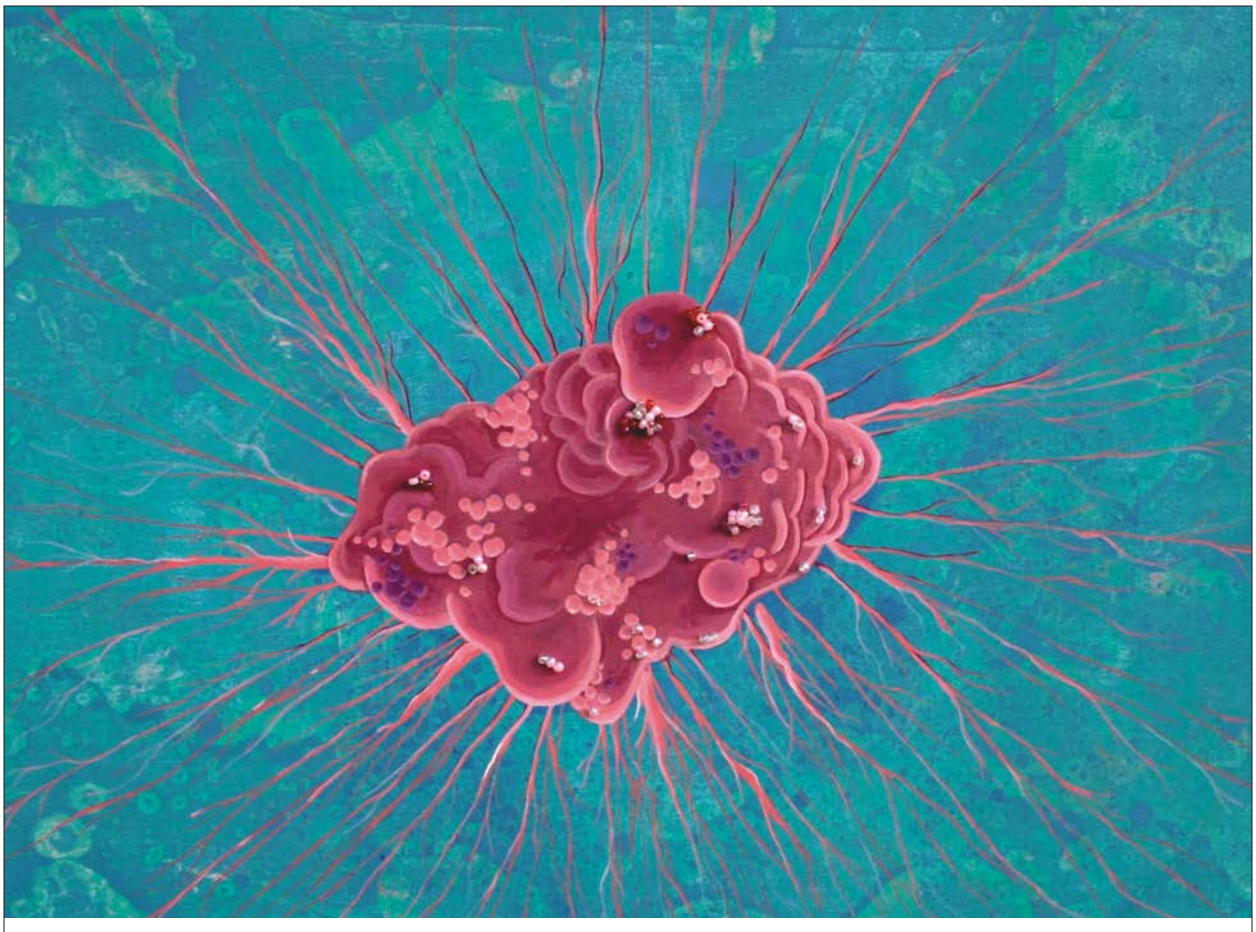

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Molecular Modelling Analysis of the Metabolism of Anagrelide

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Abstract: Anagrelide is an orally active imidazoquinazoline derivative used for the treatment of thrombocytosis in patients with chronic myeloproliferative disorders. AG is extensively metabolized by the liver into two major metabolites BCH24426 and RL603. AG and its active metabolite RL603 reversibly block the maturation of late-stage megakaryocytes in a dose-dependent manner, thus reducing platelet counts in patients with essential thrombocythaemia. The most common adverse effects associated with anagrelide are headache, palpitations, diarrhoea, asthenia, oedema, nausea, abdominal pain and dizziness. AG is not mutagenic and to date there is no evidence to suggest it is leukaemogenic. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that AG and its metabolites BCH24426 and RL603 have moderately large LUMO-HOMO energy differences so that neither is expected to be highly labile. The active metabolite RL603 has the largest LUMO-HOMO energy difference so that it would be most inert kinetically. Thus although the molecular surfaces of neither AG and its metabolites are found to have some electron-deficient (blue) regions so that they can react with glutathione and nucleobases in DNA, the rates of such adverse reactions are not expected to be significant.

