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Molecular Modelling Analysis of the Metabolism of Chlorpromazine

Fazlul Huq* & Zahed Hossain

School of Biomedical Sciences, Faculty of Health Sciences, The University of Sydney

ABSTRACT: Chlorpromazine (CPZ) is an antipsychotic drug that is said to have revolutionized the practice of psychiatry. Before its introduction in mid-1950s, there was no regularly efficacious treatment for the most common psychotic disorders. Neuroleptics also play an ill-defined and some times controversial role in the treatment of disturbed children. The most common side effects of CPZ are dry mouth, constipation, blurred vision and tachycardia. The two major routes for the metabolism of CPZ and other tricyclic antidepressants are through the cytochrome P450 mixed-function oxidase system. These include hydroxylation of either or both aromatic rings and N-demethylation of one or both of the methyl groups associated with amine side chain, N-oxidation and sulfoxidation. Hydroxylated and N-demethylated products are found to be pharmacologically active. Molecular modelling analyses show although CPZ and its metabolites have similar difference kinetic lability except CPZNOSO which is expected to be significantly more labile. That none of the compounds is extremely labile or highly inert may mean that a clear choice on the toxicity due to the drug cannot be made. The calculated dipole moment and the solvation energy values of CPZ and its metabolites differ widely, indicating a significant difference in their aqueous solubility. CPZ is expected to be least soluble in water whereas CPZNOSO which is found to be most kinetically labile is expected to be most soluble as it has the highest solvation energy and therefore more easily excreted.

Key words: Chlorpromazine, neuroleptic, chlorpromazine N-oxide, cytochrome P450, molecular modelling

Introduction

Chlorpromazine (CPZ) is an antipsychotic drug that is said to have revolutionized the practice of psychiatry [1]. Before its introduction in mid-1950s, there was no regularly efficacious treatment available for the most common psychotic disorders. The compound was initially examined by Laborit as a part of his studies on the role of histamine in surgical shock. Its possible use in psychiatry was suggested when the drug was found to produce a state of calmness and indifference. Psychosis is the condition that is most often treated with antipyschotic drugs also known as neuroleptic. An essential feature of psychosis is the existence of state of partial or complete separation from the reality [1]. Antipyschotic drugs are used to treat a wide variety of mental disorders including schizophrenia, delirium and dementia [2]. Schizophrenia, the most common forms of psychosis, is characterized by chronicity, impaired function and disturbances of thinking. CPZ and other antipsychotic drugs also play an ill-defined and some times controversial role in the treatment of disturbed children [1]. The most common adverse side effect of CPZ and other tricyclic antidepressants is cholinergic blockade, including dry mouth, constipation, blurred vision and tachycardia. Other side effects include allergic reactions and drug-drug interactions [1]. The precise mechanisms by which antipsychotic drugs including CPZ exert their beneficial effects remain unknown. One common characteristic of the drugs is however their antagonism of striatal dopamine functions that resembles Parkinson's disease, a disorder caused by the loss of dopaminergic neurons in the substantia nigra and the axial projections to the striatum. According to the dopamine hypothesis for schizophrenia, hyperactivity in dopaminergic systems causes both the signs and symptoms characteristic of the disease - an idea which is supported by the fact that all clinically effective antipsychotic drugs block an aspect of dopaminergic function.

* *Corresponded Authors* C42, The University of Sydney, PO Box 170, Lidcombe, NSW 1825, Australia.
Telephone: +61 2 9351 9522 Fax: +61 2 9351 9520
E-mail: *f.huq@fhs.usyd.edu.au.*

The two major routes for the metabolism of CPZ and other tricyclic antidepressants are through the cytochrome P450 mixed-function oxidase system [1,3]. These include hydroxylation of either or both aromatic rings and N-demethylation of one or both of the methyl groups associated with amine side chain, N-oxidation and sulfoxidation. Hydroxylated and N-demethylated products are found to be pharmacologically active. The hydroxylated aromatic rings can be conjugated with glucuronic acid or sulfate that results into inactivation of the drug. CPZ is highly lipid soluble and bound to serum proteins. CPZ has been known to accumulate in the lung tissue [4] where the principal metabolic pathway involves the N-oxidation.

