IJPAC : **13(1-2),** *(January-June 2018)*

Molecular Modelling Analysis of the Metabolism of Dacarbazine

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ABSTRACT: Dacarbazine (DTIC) that is routinely used in the treatment of malignant melanoma, Hodgkin's disease, renaladenocarcinoma, soft tissue sarcoma, solid tumours and malignant lymphomas, is a produg that is metabolized in the liver to produce the active species responsible for causing DNA methylation. Hydroxylation one of the methyl groups first produces HMMTIC that yields the unstable metabolite MTIC following the loss of the hydroxymethyl moiety as formaldehyde. Spontaneous cleavage of MTIC produces AIC, and the methylating agent MHAZ. The methylation of DNA is believed to be responsible for both antineoplastic activity and carcinogenicity. The adverse effects of DTIC include nausea, vomiting, myelosuppression, flu-like syndrome and facial flushing. In this study, molecular modelling analyses have been carried out using the program Spartan '02 to investigate the relative stability of DTIC and its metabolites, to locate the positions of negative electrostatic potential and the HOMOS with high electron density as applied to DTIC and its metabolites, with the aim of providing a better understanding of toxicity due to the drug. The results of analyses show that DTIC and its metabolites differ significantly in their kinetic lability with most having relatively small LUMO-HOMO energy differences and hence quite labile. The results of the analyses also show that most of the metabolites may be subject to electrophilic attack at a number of sites. The location of HOMOs with high electron density close to O, C and in between the two nitrogen atoms of MDAZH indicates that the reaction with the molecule may take place at a number of sites. The presence of HOMOs with high electron density close to O, C and in between the two nitrogen atoms of MDAZH indicate that the electrophilic attack on the molecule may take place at a number of sites.

Key words: Dacarbazine, antitumour activity, Hodgkin's disease, methylation, molecular modelling

Introduction

Dacarbazine (DTIC) that has been used in cancer therapy since 1970, is routinely used as a single agent in the treatment of malignant melanoma and in combination with other drugs such as doxorubicin, bleomycin and vinblastine for the treatment of Hodgkin's disease, renaladenocarcinoma, soft tissue sarcoma, solid tumours and malignant lymphomas [1]. A recent review shows that DTIC monotherapy is equivalent in terms of antitumour efficacy and survival to combination therapy [2]. Chemically, DTIC is 5-(3,3-dimethyltriazen-1-yl) imidazole-4-carboxamide, belonging to the class of compounds known as dimethyl triazenes.

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The adverse effects of DTIC include nausea, vomiting, myelosuppression, flu-like syndrome and facial flushing. Hepatotoxicity has also been recorded with the drug. DTIC may cause severe pain and tissue necrosis if infiltration occurs and has been found to be carcinogenic in experimental animals [3]. Chronic oral and i.p. administration of DTIC to rats and mice causes mammary adenocarcinomas, and thymic and splenic lymphomas. DTIC also shows delayed and cumulative haematological toxicity [4]. In sporadic cases DTIC has been found to lead to irreversible hepatic vascular toxicity that is characterized by fever, eosinophilia and hepatic necrosis [5]. The half-life for excretion of DTIC is about 5 hours [3].

DTIC is a produg that that is metabolized in the liver to produce the active species responsible for causing DNA methylation [6]. Bioactivation of DTIC is initiated by the hydroxylation one of the methyl groups catalyzed

by CYP1A2, that leads to the formation of MTIC which is 5-(3-methyl-1-triazeno)imidazole-4-carboxamide. Hydroxylation first produces the intermediate carbinolamine 5-(3-hydroxymethyl-3-methyl-1-triazeno) imidazole-4-carboxamide (HMMTIC) which yields the reactive metabolite monomethyl triazene imidazole carboxamide (MTIC) following the loss of the hydroxymethyl moiety as formaldehyde (FDH). The two tautomeric forms of DTIC (denoted as MTIC1 and MTIC2) that differ in the position of N=N bond exist in equilibrium. Spontaneous cleavage of MTIC produces the stable metabolite, 4-amino-5-imidazole-carboxamide (AIC), and methanediazohydroxide (MDAZH) which is a known methylating agent (MDAZH ionizes to form methanediazonium ion that methylates DNA). Not much is known about the plasma disposition of the intermediate metabolites, HMMTIC and MTIC because of their high reactivity [7]. The established pathways for DTIC metabolism are shown in Figure 1.

