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Cyclophosphamides as hypoxia-activated diffusible cytotoxins: A theoretical study

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Summary

Cyclophosphamides have been in clinical use as anti-cancer drugs for a long time and much research has been directed towards reducing their side effects. Here we have performed a theoretical investigation into the possibility of designing bioreductive analogues of cyclophosphamides. Our calculations have employed semiempirical molecular orbital AM1-SM2 and PM3-SM3 calculations, as implemented in MOPAC 93, which include a modified Born method for the treatment of solvation. We have investigated the effect of bioreductive activation on the β-elimination reaction that is central to the activation of cyclophosphamides. The approach was tested on two known bioreductive agents, including CB1954, and gave results in agreement with experiment. Non-local density functional calculations on CB1954 and its metabolites, including the radical anion, were in agreement with the semiempirical calculations. The calculations have identified a number of potentially novel bioreductive cyclophosphamides. In particular, our calculations identified compounds in which the initial one-electron reduction was not activating. Such compounds are likely to be more effective bioreductive agents, as the β-elimination will not compete under oxic conditions with the important re-oxidation required for the protection of oxic tissue.

Introduction

Cyclophosphamide [1] (CP, 1a, Figure 1) has been in clinical use for cancer therapy for a long time. However, serious side effects such as immunosuppression, bone marrow depression, nausea, vomiting, haemorrhage, cystitis and the development of drug resistance have prompted research on the development of more effective cyclophosphamide analogues. A number of these analogues have indeed shown promising properties [2,3]. Moreover, since encouraging results have also been obtained for nitrobenzylphosphamides [4], it appeared appropriate to initiate a theoretical investigation of bioreductive analogues of cyclophosphamide. Indeed, bioreductive activation offers the potential for reducing side effects and at the same time targeting the active cytotoxins to the particularly resistant hypoxic tumour cells. Bioreductive anticancer drugs are ideally only activated under the reducing conditions of oxygen deficient cancer cells – the principles of bioreductive activation have been described elsewhere [5,6]. A number of bioreductive agents, RSU 1069, EO9 and SR4233, which represent the main classes of bioreductive agents, namely nitroimidazoles, quinone-based compounds and di N-oxides have shown sufficient promise to enter phase II clinical trials but these compounds all produce reactive free radicals with a limited diffusion range [7]. A possible way forward would be to design a diffusible hypoxia-activated cytotoxin that could also kill cells in the oxic fraction. Progress in this area has been achieved using cobalt complexes as carriers for nitrogen mustard, but the complexes were too toxic [9]. Theoretical studies have given novel insights that may lead to improvements in the cobalt systems [10] and it seemed appropriate to extend this theoretical investigation to the cyclophosphamides.

The mechanism of activation [11,12] of cyclophosphamide (1a) is shown in Figure 1. The initial step involves the hepatic microsomal oxidation of

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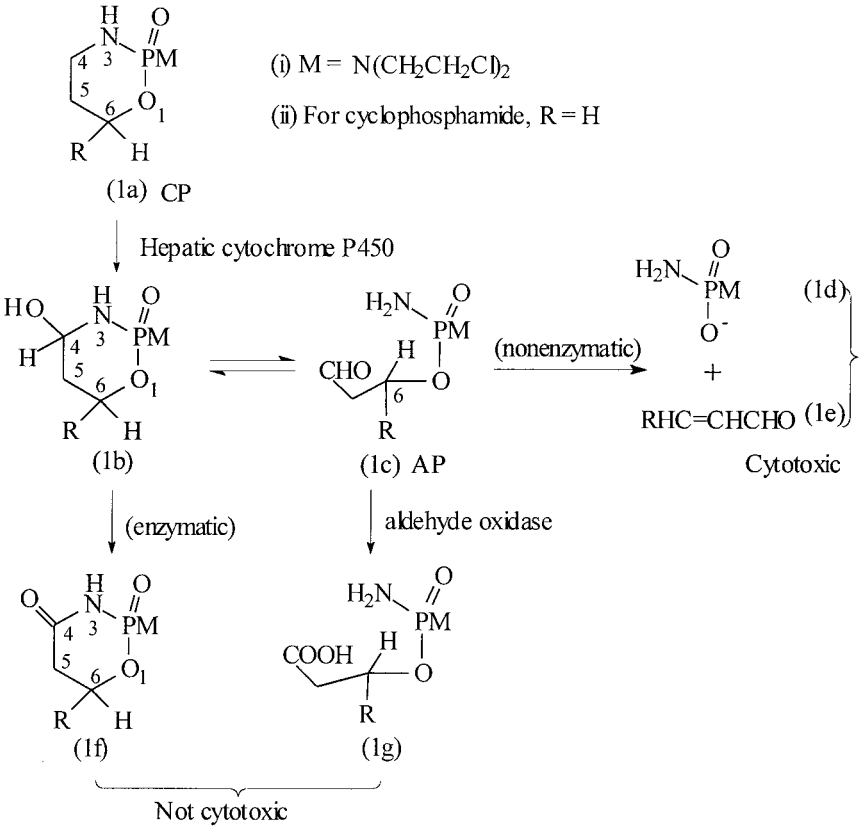