The metabolism of CPZ is found to differ strikingly in between species and also between hepatitic and intestinal tissues of the same species [5]. For example, hepatitic microsomes of guinea pigs were to found to produce relatively large amounts of N-oxide whereas the human hepatitic microsomes produced predominantly 7-hydroxy metabolites with minimal formation of N-oxides. The kinetics of the formation of metabolites and studies with inhibitors of cytochrome P450 suggested that multiple microsomal enzymes are involved in the metabolism of CPZ. CPZ metabolizing activity of the rat lung was found to be 10-fold higher than that of the rabbit lung [5]. In vitro and in vivo studies involving humans and experimental animals have identified a number of metabolites of CPZ including chlorpromazine N-oxide (CPZNO), chlorpromazine sulfoxide (CPZSO), 7-hydroxychlorpromazine (7OHCPZ), N-monodemethy lchlorpromazine (NMDMCPZ), N-monodemethy lchlorpromazine sulfoxide (NMDMCPZSO), N-monodemethy lchlorpro- mazine sulfdioxide (NMDMCPZSDO), N,N-didemethylchlorpromazine (NNDDMCPZ), N,N-didemethylchlor promazine sulfoxide (NNDDMCPZSO), N,N-didemethylchlorpro-mazine sulfdioxide (NNDDMCPZSDO) and chlorpromazine N-oxide sulfoxide (CPZNOSO). Chlorpromazine sulfoxide was not found to undergo photodechlorination but instead it generated high yield of the *OH radical [6] which can cause damage to biomolecules including proteins, enzymes and DNA. The intake of chlorpromazine hydrochloride (1 mg/kg/day, orally, for 30 days) was found to cause a decrease in triglycerides and alkaline phosphatase levels and an increase in blood sugar [7].

In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 [8] and Spartan '02 [9] to investigate the relative stability of CPZ and its metabolites with the aim of providing a better understanding of toxicity due to CPZ and its metabolites.

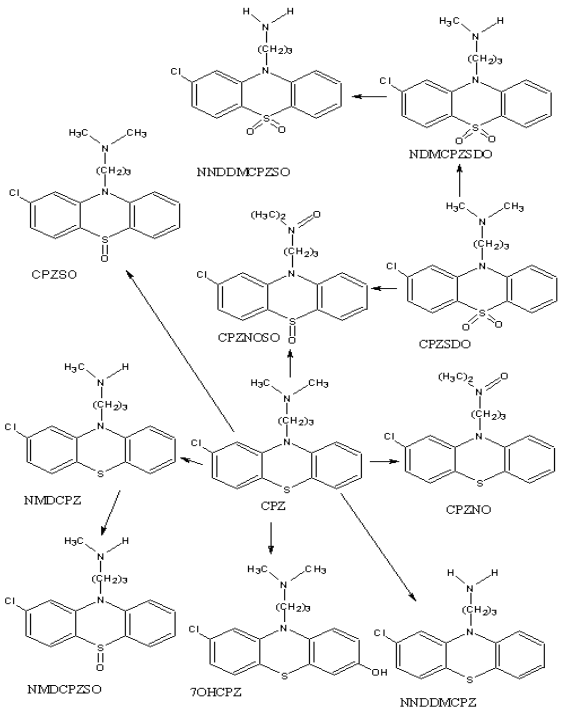

Figure 1: Proposed metabolic pathways for chlorpromazine and its metabolites [Based on references 4 and 5]

Computation methods

The geometries of CPZ and its metabolites CPZNO, CPZSO, 7OHCPZ, NMDMCPZ, NMDMCPZSO, NMDMCPZSDO, NNDDMCPZ, NNDDMCPZSO, NNDDMCPZSDO and CPZNOSO have been optimised based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling programs Spartan '02 and HyperChem 7.0. Molecular mechanics calculations were carried out using MM+ 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 0.01 was set 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 and LUMO.

Results and Discussion

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, dipole moment, energies of HOMO and LUMO as per both PM3 and DFT calculations for CPZ and its metabolites CPZNO, CPZSO, 7OHCPZ, NMDMCPZ,

NMDMCPZSO, NMDMCPZSDO, NNDDMCPZ, NNDDMCPZSO NNDDMCPZSDO and CPZNOSO. Figures 2-12 give the optimised structures of CPZ and the metabolites CPZNO, CPZSO, 7OHCPZ, NMDMCPZ, NMDMCPZSO, NMDMCPZSDO, NNDDMCPZ, NNDDMCPZSO NNDDMCPZSDO and CPZNOSO as per PM3 calculations using the program HyperChem 7.0. The structures also give (a) 2D contours of total electrostatic potential and (b) 2D HOMO plots. The dotted arrows in (a) indicate positions of most negative electric potential and in (b) the locations of HOMOs with the greatest electron densities.

