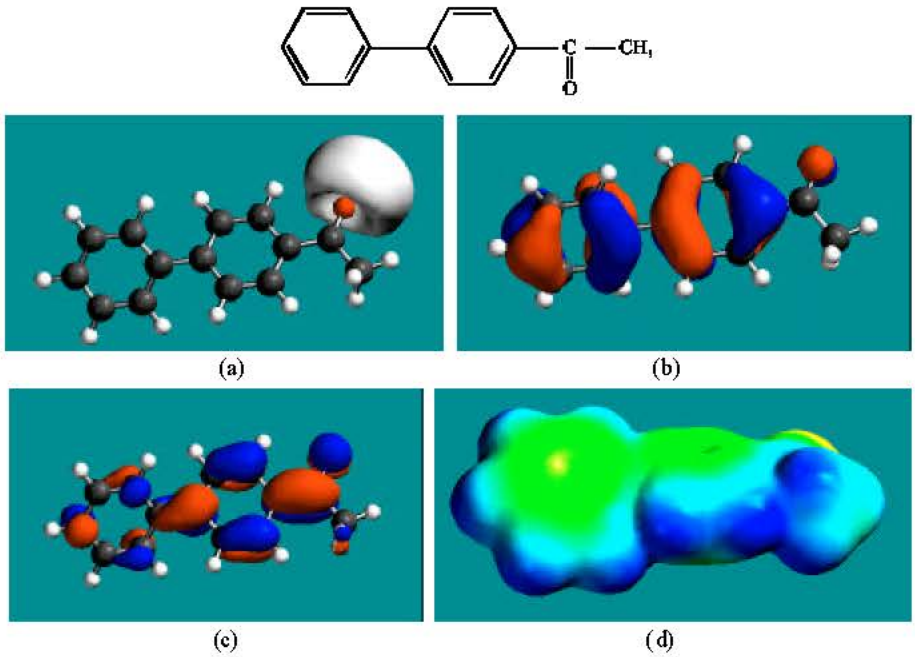

Fig. 7: Structure of BPEN giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

found to abound most in electron-deficient regions so that it would be most likely to react with cellular nucleophiles such as glutathione and nucleobases in DNA. This means that BPEN would be most likely to cause depletion of cellular glutathione and oxidation of nucleobases in DNA. Depletion of glutathione would induce cellular toxicity by compromising the anti-oxidant status of the cell whereas oxidation of nucleobases in DNA would result into DNA damage.

When the surface areas and volumes of FN and its metabolites are compared, it is found that the values for FN differ significantly from those of its pharmacologically active metabolite BPAA, giving support to the idea that the two compounds may not act as substrate for the same binding site. Although the metabolites BPAA and BPEN have similar values for surface area and volume, they may still not act as substrate for the same binding site because of the differences in their nature e.g., as noted earlier the surface BPEN abounds more in electron-deficient regions than that of BPAA.

Conclusions

Molecular modelling analyses based on molecular mechanics, semi-empirical and DFT calculations show that FN and its metabolites differ in their LUMO-HOMO energy differences indicating that they would differ in their kinetic lability. The molecular surface of BPEN is found to abound most in electron-deficient regions so that the metabolite is more likely to react with cellular antioxidant glutathione and nucleobases in DNA resulting into glutathione depletion and DNA damage. Depletion of reduced form of glutathione will introduce cellular toxicity by compromising the antioxidant status of the cell.

Acknowledgments

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

References

Boelsterli, U.A., H.J. Zimmerman and A. Kertz-Rommel, 1995. Idiosyncratic liver toxicity of nonsteroidal antiinflammatory drugs: Molecular mechanisms and pathology. Crit. Rev. Toxicol., 25: 207-235.

Chiccareli, F.S., H.J. Eisner and G.E. Van Lear, 1980. Disposition and metabolism of fenbufen in several laboratory animals. Arzneimittel-Forschung, 30: 707-715.

Di Martino, P., C. Barthelemy, F. Piva, E. Joiris, G.F. Palmieri and S. Martelli, 1999. Improved Dissolution Behavior of Fenbufen by Spherical Crystallization. Drug Dev., 25: 1073-1081.

Huq, F. and A. Alshehri, 2006. Molecular modelling analysis of the metabolism of diclofenac. IJPAC.,

Levoin, N., C. Blondeau, C. Guillaume, L. Grandcolas and F. Chretien *et al*., 2004. Elucidation of the mechanism of inhibition of cyclooxygenases by acyl-coenzyme A and acylglucuronic conjugates of ketoprofen, Biochem. Pharmacol., 68: 1957-1969.

Navaratnam, S. and S.A. Jones, 2000. Primary process in the photochemistry of fenbufen in acetonitrile, J. Photochem. Photobiol., 132: 175-180.

Siluveru, M. and J.T. Stewart, 1996. Determination of fenbufen and its metabolites in serum by reversed-phase high performance liquid chromatography using solid-phase extraction and online post-column ultraviolet irradiation and fluorescence detection. J. Chormatogr., 682: 89-94.

Sidenius, U., C. Skonberg, J. Olsen and S.H. Hansen, 2004. *In vitro* reactivity of carboxylic acid-coa thioesters with glutathione. Chem. Res. Toxicol., 17: 75-81.

Siraki, A.G., T. Chevaldina and P.J. O'Brien, 2005. Application of quantitative structure-activity relationships for acute NSAID cytotoxicity in rat hepatocytes, Chem. Biol. Interact., 151: 177-191.

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

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (New Biol.), 231: 232-235.