Key words: Anagrelide, thrombocytosis, platelet count, headache, molecular modelling

Introduction

Anagrelide (AG; Agrylin®, Xagrid®) is an orally active imidazoquinazoline derivative used for the treatment of thrombocytosis in patients with chronic myeloproliferative disorders (Silverstein *et al*., 1988). It was initially developed as an inhibitor of platelet aggregation (Fleming and Buynisski, 1979). However, when tested it showed profound thrombocytopenic effects (Abe *et al*., 1984) and this prompted its evaluation in the treatment of thrombocytosis.

Essential thrombocythaemia is a myeloproliferative disorder associated with a sustained increase in the number of platelets in peripheral blood (Wagstaff and Keatng, 2006). The thrombocytopenic activity of AG results primarily from an inhibitory effect on post mitotic phase of megarkaryocyte (MK) maturation (Mazur *et al*., 1992). However, the mechanism by which AG reduces platelet count remains largely unclear (Hong and Erusalimsky, 2002). The only known primary target of anagrelide is a type III phosphodiesterase (PDEIII) found in platelets and myocardium (Gillespie, 1988; Beavo, 1995). The lack of animal models of the thrombocytopenic effects of anagrelide (which are more evident in humans than in laboratory animals) has limited the study of the pharmacodynamic effects of AG (Birgerard *et al*., 2004).

AG is extensively metabolized by the liver (Prescatore and Lindley, 2000) into two major metabolites 6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo (2,1-b) quiazolin-2-one (BCH24426) and 2-amino-5,6-dichloro-3,4-dihydroquinazoline (RL603). AG and its active metabolite RL603 reversibly block the maturation of late-stage megakaryocytes in a dose-dependent manner, thus reducing platelet

counts in patients with essential thrombocythaemia. The most common adverse effects associated with oral anagrelide are headache, palpitations, diarrhoea, asthenia, oedema, nausea, abdominal pain and dizziness (Wagstaff and Keating, 2006). AG is not mutagenic and to date there is no evidence to suggest it is leukaemogenic.

In this study molecular modelling analyses have been carried of AG and its metabolites in order to obtain a better understanding of toxicity due to AG and its metabolites. The study was carried out in the School of Biomedical Sciences, The University of Sydney during March to September 2006.

Materials and Methods

The geometries of AG and its metabolites have been optimised based on molecular mechanics (Fig. 1), semi-empirical and DFT calculations, using the molecular modelling program Spartan '02 (2002). Molecular mechanics calculations were carried out using MMFF 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 0.001 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. The order of calculations: molecular mechanics followed by semi-empirical

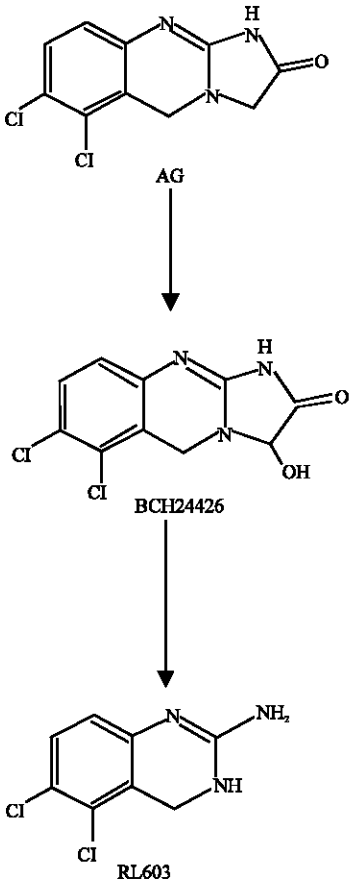

Fig. 1: Metabolic pathways of Anagrelide (Based on Wang *et al*., 2005)

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 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 AG and its metabolites BCH24426 and RL603. Figure 2-4 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 surfaces (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimised structures of AG and its metabolites BCH24426 and RL603.

