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Molecular Modelling Analysis of The Metabolism of Hydralazine

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ABSTRACT: Hydralazine, widely used in the long term treatment of hypertension, can cause a disease with auto immune features known as lupus erythematosus that is characterized by connective tissue alteration and damage similar to that found in rheumatoid arthritis. However the molecular mechanism of toxicity due to hydralazine remains unclear. Hydralazine is metabolized by N-acetylation, glucuronidation and hydroxylation. Hydralazine-induced lupus occurs in individuals with a low capacity for N-acetylation. Acetylation is the major route of hydralazine metabolism that yields the non-toxic metabolite 3-methyl-s-triazolo[3,4-a]phthalazine. This suggests that either the parent drug or a different metabolic pathway is involved in the induction of lupus. Molecular modelling analyses using semi-empirical and DFT calculations show that a number of metabolites of hydralazine are kinetically labile with relatively low values for HOMO-LUMO energy differences; the most labile one being phthalazine dimer that is believed to be formed from the free radicals produced from hydralazine. It is logical to assume that the free radicals produced would be even more reactive that can damage DNA and other biomolecules.

Key words:Hydralazine, acetylation, toxicity, molecular modelling

Introduction

Hydralazine (1-hydrazinophthalazine, also known as Apresoline) (HP) is widely used in the long term treatment of hypertension because of its peripheral vasodilator effect [1,2]. Hypertension often coexists with hyperlipidemia, insulin resistance and glucose intolerance, commonly known as syndrome X [3]. HP acts directly on the arteriolar smooth muscle to induce relaxation with little or no effect on veins [4]. However, it can cause a disease with auto immune features known as lupus erythematosus that is characterized by connective tissue alteration and damage [5], similar to that in rheumatoid arthritis. The drug has also a number of side effects including headache, tachycardia, and other manifestations of baroreflex stimulation and marked with sodium and water retention., diarrhoea, constipation, nasal congestion, flushing and rashness. HP has a relatively short half-life of about 2.2–2.6 h with peak concentration in plasma reaching during 30–120 min after dosing.

Drug-induced immune reactions are thought to be caused by reactive metabolites [6]. The cellular response to hydralazine includes production of free radicals and induction of DNA damage. HP is metabolized by N-acetylation, glucuronidation and hydroxylation. Hydralazine-induced lupus occurs in individuals with a low capacity for N-acetylation [7]. Acetylation is the major route of hydralazine metabolism [8] that yields the inactive, non-toxic metabolite 3-methyl-s-triazolo [3, 4-a] phthalazine (MTP). This suggests that either the parent drug or a different metabolic pathway is involved in the induction of lupus. Metabolism of HP by the rat liver microsomes leads to the formation of phthalazine (P), phthalazinone (PZ) and reactive metabolites that bind covalently to microsomal protein [9] and may be involved in hydralazine-induced lupus [10]. There is some evidence to suggest that the oxidative metabolism of hydralazine may be involved in the adverse effects of the drug. For example, it has been demonstrated that phthalazinone (PZ), an oxidative product of hydralazine, is excreted in greater amounts by slow acetylators than by fast acetylators [11]. Formation of the dimer provides indirect evidence that free radicals are produced during microsomal metabolism of HP. It should be noted that the molecular mechanism of hydralazine-induced toxicity remains unclear although several studies provided evidence for the generation of free radicals [12,13].

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Figure 1 summarizes the metabolic pathway for hydralazine. In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 [14] and Spartan '02 [15] to investigate the relative stability of hydralazine and its metabolites with the aim of providing a better understanding of toxicity due to hydralazine and its metabolites.