Figure 1. The mechanism of activation of cyclophosphamide, CP.

(1a) to 4-hydroxycyclo-phosphamide (1b), which is in equilibrium with its tautomer, aldophosphamide (AP, 1c). These intermediates undergo further enzyme reactions to produce the non-toxic products: 4-ketocyclophosphamide (1f) and carboxyphosphamide (1g). Here, hepatic cytochrome P450 is responsible for the initial hepatic transformation of the parent drug [13] while aldehyde dehydrogenase is thought to be responsible for the oxidation of (1c) to (1g) [11]. The aldophosphamide (1c) is chemically unstable and undergoes β-elimination to yield phosphoramide mustard (1d), the diffusible cytotoxin, and acrolein (1e), which is thought to be responsible for the serious kidney toxicity in cyclophosphamide therapy [14]. One possible reason for the selective action of cyclophosphamide is that the aldehyde dehydrogenase detoxification reactions occur to a greater extent in normal cells than in cancer cells [11]. Aldehyde dehydrogenase may also be involved in cyclophosphamide resistance, since it is associated with reduced levels of DNA interstrand cross-linking [15]. Moreover, sensitivity to cyclophosphamide can be restored by inhibitors of aldehyde dehydrogenase [16].

Of the CP analogues studied to date, the majority are substituted at the C-4, C-5 and C-6 positions [2,17,18] (see Figure 2). The 4-substituted analogues (2a) [19] and (2b) [20] were introduced to bypass the need for the initial enzymatic activation step, while N3-substituted derivatives (2c–2e) of (2a) and (2b) [3]were developed with some success to avoid the premature hydrolysis of the activated CP analogues. The less successful 5-substituted cyclophosphamide analogues (2f and 2g) were designed to diminish both the affinity of the aldophosphamide analogues for aldehyde dehydrogenase and the electrophilicity of the unsaturated aldehyde (1e) [2].

The 6-substituted CP analogues (2h, 2i) [2] were equally disappointing but 6-substituted 4HC analogues [2], (2j–2l), have shown improvements over 4HC itself. The para-nitrobenzyl group has also been used to develop hypoxia-selective phosphamides (2j). Compounds (3a, 3h and 3i) (Figure 3) [4,21] have shown selective toxicities towards hypoxic cells of