The calculated solvation energies of AG and its metabolites BCH24426 and RL603 from PM3 calculations in kcal mol^{-1} are, respectively -12.24, -11.12 and -14.56 and their dipole moments from DFT calculations are 4.6, 1.5 and 5.1, respectively. The values suggest that AG and its metabolites would be moderately soluble in water with RL603 having moderately higher solubility.

The LUMO-HOMO energy differences from DFT calculations of AG and its metabolites BCH24426 and RL603 are 4.79, 4.74 and 5.00 eV, respectively, indicating that the compounds would be moderately inert with RL603 being the most.

The molecular surfaces of AG and its metabolites (Fig. 2d-4d) are found to have electron-rich (yellow and red), electron-deficient (blue) and neutral (green) regions so that they may be subject to electrophilic, nucleophilic and hydrophobic interactions. The surface of AG is found to abound most in electron-rich regions whereas that of RL603 abounds most in electron-deficient regions so that RL603 may be most subject to nucleophilic attacks. However, the high kinetic inertness of the RL603 means that its reaction with glutathione and nucleobases in DNA may not be significant.

In the case of AG, the electrostatic potential is found to be more negative around the nitrogen and carbonyl oxygen centres, indicating that the positions may be subject to electrophilic attack. In the case

Table 1: Calculated thermodynamic and other parameters of AG 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})
AG	PM3	-16.72	-4.47	107.76	114.29	-12.24
	DFT	-1545.19		109.61	113.81	-13.19
BCH24426	PM3	-18.06	-6.94	111.23	120.62	-11.12
	DFT	-1620.33		112.49	121.83	-9.87
RL603	PM3	12.94	27.50	97.13	105.73	-14.56
	DFT	-1393.74		98.17	106.41	-13.17

Continued

Molecule	Calculation type	Free energy (kcal mol^{-1})	Area ($Å^2$)	Volume ($Å^3$)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO HOMO (eV)
AG	PM3	73.68	227.09	211.03	1.5	-8.81	-0.80	8.01
	DFT	75.68	229.11	211.21	4.6	-5.97	-1.18	4.79
BCH24426	PM3	75.27	238.51	220.21	2.2	-8.94	-0.88	-8.06
	DFT	76.17	237.31	219.41	1.5	-6.06	-1.32	4.74
RL603	PM3	65.61	199.82	179.82	3.4	-8.45	-0.48	7.97
	DFT	66.44	201.21	180.40	5.1	-5.53	-0.53	5.00

