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Molecular Modelling Analysis of the Metabolism of Aniracetam

Fazlul Huq
Discipline of Biomedical Science, School of Medical Sciences, Faculty of Medicine, Cumberland Campus, C42, The University of Sydney, Lidcombe, NSW, Australia

Abstract: Aniracetam (ACM) is a pyrrolidinone-type cognition enhancer that has been used in the treatment of behavioural and psychological symptoms of dementia following stroke and Alzheimer's disease. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that both ACM and its major metabolites have LUMO-HOMO energy differences ranging from 4.89 to 7.4 eV, indicating that the compounds would all be kinetically inert with SD being the most inert and the parent drug being the least inert one. The molecular surface of ACM is found to posses significant amounts of 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 ACM is expected to be to some extent kinetically inert, the rates of such adverse reaction are expected to be low unless speeded up enzymatically.

Key words: Aniracetam, schizoprenia, dementia, Alzheimer's disease, molecular modelling

INTRODUCTION

Schizoprenia is a complex psychiatric disorder characterized by variable expression of three major categories of symptoms termed positive, negative and cognitive symptoms. Positive symptoms (also called psychotic symptoms) include hallucinations and delusions whereas negative symptoms include apathy, anhedonia and social and emotional withdrawal (Rogers and Schmidt, 2006). Schizoprenia is now viewed as being the result of a neurochemical imbalance across multiple transmitter systems (Larulle *et al*., 1999). It is believed that reduced glutamergic neurotransmission, particularly at the N-methyl-d-aspartate (NMDA) receptor subtype, underlies even the dopaminergic abnormalities that are common in schizophrenic patients (Javitt, 2004). This growing understanding of neurochemical deficits underlying the disease has led to a corresponding broadening in search for novel treatment approaches. Aniracetam (1-p-anisoyl-2-pyrrolidinone; ACM) is a pyrrolidinone-type cognition enhancer that has been clinically used in the treatment of behavioural and psychological symptoms of dementia following stroke and Alzheimer's disease (Nakamuara, 2002). ACM has been found to preferentially increase extracellular levels of dopamine (DA) and serotonin in the prefrontal cortex (PFC), basolateral amugdala and dorsal hippocampus of the mesocorticolimbic system in stroke-prone spontaneously hypersensitive rats. The drug is recognized to be an allosteric modulator of α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA) receptors. It is believed to block desentization of the AMPA receptors in the suprachiasmatic nucleus (SCN) that has been activated by glutamate (Lee and Benfield, 1994).

After oral adninistration, ACM is rapidly absorbed from the intestinal tract (Ogiso *et al*., 1998). The metabolites of ACM include 2-pyrrolidinone (PD), *p*-anisic acid (AA), 4-p-anisamidobutanoic acid (ABA), succinimide (SD), 5-hydroxy-pyrrolidinone (5HPD), succinimide (SD) (Yoshimoto *et al*., 2000). ACM is first metabolized to ABA, AA and PD. PD is further metabolized into 5-hydroxy-pyrrolidinone (5HPD). 5HPD is metabolized into succinimide (SD) which enters Krebs

cycle producing carbon dioxide. AA is conjugated with glycine and glucuronic acid to produce AA-glycine conjugate and AA-glucuronide, respectively. It is believed that the metabolite PD enhances activity of activated Protein Kinase C (PKC), thereby potentiating α7 receptor responses and then leading to facilitation of hippocampal synaptic responses.

In this study, molecular modelling analyses have been carried out using the program Spartan '02 (Spartan, 2002) to provide information on the relative toxicity of ACM and its metabolites.

COMPUTATIONAL METHODS

The geometries of ACM and its metabolites PD, ABA, AA and 5HPD have been optimized based on molecular mechanics (Fig. 1), semi-empirical and DFT (Density functional theory)