Methylation of DNA by the activated metabolite of DTIC is believed to be responsible for both antineoplastic activity and carcinogenicity of DTIC [7-8]. Methylation of DNA by MTIC produces N^{7}-methylguanine (N^{7}-meG) and O^{6}-methylguanine (O^{6}-meG), among other lesions [9]. MTIC has been found to have antiproliferative activity in vitro experiments [10]. O^{6}-methylguanine, produced in chromosomal DNA by alkylating carcinogens can induce both apoptosis and mutagenesis [11].

In this study, molecular modelling analyses have been carried out using the program Spartan '02 [12] to investigate the relative stability of DTIC and its metabolites, to locate the positions of negative electrostatic potential and the HOMOS with high electron density as applied to DTIC and its metabolites, with the aim of providing a better understanding of toxicity due to the drug.

Computation methods

The geometries of DTIC and its metabolites HMMTIC, MTIC, AIC, MHAZ, MDAZH and FDH have been optimised 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 0.01 was set as the terminating 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.

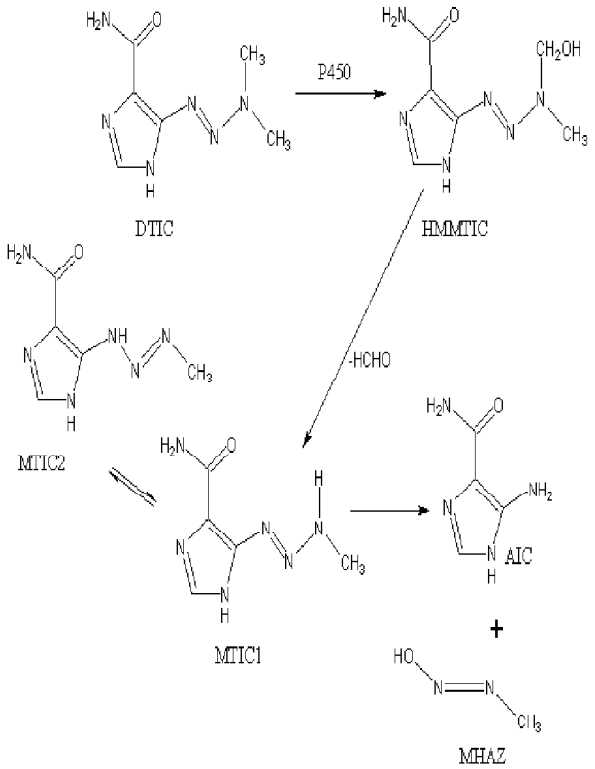


Figure 1: Proposed metabolic pathways for DTIC and its metabolites [Based on reference 6]

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 DTIC and its metabolites HMMTIC, MTIC, AIC, MDAZH and FDH. Figures 2, 3, 4, 5, 6, 7 and 8 give the optimised structures of DTIC and its metabolites HMMTIC, MTIC, AIC, MDAZH and FDH as per DFT calculations. The structures also give regions of negative electrostatic potential (greyish-white envelopes) and HOMOs (blue and red where red indicates HOMOs with high electron density).