The calculated solvation energies of CPZ and its metabolites CPZNO, CPZSO, 7OHCPZ, NMDMCPZ, NMDMCPZSO, NMDMCPZSDO, NNDDMCPZ, NNDDMCPZSO NNDDMCPZSDO and CPZNOSO from PM3 calculations in kcal mol^{-1} are respectively -5.97, -13.37, -13.62, -10.72, -8.19, -15.88, -13.12, -8.99, -17.08, -20.12 and -22.90 indicating that the compounds would differ in their solubility in water. CPZ would be least soluble in water (and therefore most soluble in lipid) followed by NMDMCPZ and NNDDMCPZ and the metabolite CPZNOSO is expected to be most soluble in water as it has the highest solvation energy. The increase in solvation energy and hence solubility in water in going CPZ to NMDCPZ is due to the loss of a methyl group which is non-polar. The solubility of NNDDMCPZ is expected to be higher than that of NMDCPZ as a further methyl group has been replaced by a hydrogen atom in the reaction:

NMDCPZ → NNDDMCPZ.

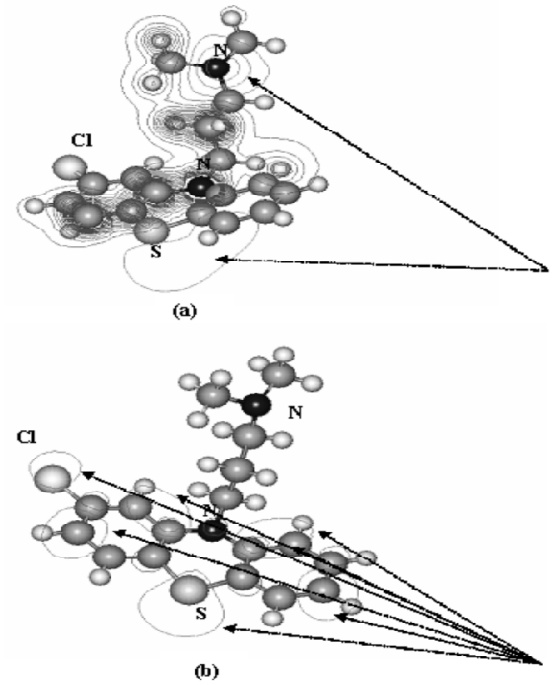

Figure 2: Structure of CPZ giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

Table 1
Calculated thermodynamic and other Parameters of Chloropromazine and its Metabolites

Molecule	*Calculation type*	*Total energy (kcalmol^{-1})*	*Heat of formation (kcalmol^{-1})*	*Enthalpy (kcalmol^{-1} K^{-1})*	*Entropy (calmol^{-1} K^{-1})*	*Solvation (calmol^{-1} K^{-1})*	*Free energy (calmol^{-1}*	*Dipole moment (debye)*	*HOMO (eV)*	*LUM 0 (eV)*	*LUMO HOMO*
CPZ	PM3	-41.33	47.30	214.46	145.78	-597	171.00	1.84	-8.13	-0.41	7.72
	DFT	-1627.13		213.22	144.23	-534	170.24	2.24	-5.07	-0.65	4.42
CPZNO	PM3	48.94	62.31	217.75	148.32	-13.37	173.53	4.14	-8.21	-0.57	7.64
	DFT	-1702.28		216.12	150.34	-12.45	171.32	6.03	-4.80	-0.44	4.36
CPZSO	PM3	8.58	20.56	217.32	147.75	-11.98	173.27	5.70	-8.62	-0.48	8.14
	DFT	-1702.30		215.76	145.89	-11.02	172.28	6.04	-5.54	-0.84	4.70
OHCPZ	PM3	-8.31	2.40	219.02	149.30	-10.72	174.50	1.43	-8.05	-0.39	7.66
	DFT	-1702.34		217.85	147.67	-9.87	173.84	1.21	-4.94	-0.58	4.36
NMDMCPZ	PM3	41.24	49.43	196.89	138.27	-8.19	155.67	2.15	-8.08	-0.39	7.69
	DFT	-1587.82		195.22	136.45	-7.76	154.57	1.97	-5.07	-0.66	4.41
NMDMCPZSO	PM3		17.94	200.56	139.90	-15.88	158.85	4.97	-8.70	-0.52	8.18
	DFT	-1662.99		198.89	137.98	-14.54	157.77	5.18	-5.63	-0.92	4.71
NMDMCPZSDO	PM3	-32.79	-13.12	202.23	145.68	-13.12	158.80	5.99	-8.95	-0.70	8.25
	DFT	-1738.20		200.98	144.24	-12.22	158.00	6.43	-6.01	-1.30	4.71
NNDDMCPZ	PM3	43.32	52.23	178.99	133.14	-8.99	139.30	1.68	-8.14	-0.41	7.73
	DFT	-1548.51		177.22	132.02	-796	137.88	2.04	-5.06	-0.63	4.43
NNDDMCPZSO	PM3	8.46	25.53	181.98	135.41	-17.08	141.60	5.41	-8.70	-0.50	8.20
	DFT	-1623.68		180.23	134.12	-15.89	140.26	5.83	-5.64	-0.91	4.73
NNDDMCPZSDO	PM3	-31.06	-10.94	184.62	139.77	-20.12	142.95	5.99	-8.95	-0.71	8.24
	DFT	-1698.89		182.78	138.24	-19.02	141.58	6.17	-6.03	-1.32	4.71
CPZNOSO	PM3	13.31	36.21	220.87	149.59	-22.90	176.27	1.48	-8.93	-0.73	8.20
	DFT	-1777.44		219.56	147.98	-20.88	175.46	2.34	-5.20	-1.14	4.06