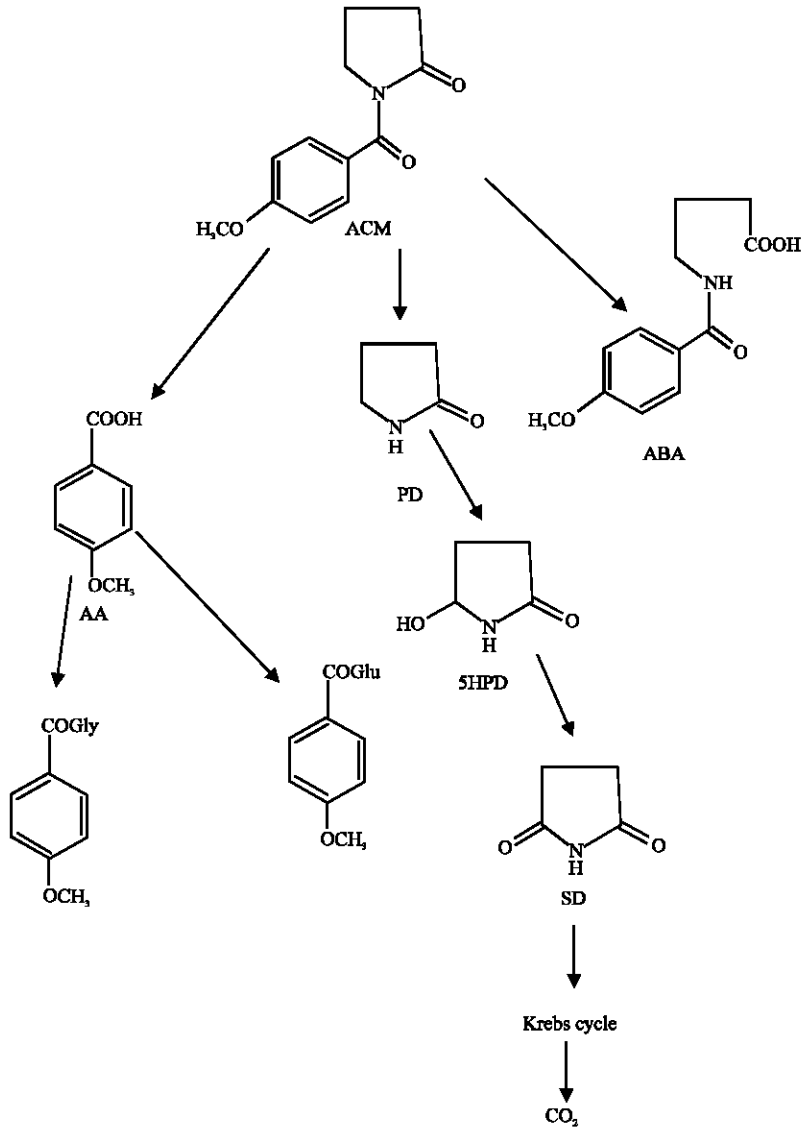

Fig. 1: Metabolic pathways for ACM in rats and humans (Nakamura and Shirane, 1999)

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 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 (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.

RESULTS AND DISCUSSION

Table 1 shows 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 ACM and its metabolites PD, ABA, AA and 5HPD. 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 on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to optimized structures of ACM and its metabolites PD, ABA, AA and 5HPD.

Table 1: Calculated thermodynamic and other parameters of ACM 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})	Free energy (kcal mol^{-1})	Solvation energy (kcal mol^{-1})
ACM	PM3	-103.34	-94.98	153.53	126.45	115.83	-8.36
	DFT	-745.55		156.61	120.29	120.74	-7.76
AA	PM3	-114.75	-103.19	98.61	99.27	69.01	-11.56
	DFT	-535.35		99.19	97.52	70.11	-9.62
ABA	PM3	-166.05	-150.24	171.61	135.77	131.13	-15.80
	DFT	-821.99		174.93	136.68	134.18	-11.64
PD	PM3	-56.11	-47.82	71.25	74.93	48.90	-8.29
	DFT	-286.63		73.37	74.09	51.28	-7.32
5HPD	PM3	-105.55	-99.53	74.91	80.44	50.93	-13.02
	DFT		-361.84				
SD	PM3	-98.14	-87.83	60.18	80.52	36.17	-10.31
	DFT	-360.68		61.70	77.80	38.51	-8.61