The calculated solvation energies of DTIC and its metabolites HMMTIC, MTIC, AIC, MDAZH and FDH from DFT calculations in kcal mol^{-1} are respectively -20.36, -25.45, -22.10, -22.32, -17.03, -12.90 and -2.44 and their dipole moments also from DFT calculations are 5.20, 3.82, 5.07, 3.55, 3.70, 1.79 and 2.19 respectively. The values indicate that DTIC and all of its metabolites would be soluble in water with FDH being the least soluble metabolite. When the calculated

Table 1
Calculated thermodynamic and other parameters of DTIC and its metabolites

Molecule	*Calculation type*	*Total energy (kcal mol^{-1})*	*Heat of formation (kcal mol^{-1})*	*Enthapy (kcal mol^{-1}K^{-1})*	*Entropy (cal mol^{-1} K^{-1})*	*Solvation energy (kcal mol^{-1})*	*Free energy (kcal mol^{-1})*	*Dipole moment (debye)*	*HOMO (eV)*	*LUMO (eV)*	*LUMO HOMO (eV)*
DTIC	PM3	27.95	47.07	119.39	115.50	-19.12	84.95	5.11	-9.04	-0.70	8.34
	DFT	-638.85		121.34	117.12	-20.36	86.44	5.20	-5.45	-1.27	4.18
HMMTIC	PM3		3.81	122.90	121.63	-24.25	86.64	4.09	-9.19	-0.69	8.50
	DFT	-713.56		124.45	123.73	-25.45	87.58	3.82	-5.66	-1.43	4.23
MTIC1	PM3	26.52	48.10	101.72	108.54	-21.59	69.36	4.92	-9.12	-0.73	8.39
	DFT	-599.04		103.39	110.50	-22.10	70.44	5.07	-5.66	-1.50	4.29
MTIC2	PM3	23.96	41.71	101.45	108.86	-17.75	69.30	4.40	-9.05	-0.20	8.85
	DFT	-599.05		103.14	109.08	-22.32	70.62	3.55	-5.54	-1.01	4.53
AIC	PM3	-28.67	-10.06	79.96	89.63	-18.61	50.23	3.61	-9.44	0.01	9.45
	DFT	-450.29		77.66	88.68	-17.03	51.23	3.70	-5.47	0.27	5.74
MDAZH	PM3	9.56	14.91	41.06	68.67	-5.35	20.58	1.56	-10.14	-0.41	10.55
	DFT	-225.15		41.42	67.41	-12.90	21.32	1.79	-6.94	-0.74	6.20
PDH	PM3	-36.94	-34.08	2.23	53.64	-2.86	2.23	2.16	-10.63	0.83	11.46
	DFT	-114.50		18.63	53.61	-2.44	2.65	2.19	-7.31	-1.15	6.16

solvation energies are compared with the corresponding dipole moments, it is found that generally a high dipole moment corresponds to a high solvation energy value. This is different from that observed in the case of chlorpromazine and its metabolites where some metabolites were reported to have high solvation energy values even thtough the dipole moments are small [13]. It was pointed out that the solvation energy and the process of solution can be quite complex as compounds may hydrolyse in solution in water.

The calculated heat of formation of HMMTIC is 3.81 kcal mol^{-1} as compared to that for DTIC of 47.07 kcal mol^{-1}. The much larger value of heat of formation for DTIC as compared to that for HMMTIC suggests the Gibb's free energy change (DG) for the reaction: DTIC $\rightarrow$ HMMTIC would be negative so that the process could be thermodynamically spontaneous. From similar considerations we may conclude that the conversion of MTIC (more exactly the tautomeric forms MTIC1 and MTIC2 in equilibrium) into AIC would also be spontaneous. The differences in heats of formation of MTIC1 and MTIC2 suggest that MTIC2 would be slightly more stable than MTIC1.