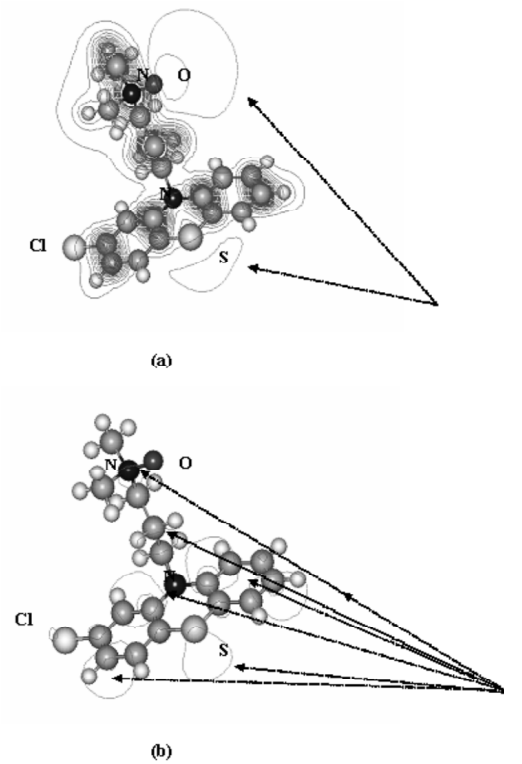

Figure 3: Structure of CPZNO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

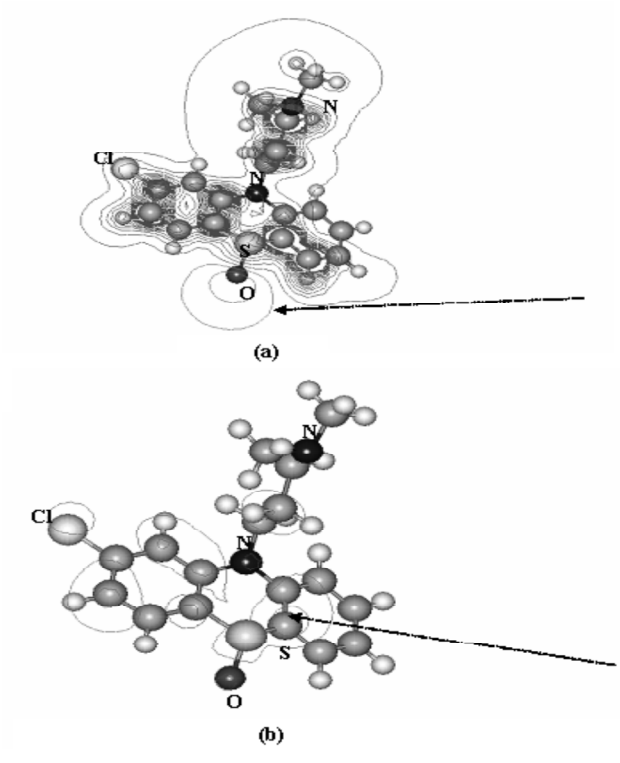

Figure 4: Structure of CPZSO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