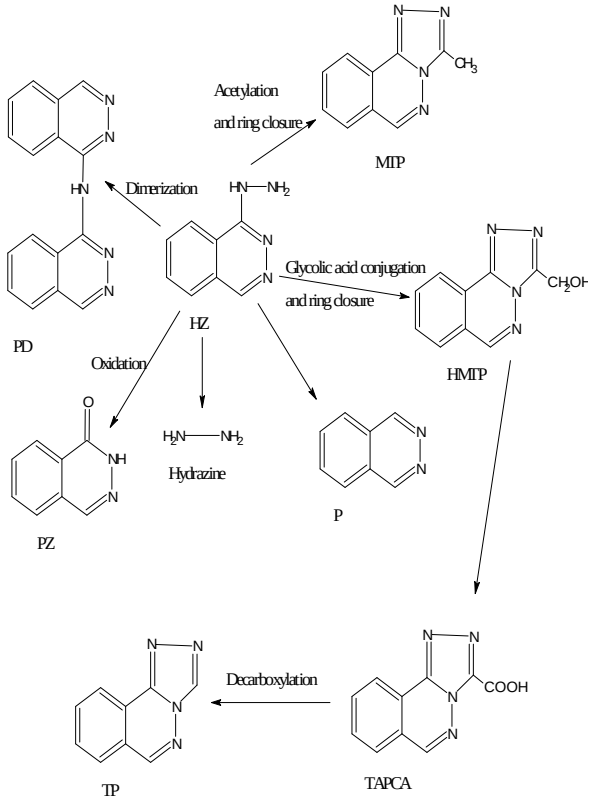

Figure 1: Schematic representation of metabolism of HP [Based on reference 7]

Computational methods

The geometries of HP and its metabolites P, PZ, s-triazolo [3,4-α] phthalazine (TP), MTP,

3-hydroxymethyl-s-triazolo[3,4-a]phthalazine (HMTP), s-triazolo[3,4-a]phthalazine-3-carboxylic acid (TAPCA), phthalazine dimer (PD) and hydrazine 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 as the termination condition. For the optimised structures, single point calculations were carried 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 HP, P, PZ, TP, MTP, HMTP, TAPCA, PD and hydrazine. Figures 2-10 give the optimised structures of HP, P, PZ, TP, MTP, HMTP, TAPCA, PD and hydrazine, respectively, 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 indicate positions of most negative electric potential and HOMOs with the greatest electron densities.

Table 1
Calculated thermodynamic and other parameters for hydralazine and its metabolites

Molecule	*Calculation type*	*Total energy (kcalmol)*	*Heat of formation*	*Enthalpy (Kcalmol K)*	*Entropy (kcalmol K)*	*Solvation energy (kcalmol K)*	*Free energy (kcalmol)*	*Dipole-moment (debye)*	*HOMO (eV)*	*LUMO (eV)*	*LUMO-HOMO eV*
HP	PM3	84.57	94.57	103.77	95.20	-10.00	75.38	4.03	-9.00	-1.04	7.96
	DFT	-528.62		105.00	94.51	-10.06	76.82	4.35	-6.03	-1.51	4.52
P	PM3	64.82	72.26	81.03	82.00	-7.44	56.58	4.46	-9.66	-1.02	8.64
	DFT	-417.95		81.71	81.65	-7.44	57.37	3.41	-6.34	-1.76	4.58
PZ	PM3	12.05	20.87	84.67	88.31	-8.82	58.34	3.20	-9.20	-0.88	8.32
	DFT	-493.20		85.92	86.52	-8.05	60.13	3.41	-6.37	-1.51	4.86
TP	PM3	83.00	91.18	99.03	90.62	-8.18	72.02	5.23	-9.34	-1.05	8.29
	DFT	-565.54		93.91	90.14	-10.40	67.03	5.61	-6.59	-1.96	4.63
MTP	PM3	94.89	105.34	111.48	99.46	-10.46	81.83	5.28	-9.30	-1.24	8.06
	DFT	-604.88		112.49	98.90	-10.63	83.01	5.17	-6.33	-1.87	4.46
HMTP	PM3	5151	65.93	115.20	105.79	-14.33	83.66	5.43	-9.45	-1.35	8.10
	DFT	-680.06		116.28	105.08	-12.86	84.95	6.72	-6.57	-2.08	4.49
TAPCA	PM3	13.67	28.93	116.19	100.91	-15.25	86.10	7.23	-9.87	-1.61	8.26
	DFT	-754.12		105.04	105.21	-10.81	73.63	7.76	-7.08	-2.31	4.77
PD	PM3	133.93	148.58	161.88	124.79	-14.65	124.67	3.54	-8.96	-1.09	7.87
	DFT	-890.04		160.02	125.34	-12.86	77.60	4.74	-6.08	-1.82	4.26
hydrazine	PM3	16.75	20.65	35.33	55.61	-3.90	18.75	0.00	-9.02	2.76	10.06
	DFT	-111.85		35.62	57.89	-5.74	18.35	0.00	-5.47	2.07	7.54