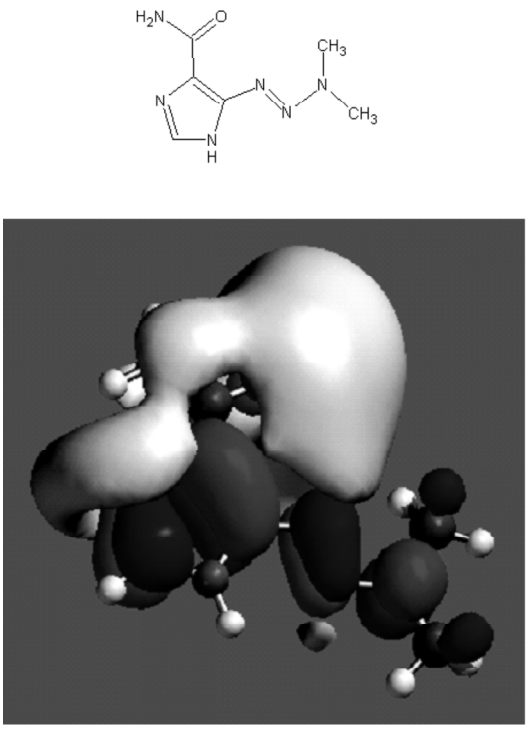
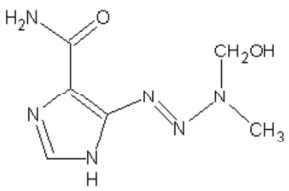


Figure 2: Structure of DTIC giving the regions of negative electrostatic potential and the HOMOs



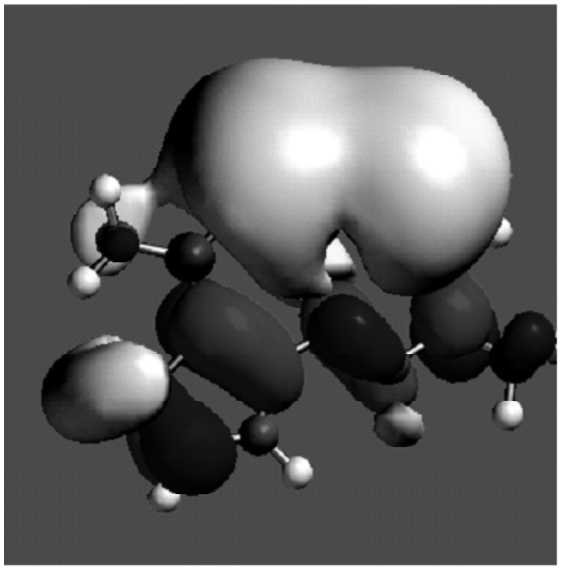
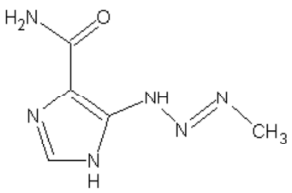


Figure 3: Structure of HMMTIC giving the regions of negative electrostatic potential and the HOMOs



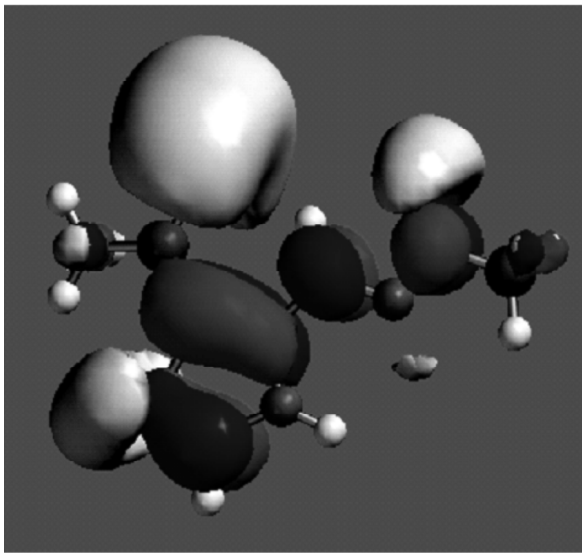
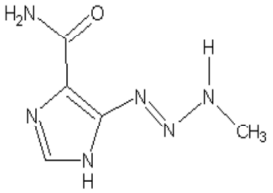


Figure 5: Structure of MTIC2 giving the regions of negative electrostatic potential and the HOMOs