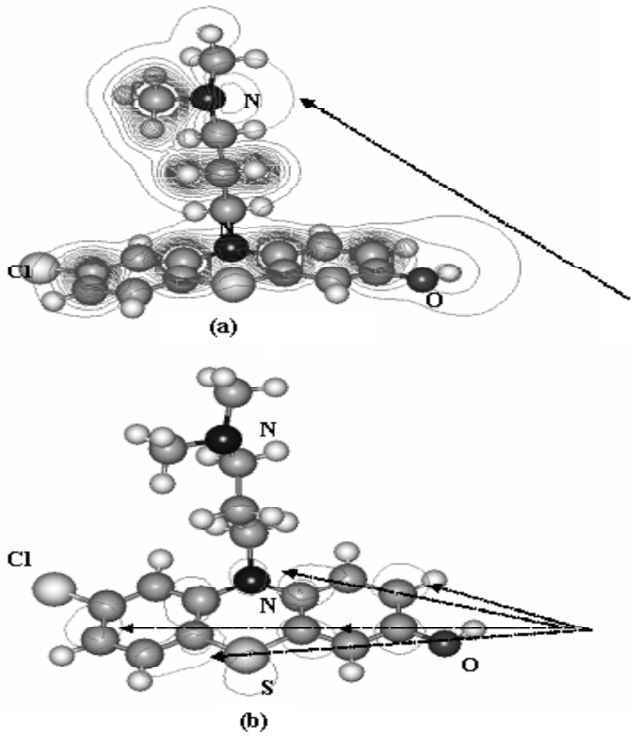

Figure 5: Structure of 7OHCPZ giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

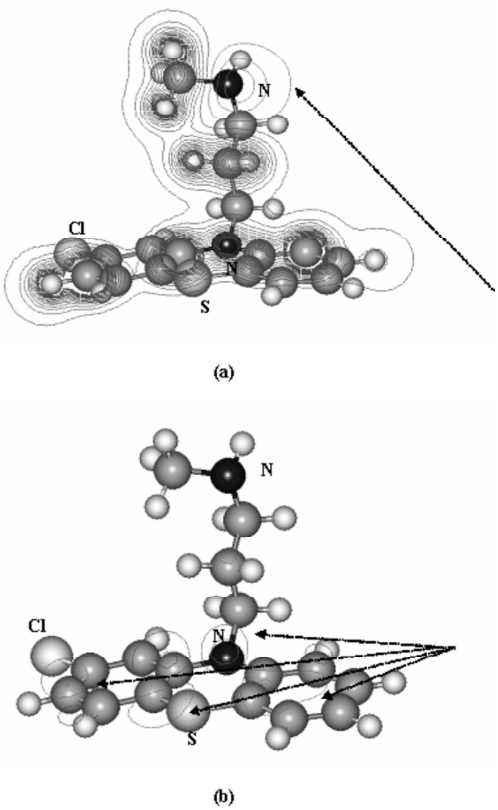

Figure 6: Structure of NMDMCPZ giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

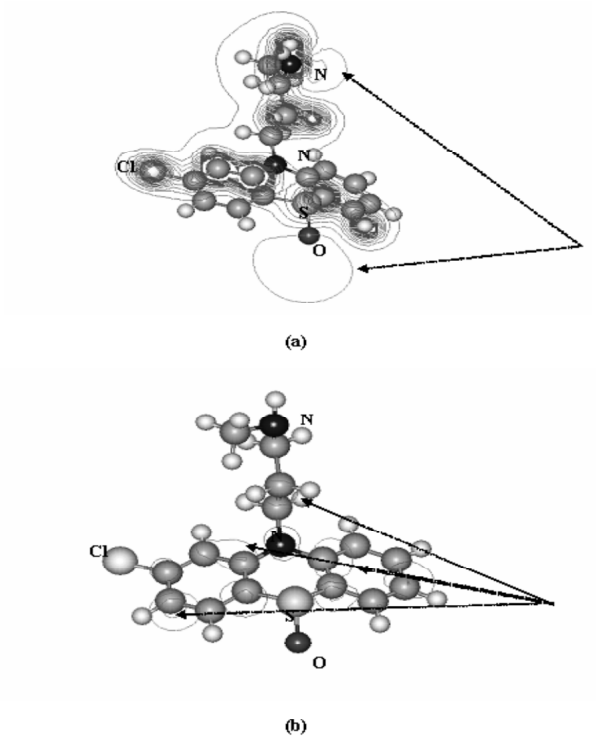

Figure 7: Structure of NMDMCPZSO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