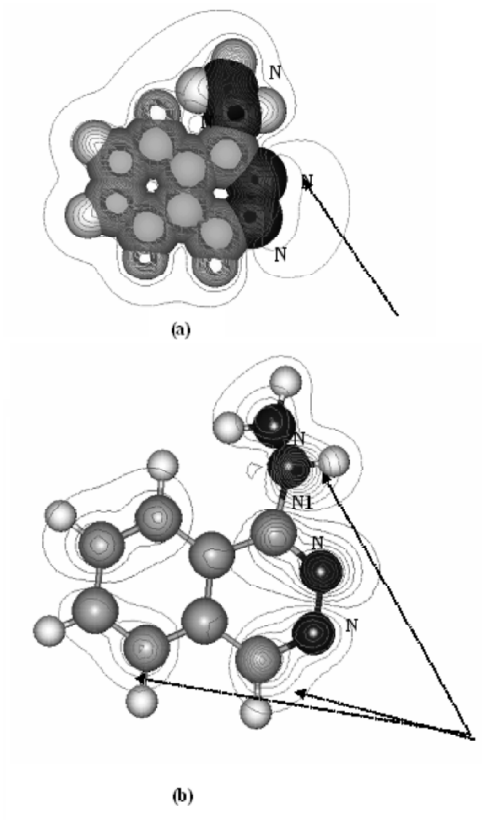

Figure 2: Structure of HP giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