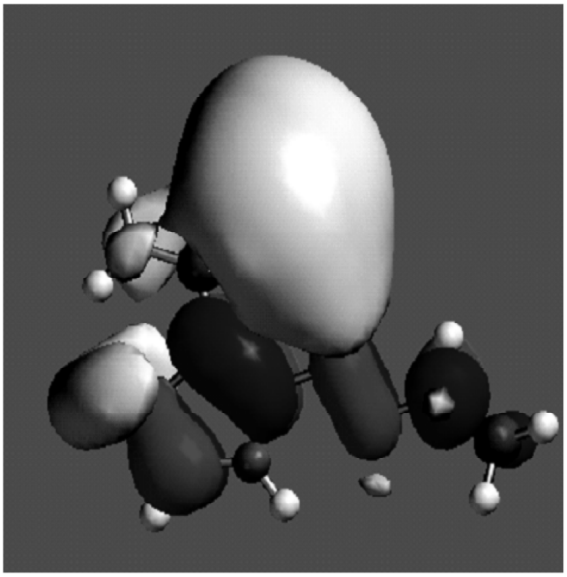
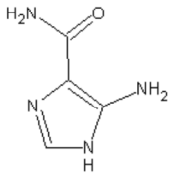


Figure 4: **S**tructure of MTIC1 giving the regions of negative electrostatic potential and the HOMOs



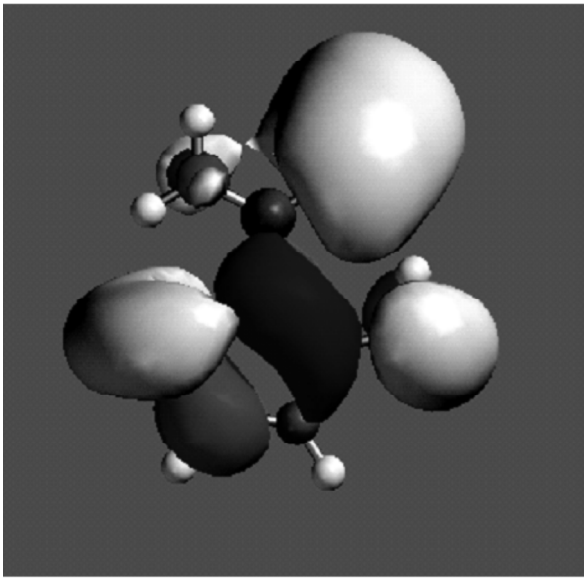
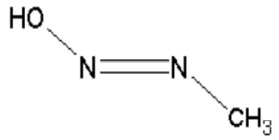


Figure 6: Structure of AIC giving the regions of negative electrostatic potential and the HOMOs



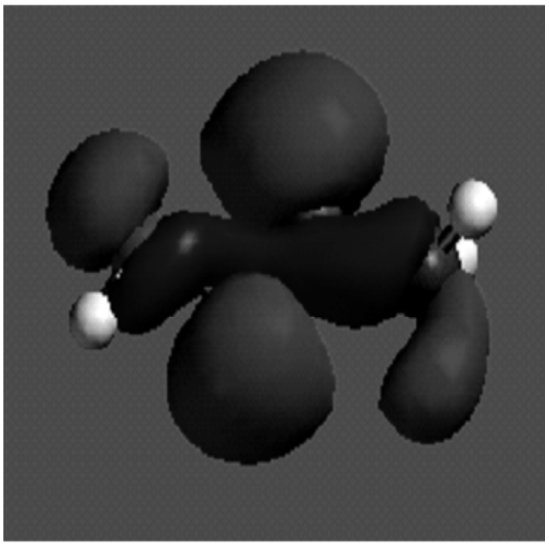
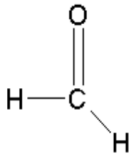


Figure 7: Structure of MDAZH giving the regions of negative electrostatic potential and the HOMOs