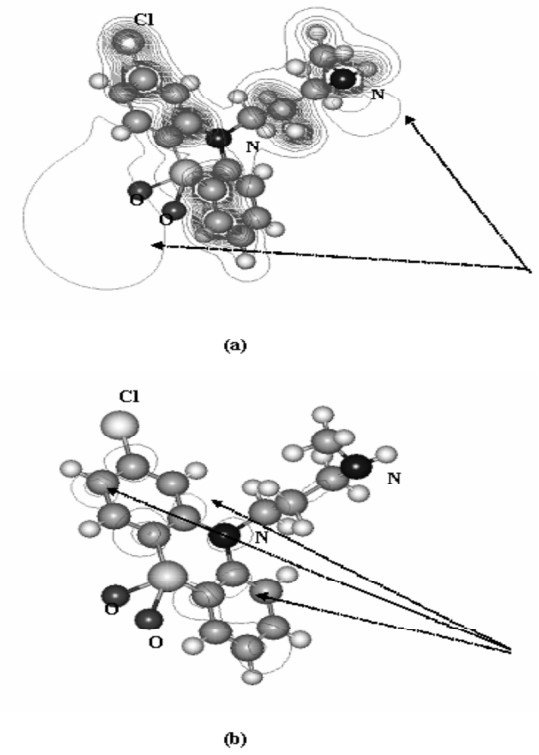

Figure 8: Structure of NMDMCPZSDO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

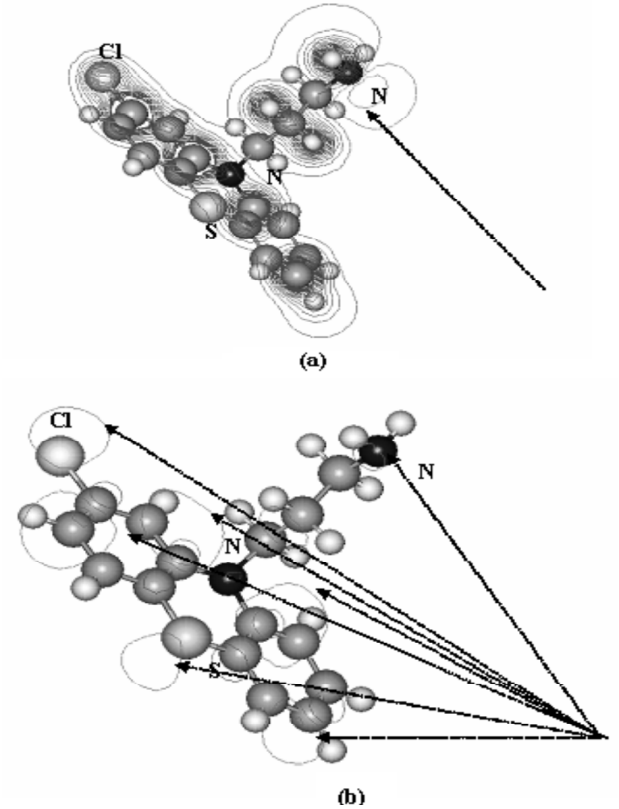

Figure 9: Structure of NNDDMCPZ giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