* in atomic units from DFT calculations

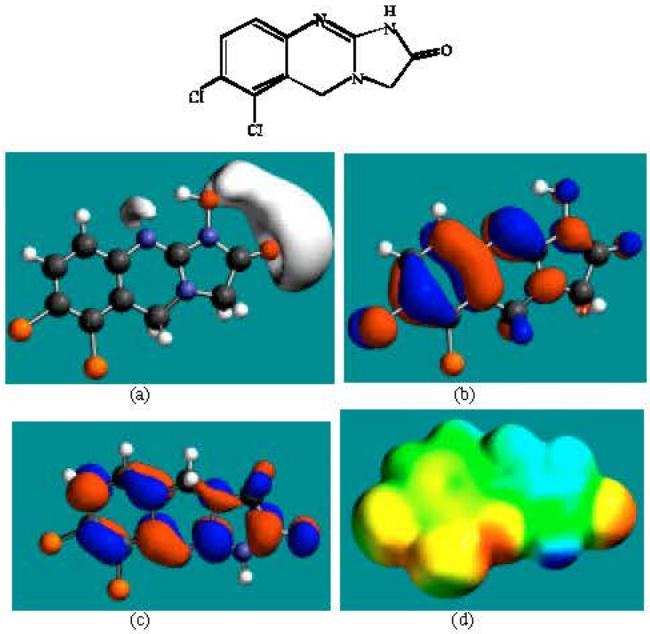

Fig. 2: Structure of AG giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

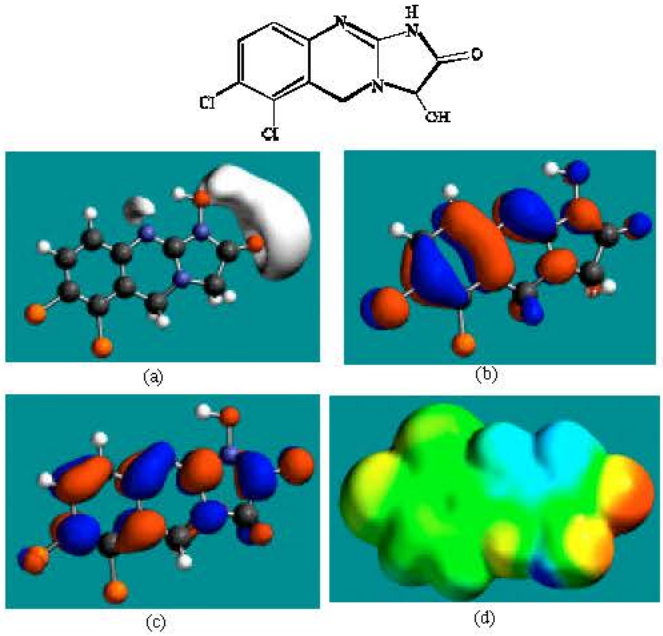

Fig. 3: Structure of BCH24426 giving in: (a) the electrostatic potential (greyish envelope enotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

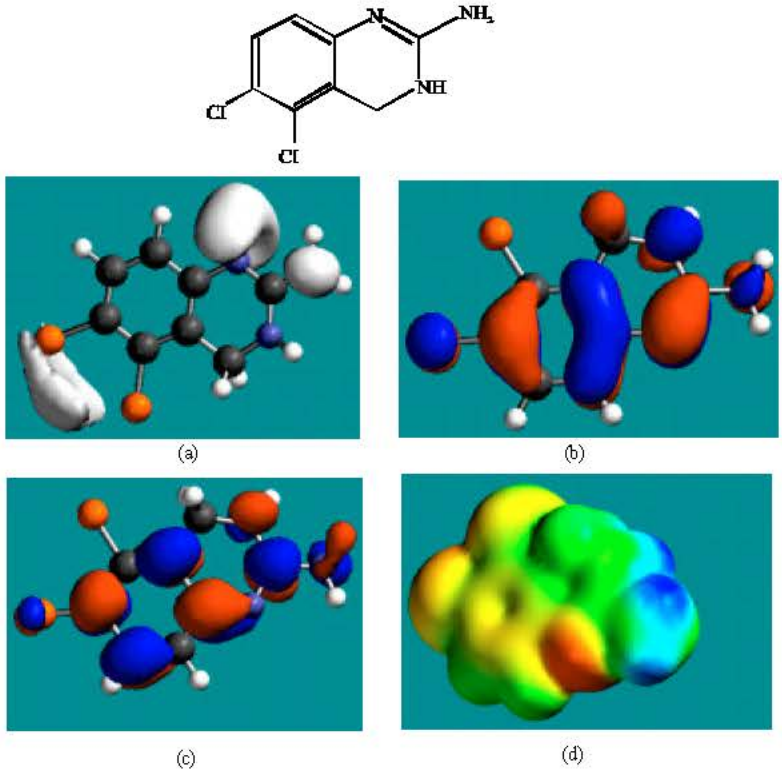

Fig. 4: Structure of RL603 giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

of BCH24426, the electrostatic potential is found to be more negative around the carbonyl and hydroxyl oxygen centres indicating that the positions may be subject to electrophilic attack. In the case of RL603, the electrostatic potential is found to be more negative around the amino nitrogen atom and two chlorine atoms indicating that the positions may be subject to electrophilic attack.

In the case of AG, BCH24426 and RL603, both the HOMOs with high electron density and the LUMOs are found close to most of the non-hydrogen atoms.

When surface area and volume of AG are compared with those of its metabolites BCH24426 and RL603, it is found to that the values for BCH24426 are more different from of RL603 (Table 1) even though RL603 is pharmacologically like the parent drug whilst BCH24426 is not. Looking at the commonness and differences in the structures of AG, BCH24426 and RL603, it appears that a key determinant of lack of activity of BCH24426 may be the presence of hydroxyl group in it.

Conclusions

Molecular modelling analyses based on semi-empirical and DFT calculations show that among AG and its metabolites, BCH24426 and RL603 have large LUMO-HOMO energy difference so that they would be kinetically inert. Thus, although the molecular surfaces of the three compounds possess some electron-deficient regions so that they can react with cellular glutathione and nucleobases in DNA, in actual fact, the rates of such adverse reactions would be low.

Abbreviations

AG:	Anagrelide; Agrylin®, Xagrid®)
PDEIII:	Type III phosphodiesterase
BCH24426:	6,7-dichloro-3-hydroxy-1,5-dihydro-imidazo(2,1-b)quiazolin-2-one
RL603:	2-amino-5,6-dichloro-3,4-dihydroquinazoline
LUMO:	Lowest unoccupied molecular orbital
HOMO:	Highest occupied molecular orbital

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