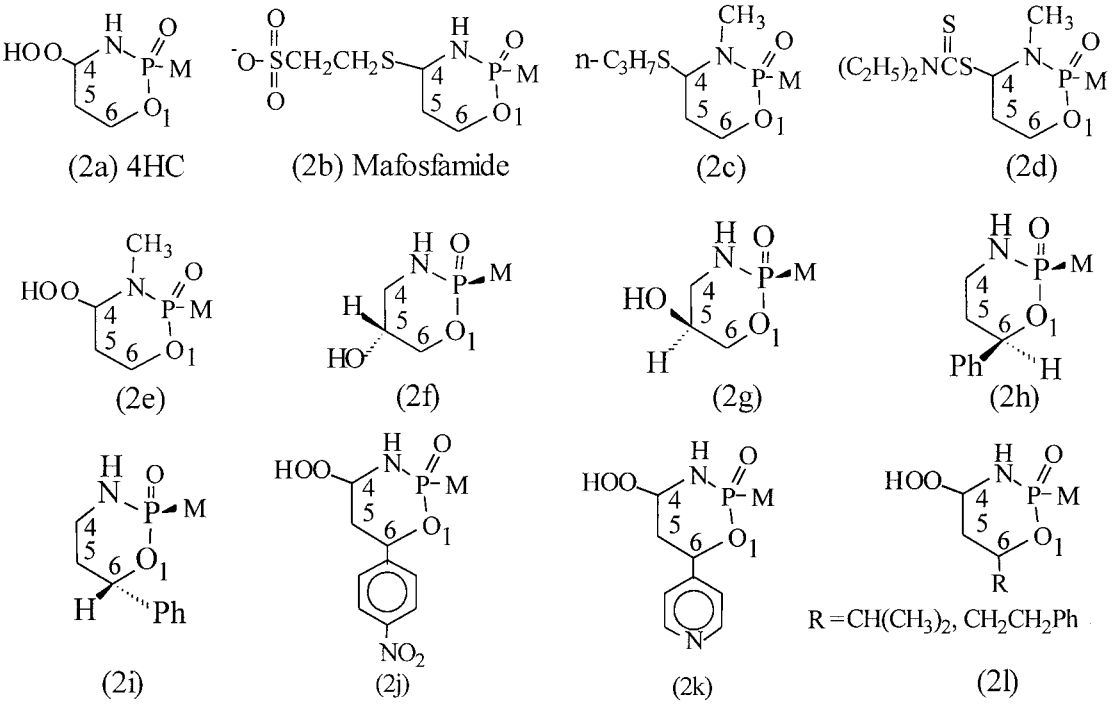

Figure 2. Various 4, 5, and 6-substituted cyclophosphamide analogues.

90:1, 11:1 and 8.1:1, respectively. These results suggest that bioreductive analogues of cyclophosphamide may be effective and provided the impetus for this theoretical study. In particular, in cyclophosphamide/P450 2B1 gene therapy, Wei et al. [22] found that the cyclophosphamide-sensitised P450-expressing C6 glioma cells transfer cytotoxicity to the P450-nonexpressing cells by releasing diffusible metabolites through the medium. This bystander effect occurs even when only 10% of the cells in culture express the P450 2B1 gene, confirming that the cytotoxic metabolites are diffusible.

Since β-elimination of the aldophosphamide analogues (1c in Figure 1) is a key step in the CP activation, we are seeking to control this step by introducing hypoxia-selective agents at the C-6 position of cyclophosphamide (1a). It is anticipated that the oxidised state of the R group (Figure 3) would deactivate the β-elimination reaction of the aldophosphamide analogues through its electron-withdrawing ability, whereas the reduced state(s) of the R group would activate the β-elimination through its electron-donating ability. Using semiempirical molecular methods, we propose to investigate key indexes of reactivity relevant to this activation process. Since the study involves novel molecules for which no experimental data are available, the method was calibrated on CB1954 (Figure 4), which is a well-studied bioreductive agent [23,24]. The method was also calibrated on compound (3a), which has a 90:1 cytotoxic selectivity toward hypoxic cells [21].

Methods

Since a large number of medium-sized drug molecules were studied, semiempirical MO methods were chosen. For CB1954 (Figure 4), SR4233 CP analogues (Figure 6) and the aziridinyl-containing compounds AT1a, AT2a-AT5a (Figure 7), AM1 [25] was the method of choice, since AM1 gives a better description of planar nitrogen than does PM3 [26]. However, since PM3 gives the best description of hyper-valent phosphorus [25], this method was used for compound (3a) and the nitroimidazole analogues (Figure 5). Non-local density functional calculations, as implemented in the DGAUSS suite of programs [27], were carried out on CB1954 analogues at the semiempirical geometry to test the reliability of the semiempirical approaches. The calculations used a DZVP basis set [27], the Becke '88 functional for exchange [28] and the Lee-Yang-Parr functional for correlation [29].