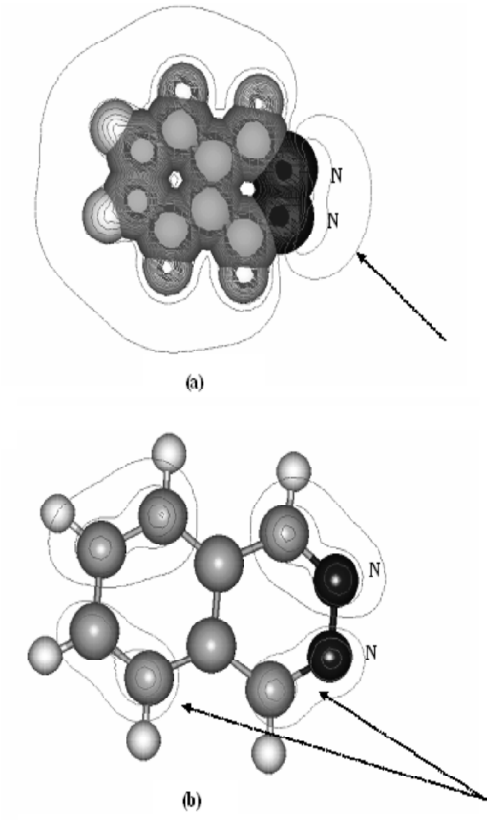

Figure 3: Structure of P giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

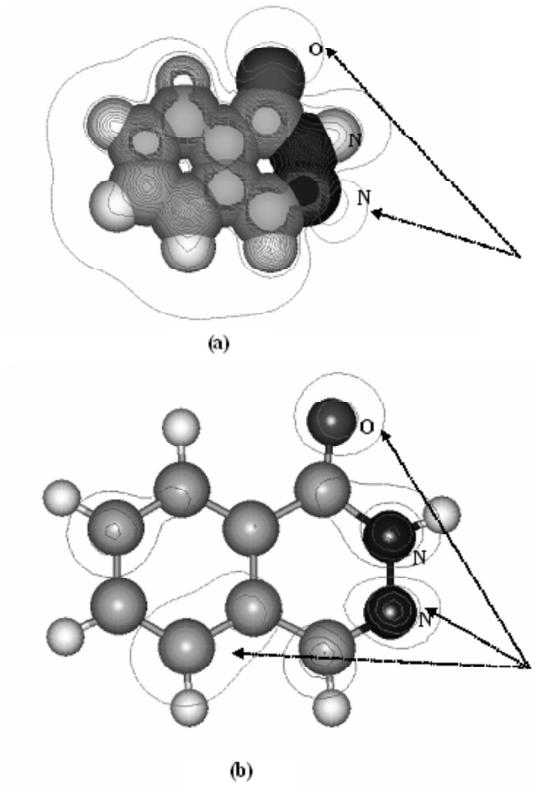

Figure 4: Structure of PZ giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

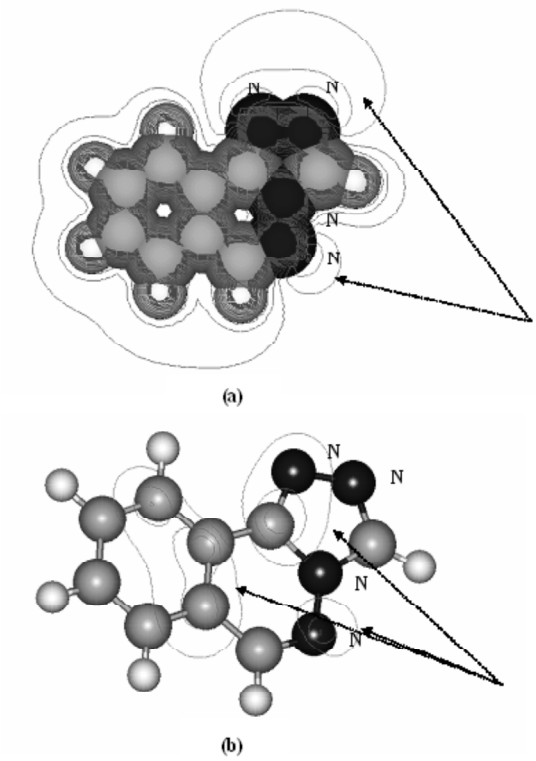

Figure 5: Structure of TP giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

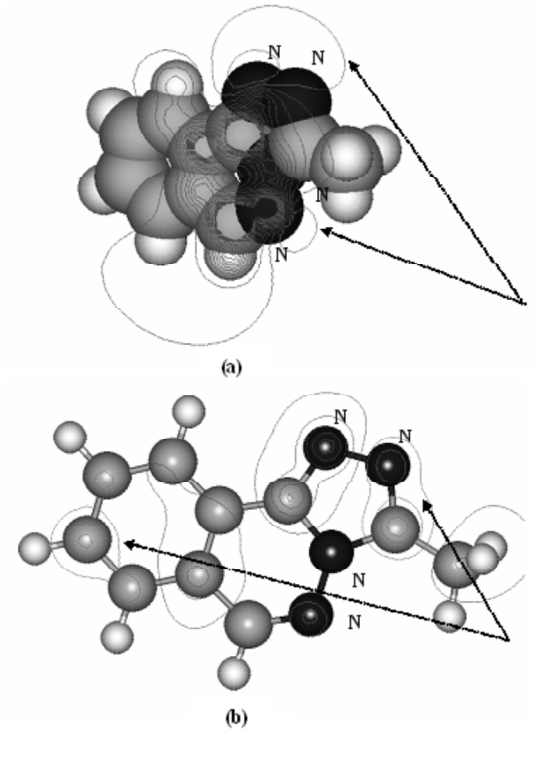

Figure 6: Structure of MTP giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