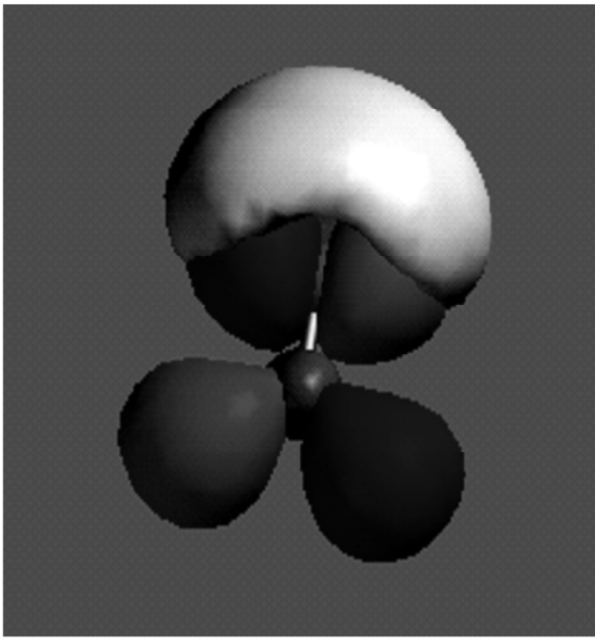


Figure 8: Structure of FDH giving the regions of negative electrostatic potential and the HOMOs

DTIC and its four metabolites have LUMO-HOMO energy differences ranging from 4.18 eV to 6.20 eV from DFT calculations suggesting that the compounds would differ in their kinetic lability. Placed in the higher of decreasing reactivity from left to right, DTIC and its metabolites are: DTIC>HMMTIC>MTIC1> MTIC2> AIC>FDH>MDAZH. It can be seen that DTIC is expected to be most labile kinetically whereas MDAZH would be least labile. HMMTIC and MTIC (MTIC1 and MTIC2) are expected to have the smaller biological half-life since they would be most easily excreted in the urine. However, the metabolites being kinetically labile may quickly change into other metabolites.

In the case of DTIC, HMMTIC, MTIC1, MTIC2, AIC and MDZAH the electrostatic potential is found to be more negative around the carbonyl oxygen, the amino (NH_2) nitrogen, the imidazole ring nitrogen involved in C=N bond and one or more of the triazene nitrogen, indicating that the positions may be subject to electrophilic attack. In the case of HMMTIC, the electrostatic potential is also found to be negative around the hydroxyl oxygen atoms. In the case of FDH also, the electrostatic potential is found to be more negative around the carbonyl oxygen, indicating that the positions may be subject to electrophilic attack.

In the case of DTIC, HMMTIC, MTIC1 and MTIC2 the HUMOs with large electron density are found close to the amino (NH_2) nitrogen, imidazole ring carbons and one or more of the triazene nitrogens. In the case of AIC, the HUMOs with high electron density are found close to the amide nitrogen, all the carbon atoms and C=N nitrogen of the imidazole ring. In the case of MDAZH, the HUMOs with high electron density are found close to each of the four non-hydrogen atoms namely O, N, N and C. In the case of FDH, the HOMOs with high electron density are found to be close to C and O atoms. The overlap of regions of negative electrostatic potential and HOMOs with large electron density close to a number of nitrogen centres reinforce the idea that the positions may be subject to electrophilic attack. The presence of HOMOs with high electron density close to O, C and in between the two nitrogen atoms of MDAZH indicate that the reaction with the molecule may take place at a number of sites.

Conclusion

Molecular modelling analyses show that DTIC and most of its metabolites have relatively small LUMO-HOMO energy differences indicating that they would be kinetically labile. The presence of HOMOs with high

electron density close to O, C and in between the two nitrogen atoms of MDAZH indicate that the electrophilic attack on the molecule may take place at a number of sites.

Abbreviations

DTIC:Dacarbazine;5-(3,3-dimethyltriazen-1-yl)imidazole-4-carboxamide

HMTIC:5-(3-hydroxymethyl-3-methyl-1-triazeno)imidazole-4-carboxamide

MTIC1:5-(3-methyl-1-triazeno)imidazole-4-carboxamide

MTIC2:5-(3-methyl-2-triazeno)imidazole-4-carboxamide

AIC:4-amino-5-imidazole-carboxamide

MDHAZ: Methanediazohydroxide

LUMO:Lowest energy unoccupied molecular orbital

HOMO:Highest energy occupied molecular orbital

Acknowledgments

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

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