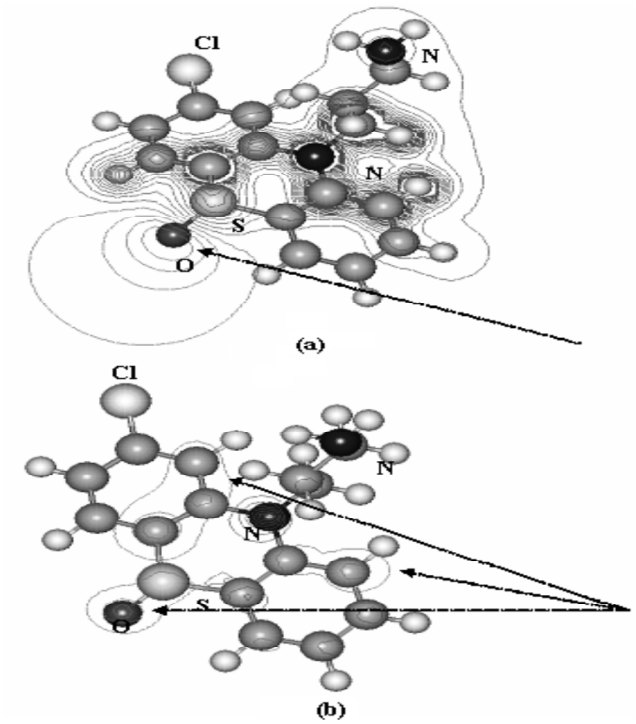

Figure 10: Structure of NNDDMCPZSO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

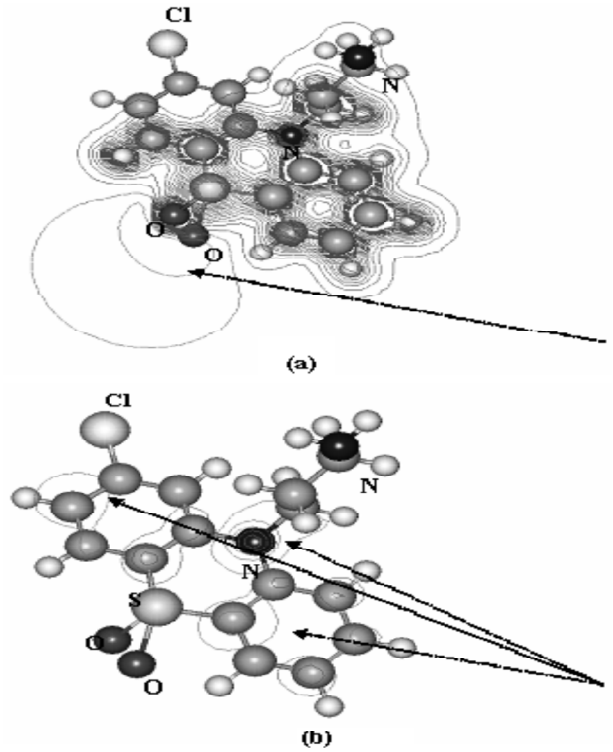

Figure 11: Structure of NNDDMCPZSDO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

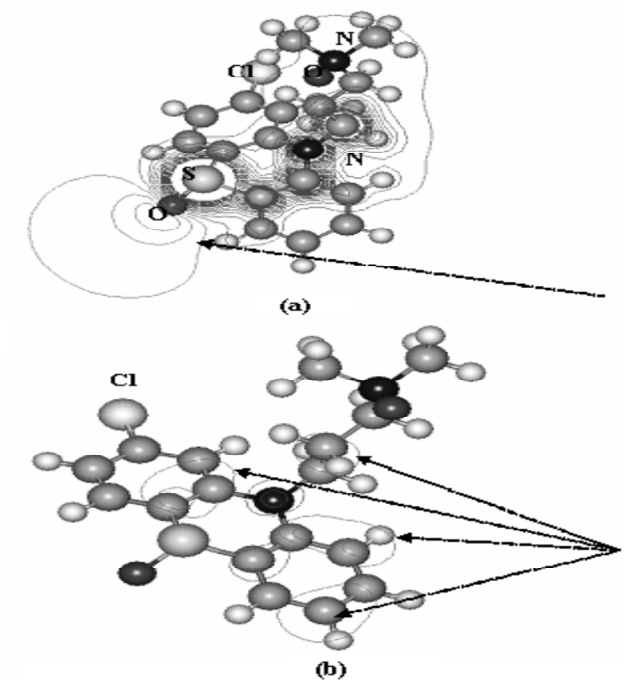

Figure 12: Structure of CPZNOSO giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

Much higher solvation energies of the metabolites CPZNOSO, NNDDMCPZSDO, NNDDMCPZSO, NDMCPZSO and NMDMCPZSDO can be explained in terms of the addition of one or more of polar groups S=O and N=O, and the oss of non-polar methyl groups. For example, CPZNOSO which has the highest solvation energy, has both N=O and S=O groups added to the CPZ molecule. When the calculated dipole moments are compared with solvation energy values, it is found that a high dipole moment does not always correlate with high solvation energy. For example, CPZNOSO which has a relatively small dipole moment (2.34 from DFT calculations) has a much higher solvation energy (-20.88 kcal mol^{-1} from DFT calculations) as compared to CPZNO which has a dipole moment of 6.03 from DFT calculations but a solvation energy value of -12.45 kcal mol^{-1} from DFT calculations. The values point out that the variation in solubility in water is a more complex process than simple dipole-dipole interactions as molecules may hydrolyse in solution in water that may affect solubility. For example, although carbon dioxide is a non-polar molecule it is highly soluble in water possibly due to hydrolysis.