Table 1: Continued

Molecule	Calculation type	Area ($Å^2$)	Volume ($Å^3$)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
ACM	PM3	247.57	225.88	2.3	-9.21	0.13	9.34
	DFT	242.72	224.49	0.7	-6.05	-1.16	4.89
AA	PM3	175.72	154.14	4.8	-9.68	-0.47	9.21
	DFT	174.44	154.01	5.4	-6.46	-1.07	5.39
ABA	PM3	273.65	243.39	1.9	-9.27	-0.05	9.22
	DFT	269.41	242.45	1.3	-5.95	-0.52	5.43
PD	PM3	113.18	92.17	3.5	-9.72	1.00	10.72
	DFT	113.02	91.95	3.9	-6.45	0.99	7.44
5HPD	PM3	122.69	99.62	4.4	-9.93	1.00	10.93
	DFT	122.59	99.37	4.7	-6.61	0.73	7.34
SD	PM3	116.22	94.62	2.4	-10.43	0.19	10.62
	DFT	115.85	94.39	1.9	-7.11	-0.62	7.73

*In atomic units from DFT calculations

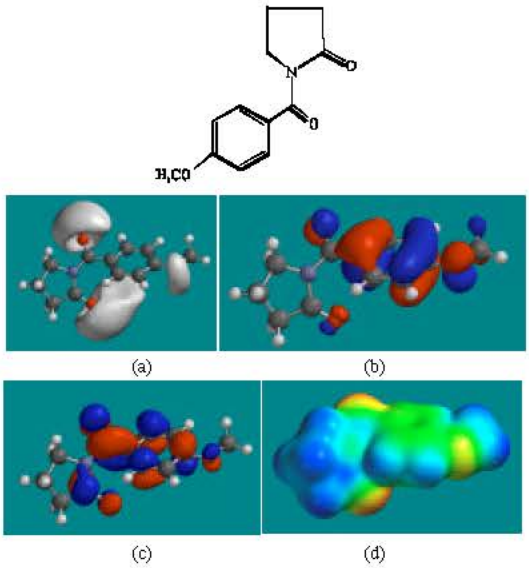

Fig. 2: Structure of ACM giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

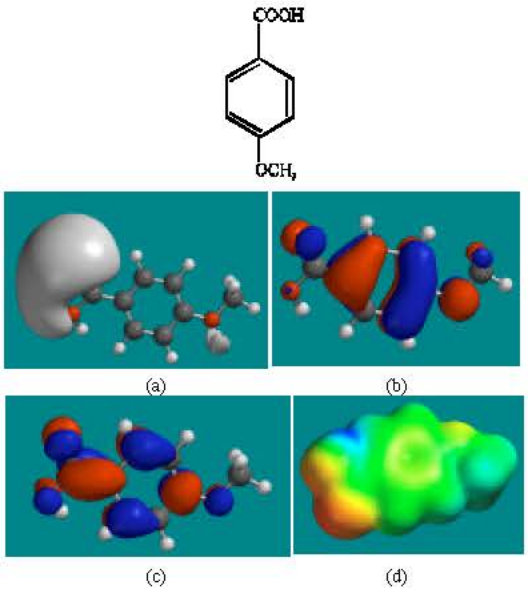

Fig. 3: Structure of AA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

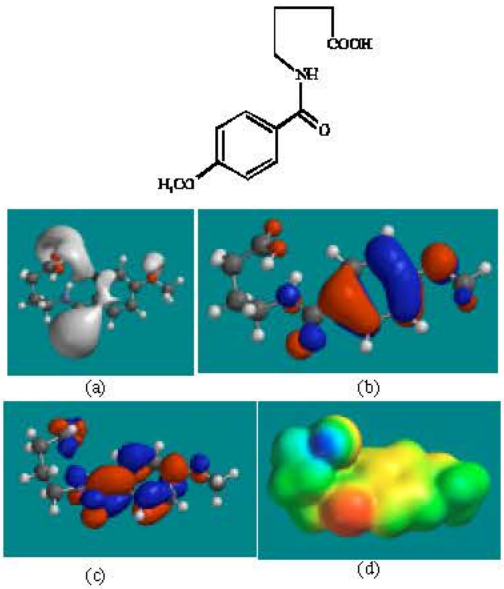

Fig. 4: Structure of ABA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

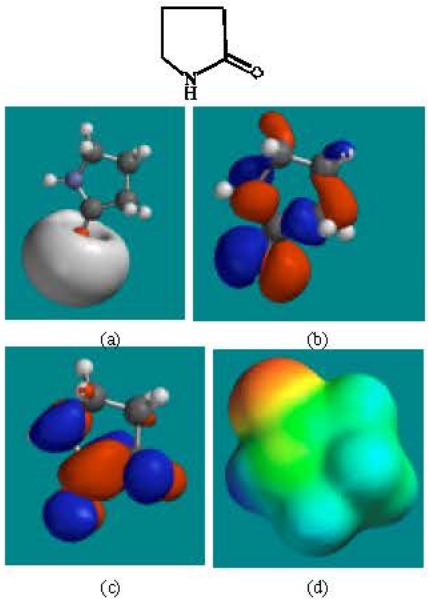

Fig. 5: Structure of PD giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

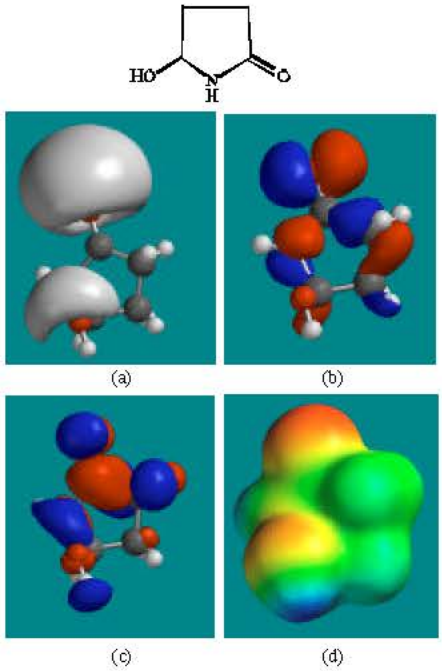

Fig. 6: Structure of 5HPD giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

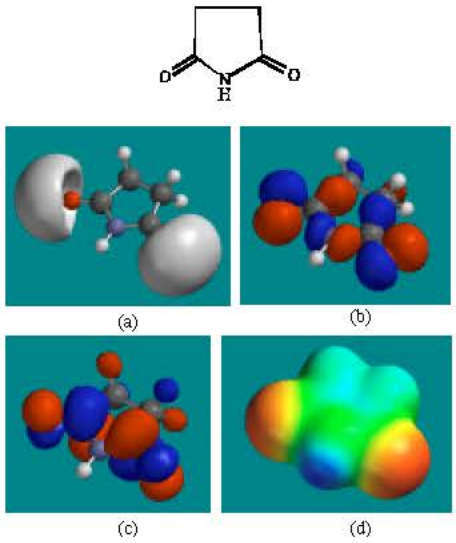

Fig. 7: Structure of SD giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

The LUMO-HOMO energy differences for ACM and its metabolites from DFT calculations are found to range from 4.89 to 7.4 eV, indicating that the compounds would all be kinetically inert with SD (Fig. 7) being the most inert and the parent drug ACM being the least inert one.

In the case of ACM, AA, ABA, PD, 5HPD and SD, the electrostatic potential is found to be more negative around oxygen atoms, indicating that the positions may be subject to electrophilic attack.