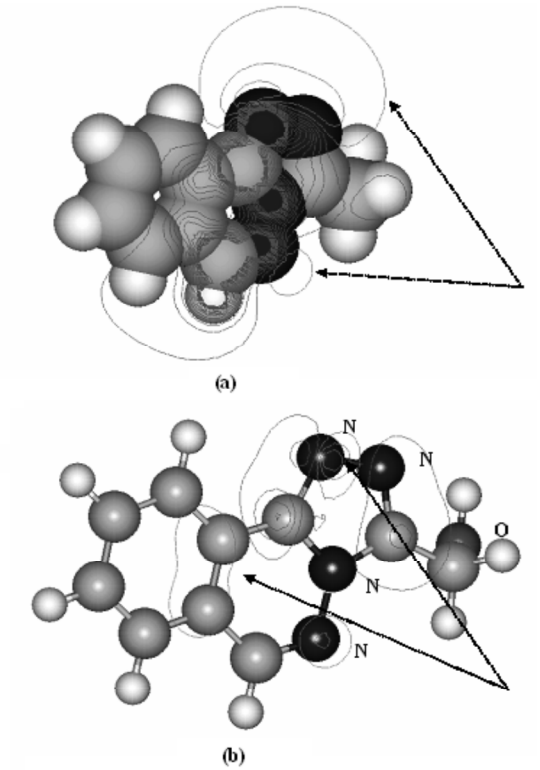

Figure 7: Structure of HMTP giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

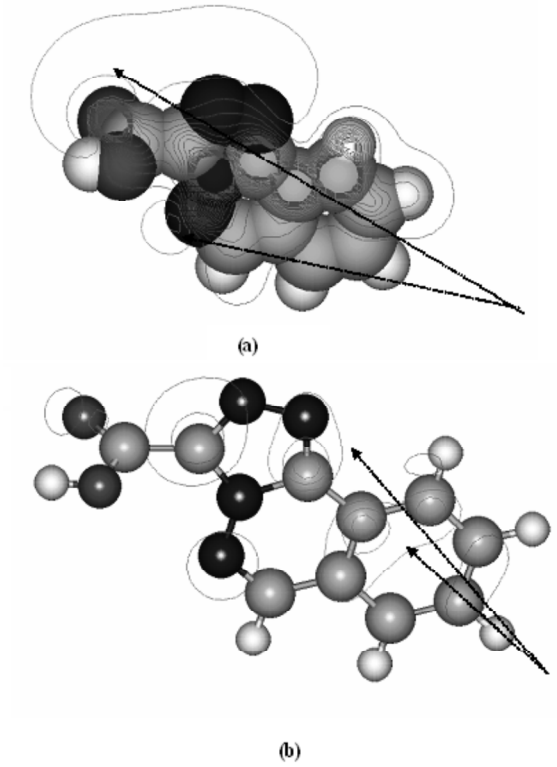

Figure 8: Structure of TAPCA giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