The calculated heats of formation of CPZ and its metabolites are found to vary widely. Except for NMDCPZSO and NNDDDMCPZSDO (which have negative heats of formation), the rest of the molecules have positive heats of formation. CPZ, CPZNO and NNDDMCPZ are found to have the larger values for heats of formation. From the heats of formation of NMDCPZ and NMDCPZSO (49.43, 17.94 kcal mol^{-1} respectively), it appears that DG for the reaction: NMDCPZ $\rightarrow$ NMDCPZSO may be negative (assuming that there is not an accompanying pronounced change in entropy) so that the reaction would be spontaneous. From similar reasoning it may be argued that the reaction: NMDCPZSO $\rightarrow$ NMDCPZSDO could also be spontaneous. It should however be noted that a more definite conclusion can only be drawn by considering all reactants and products along with entropy and enthalpy values.

CPZ and all its metabolites have moderately low values for LUMO-HOMO energy differences ranging from 4.4 to 4.7 eV from DFT calculations except CPZNOSO which has a lower value of about 4.1 eV. The values indicate that CPZ and its metabolites would have moderately low kinetic lability except CPZNOSO which would be significantly more labile.

The greater kinetic lability and the lower thermodynamic stability (as it has a large positive heat

of formation) may mean that CPZNOSO will react more easily with biomolecules such as proteins and DNA. The greater solubility in water however means that the metabolite can be excreted more readily so that the metabolite would have the smallest biological half-life.

In the case of CPZ, 7OHCPZ, NMDMCPZ, NMDMCPZSO, NMDMCPZSDO and NNDDMCPZ, the electrostatic potential is found to be more negative at the nitrogen atom of aminopropyl side chain, indicating that the position would be subject to electrophilic attack. In the case of CPZ, NNDDMCPZ and CPZNO, the electrostatic potential is also found to be negative at the positions close to the sulfur atom, indicating once again that the positions would be subject to electrophilic attack. In the case of CPZNO, CPZSO, NMDMCPZSO, NMDMCPZSDO, NNDDMCPZSO, NNDDMCPZSDO and CPZNOSO, the electrostatic potential is found to be more negative at the positions of oxygen, indicating that the positions would be subject to electrophilic attack.

For CPZ and its metabolites the HOMOs with large electron density are found centred at a number of positions including carbon, nitrogen, oxygen and sulfur centres. The overlap of region of negative electrostatic potential and the HUMO with high electron density at the sulfur centre in the case of CPZ and CPZNO give further support to the idea that the position may be subject to electrophilic attack in the two compounds. Similarly it is found that in the case of NNDDMCPZ, the region of negative electrostatic potential and the HOMO with high electron density overlap at a nitrogen centre, once again giving further support to the idea that the position may be subject to electrophilic attack.

Conclusion

Molecular modelling analyses show that although CPZ and its metabolites have similar kinetic lability except CPZNOSO which is expected to be significantly more labile. The much greater variations in energy of solvation however suggest that the compounds would differ more significantly in their solubility in water and hence in their biological half-life. That a high dipole is not always found to correlate with a high solvation energy, points to the complexity of the solution process.

Abbreviations

CPZ: Chlorpromazine

CPZNO: Chlorpromazine N-oxide

CPZSO: Chlorpromazine sulfoxide

7OHCPZ: 7-hydroxychlorpromazine

NMDMCPZ: N-demethylchlorpromazine

NMDMCPZSO: N-demethylchlorpromazine sulfoxide

NMDMCPZSDO: N-emethylchlorpromazine sulfdioxide

NNDDMCPZ: N,N-idemethylchlorpromazine

NNDDMCPZSO: N,N-idemethylchlorpromazine sulfoxide

NNDDMCPZSDO: N,N-demethylchlorpromazine sulfdioxide

CPZNOSO: Chlorpromazine N-oxide sulfoxide

LUMO: Lowest energy unoccupied molecular orbital

HOMO: Highest energy occupied molecular orbital

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