In the case of ACM (Fig. 2), the HOMOs with high electron density are found to be slightly more localised than the LUMOs. In the case of AA (Fig. 3), PD (Fig. 5) and 5HPD (Fig. 6), both the HOMOs with high electron density and the LUMOs are found to be close to nearly all the non-hydrogen atoms. In the case of ABA (Fig. 4), both the HOMOs with high electron density and the LUMOs are found to be close to essentially the same non-hydrogen atoms.

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 surfaces of ACM and all its metabolites are found to possess significant amounts of electron-rich (yellow and red) and neutral (green) regions so that they may be subject to electrophilic and lyophilic attacks. The molecular surface of ACM is also found to possess significant amounts of electron-deficient (blue) regions so that it may be subject to nucleophilic attacks such as those 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, since ACM is expected to be moderately inert kinetically, the rate of such adverse reactions may be low unless speeded up enzymatically.

CONCLUSION

Aniracetam (ACM) is a pyrrolidinone-type cognition enhancer that has been used in the treatment of behavioural and psychological symptoms of dementia following stroke and Alzheimer's disease. Molecular modelling analyses based on semi-empirical and DFT calculations show that both ACM and all its metabolites have large LUMO-HOMO energy differences so that they would be kinetically inert, with the parent drug being least inert. The molecular surface of the parent drug ACM is found to possess significant amounts of electron-deficient regions so that it can react with glutathione and nucleobases in DNA. However, ACM being to some extent kinetically inert, the rates of such adverse reactions may be low, unless speeded up enzymatically.

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