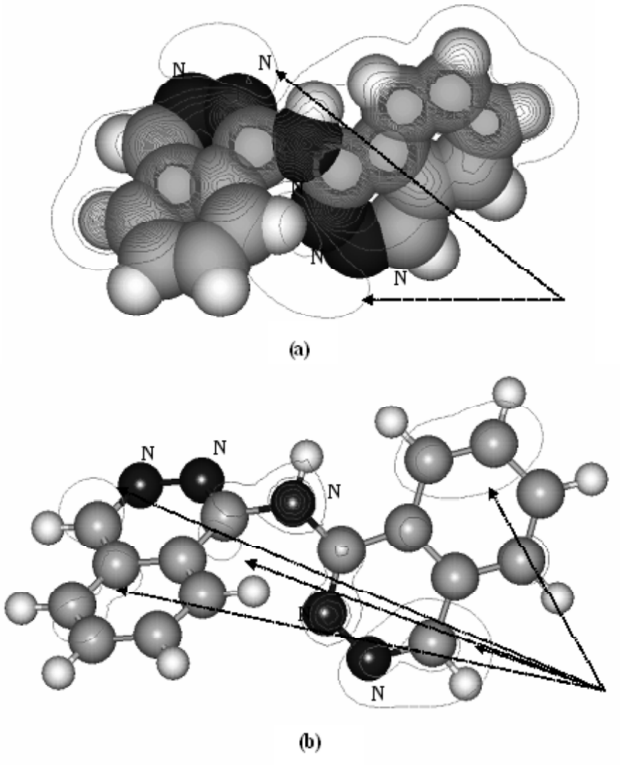

Figure 9: Structure of PD giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

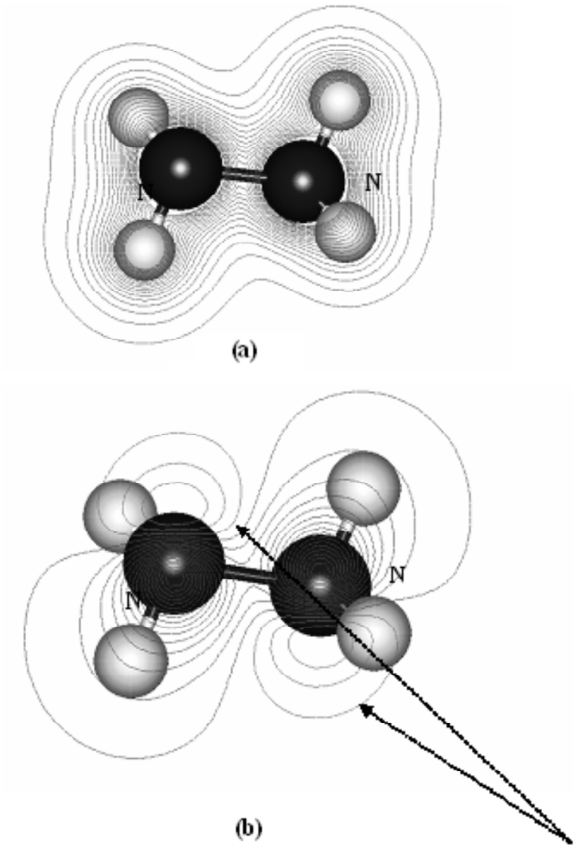

Figure 10: Structure of hydrazine giving (a) 2D contours of total electric potential and (b) 2D HOMO plot

The solvation energies of HP, P, PZ, TP, MTP, HMTP, TAPCA, PD and hydrazine from PM3 calculations in kcal mol^{-1} are respectively -10.00, -7.44, -8.82, -8.18, -10.46, -14.33, -15.25, -14.65 and -3.90 respectively indicating that hydralazine and all its metabolites would be soluble in water. The same conclusion can be drawn from DFT calculations as well.

HP and its metabolites except hydrazine have small LUMO-HOMO energy difference (of the order of 4.5 eV from DFT calculations) indicating that the compounds would be kinetically labile. It is known that according to frontier molecular orbital theory treatment, the reactivity of molecules can be controlled by the energy separation between LUMO and HOMO [16,17]. PD has the smallest LUMO-HOMO energy difference indicating that the metabolite would be most labile kinetically. It was noted earlier that the formation of phthalazine dimer provides indirect evidence for the formation of highly reactive free radicals in the metabolism of hydralazine. The low HOMO-LUMO energy difference suggests that even the dimer formed from the deactivation of free radicals itself would be quite reactive. PD has a large positive value of heat of formation (148.58 kcal mol^{-1}) indicating that the compound may also be unstable thermodynamically.

The highest HOMO-LUMO energy difference for HZ as compared to that for other metabolites and HZ indicates that the compound would be least labile kinetically. Positive values for heats of formation indicate that all the compounds would have low thermodynamic stability.