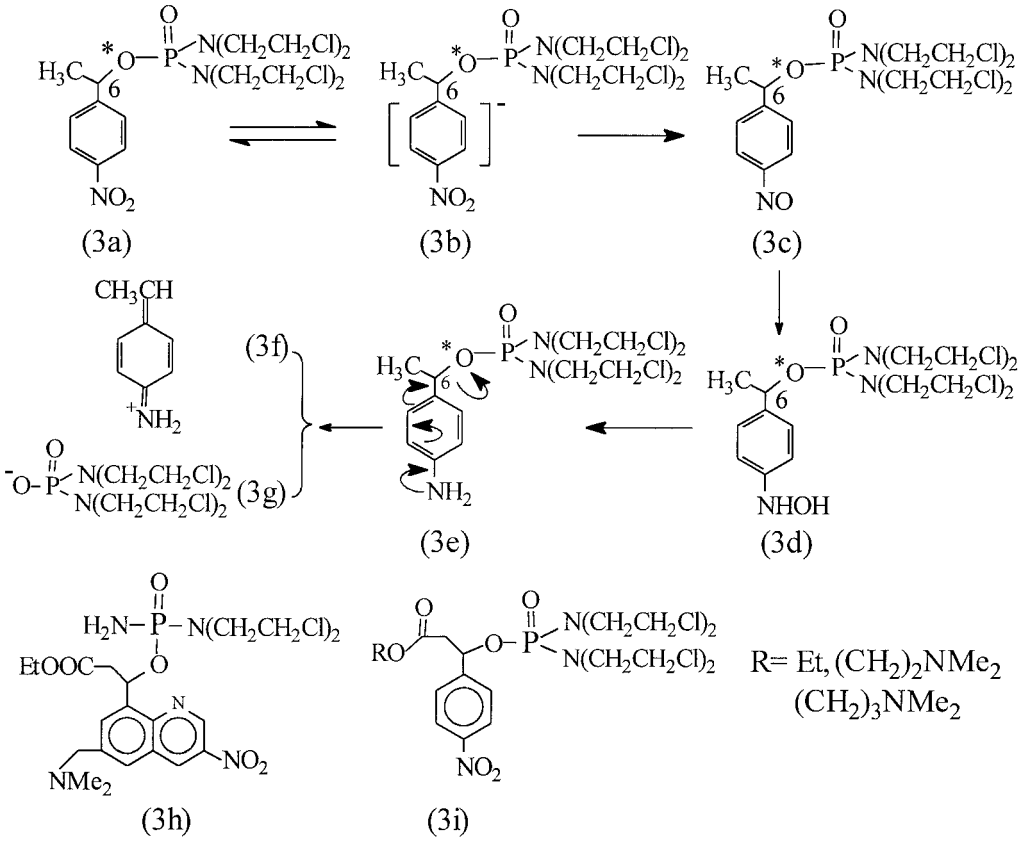

Figure 3. Bioreductive phosphamides 3a, 3h and 3i. Bioreduction of the nitro group normally passes through the radical anion (3b), through the NO and NHOH intermediates to the six-electron reduction product (3e).

Table 1. The relative AM1 and BLYP/DZVP gas-phase protonation enthalpies, $\Delta\Delta H(g)$ or $\Delta\Delta E(g)$ (in kcal mol^{-1} or Hartrees) of CB1954 and its reduced metabolites (shown in Figure 4)

Molecule	$\Delta H_f(A, g)$	$\Delta H_f(AH^+, g)$	$\Delta\Delta H(g)$	Group Y	E(BLYP,A,g)	E(BLYP(AH^+,g)	$\Delta\Delta E(g)$	Group Y
CB1954	48.8	217.4	0	NO_2	−942.623090	−942.975633	0.0	NO_2
CB1954b	−14.9	61.7	−92.5	NO_2^-	−942.661116	−943.168105	−96.9	NO_2^-
CB55c	45.5	212.3	−2.3	NO	−867.397535	−867.750776	−0.4	NO
CB55d	33.0	187.7	−14.4	NHOH	−868.607545	−868.986508	−16.6	NHOH
CB55e	33.6	187.8	−14.9	NH_2	−793.457766	−793.822488	−7.6	NH_2
CB56c	50.2	215.7	−3.8	NO	−867.401102	−867.748242	3.3	NO
CB56d	27.9	192.4	−4.6	NHOH	−868.625959	−868.971157	4.6	NHOH
CB56e	34.2	198.5	−4.8	NH_2	−793.450896	−793.803955	−3.8	NH_2

CB1954 is a highly effective bioreductive agent in rat cell lines but less effective in human cells [35]. (The calculated energies are recorded in columns 2 and 3.) The $\Delta\Delta H(g)$ or $\Delta\Delta E(g)$ values were calculated relative to the value for CB1954. Here, as in other tables, Y represents the intermediates following reduction of the NO_2 group. The structures are given in Figure 4.