In the case of both HZ and P (that is formed from the breakdown of HZ into hydrazine and P), the electrostatic potential is found to be more negative around the two heterocyclic ring nitrogen atoms indicating that the positions may be subject to electrophilic attack. The HUMOs with large electron density are found to be centred mainly on the N1 atom of the hydrazine moiety so that the position may be subject to electrophilic attack e.g. that by H^+ resulting into the formation of hydrazine. HUMOs with large electron density are also found to be centred around the meta nitrogen atom and the para carbon of the six-membered heterocyclic ring – electron density around greater on the carbon so that the atom is more susceptible to oxidation as in the formation of phthalazone. HUMOs with high electron density are also found (although less pronounced) around two carbon atoms of the aromatic carbon ring.

In the case of P, HUMOs with large electron density are found to be centred around three ring carbon atoms including the carbon at the para position of the six-membered heterocyclic ring and the adjacent nitrogen atom of the same ring.

In the case of PZ formed from the oxidation of HP, the electrostatic potential is found to be more negative around the carbonyl oxygen and the ring nitrogen atom further away from the carbonyl group, indicating that the positions would be subject to electrophilic attack. HUMOs with large electron density are also found to be centred around the same atoms plus the carbon at the other meta position of the six-membered heterocyclic ring and the carbon atom of the six-membered aromatic carbon ring to which it is bonded.

In the case of TP, MTP, HMTP and TAPCA, the electrostatic potential is found to be more negative around the adjacent nitrogen atoms of the five-membered ring and the nitrogen atom of the six-membered heterocyclic ring that is further away from these atoms, indicating that the positions would be subject to electrophilic attack. In the case of TP, HUMOs with large electron density are found to be centred on the carbon atom that is common to both the five-membered and the six-membered

heterocyclic rings, nitrogen atom belonging to the six-membered heterocyclic ring only and three carbon atoms of the six-membered aromatic ring (two of which are common to the two six-membered fused rings). In the case of MTP, HUMOs with large electron density are found to be centred on the carbon of the five-membered ring to which methyl group is attached and the adjacent nitrogen atom of the same ring and a carbon atom of the six-membered aromatic ring. In the case of HMTP these are centred on a carbon atom and the adjacent nitrogen atom of the five-membered heterocyclic ring, the nitrogen atom that belongs only to the six-membered heterocyclic ring and two carbon atoms which are common to the two six-membered fused rings. In the case of TAPCA, HUMOs with large electron density are found to be centred on a number of atoms including a nitrogen atom and an adjacent carbon atom of the five-membered heterocyclic ring and three carbon atoms of the six-membered aromatic ring.

In the case of PD, the electrostatic potential is found to be more negative around the two sets of adjacent nitrogen atoms of the two five-membered rings, indicating that the positions would be subject to electrophilic attack. HUMOs with large electron density are found to be centred mainly on a number of carbon atoms of the six-membered aromatic and heterocyclic rings. In the case of hydrazine, HUMOs with large electron density are found to be centred on the two nitrogen atoms.

Conclusion

Molecular modelling analyses show that hydralazine and most of its metabolites have relatively small LUMO-HOMO energy differences indicating that they would be kinetically labile. The phthalazine dimer that is believed to be formed from the free radicals produced from the metabolism of hydralazine by non N-acetylation pathway is the most labile metabolite, suggesting that the free radicals would be even more reactive.

Abbreviations

LUMO: Lowest energy unoccupied molecular orbital
HOMO: Highest energy occupied molecular orbital
HP: Hydralazine
P: Phthalazine
PZ: Phthalazinone
TP: s-triazolo[3,4-a]phthalazine
MTP: 3-methyl-s-triazolo[3,4-a]phthalazine
HMTP: 3-hydroxymethyl-s-triazolo[3,4-a] phthalazine
TAPCA: s-triazolo[3,4-a]phthalazine-3-carboxylic acid
PD: Thalazine dimer

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