To obtain enthalpies of protonation in aqueous solution at the optimised geometries, the corresponding AM1-SM2 and PM3-SM3 methods were used, as implemented in the AMSOL 3.0 package [30]. The initial conformations of the aldophosphamide analogues (NCP1–NCP4, Figure 5), compound (3a) (Figure 3) and their reduced intermediates are quite flexible and so the initial conformations of these species were generated by carrying out an in-depth artificial intelligence conformational analysis using the Cobra 3.0

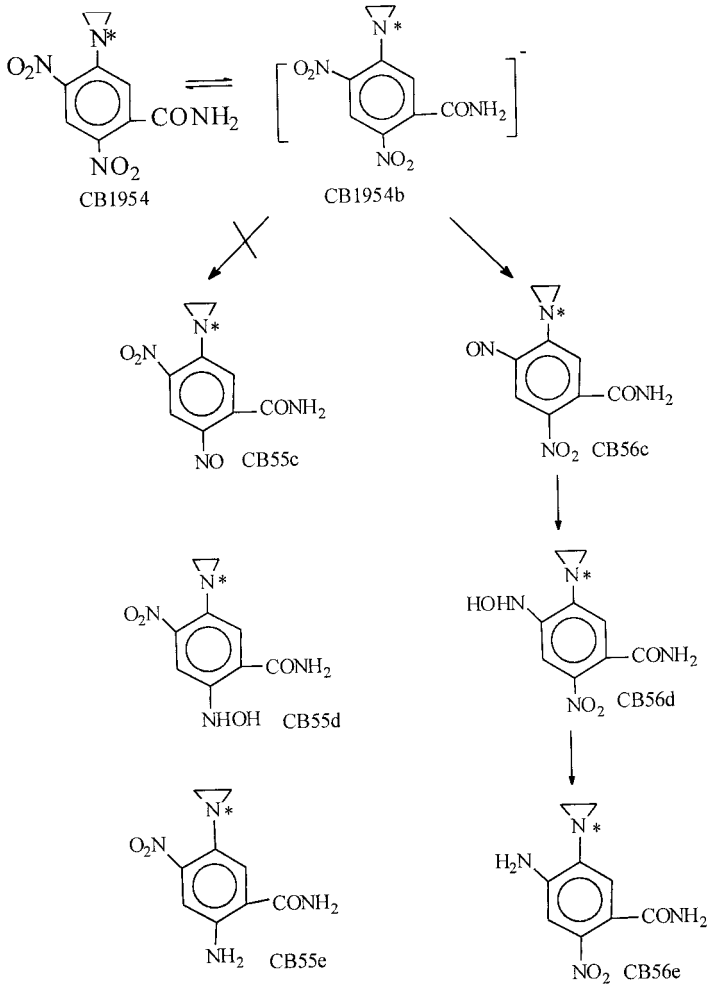

Figure 4. CB1954 and its reduction products.

program [31]. (Here, a variant of the Cosmic (90) force field [32] was used to estimate the molecular mechanics conformational energies.) The conformation with the lowest predicted energy was used in the semiempirical geometry optimisation, which was carried out using Mopac 93 with a very tight geometry convergence criterion.

Here, we assumed that $\Delta G \approx \Delta H$, since MOPAC calculates gas phase enthalpies whereas AMSOL calculates free energies of solvation. This is a minor error when comparing results across a series of similar molecules. The gas phase enthalpy, $\Delta H(g)$ (in kcal mol^{-1}) for protonation of compound A at O_1 or N* may be computed for the reaction

$$A(g) + H^+(g) \rightarrow AH^+(g),$$

$$\Delta H(g) = \Delta H_f(AH^+, g) - \Delta H_f(A, g) - \Delta H_f(H^+, g).$$

The corresponding result in aqueous solution is for the reaction

$$A(aq) + H_3O^+(aq) \rightarrow AH^+(aq) + H_2O(aq)$$

$$\Delta H(aq) = [\Delta G_f(AH^+, aq) + [\Delta G_f(H_2O, aq) - \Delta G_f(A, aq)] - \Delta G_f(H_3O^+, aq)].$$

We have tabulated the calculated $\Delta H_f(g)$ and $\Delta G_f(aq)$ but the enthalpies of protonation are presented relative to those of a specified parent compound (in the above equation, the enthalpy of protonation is relative to water).

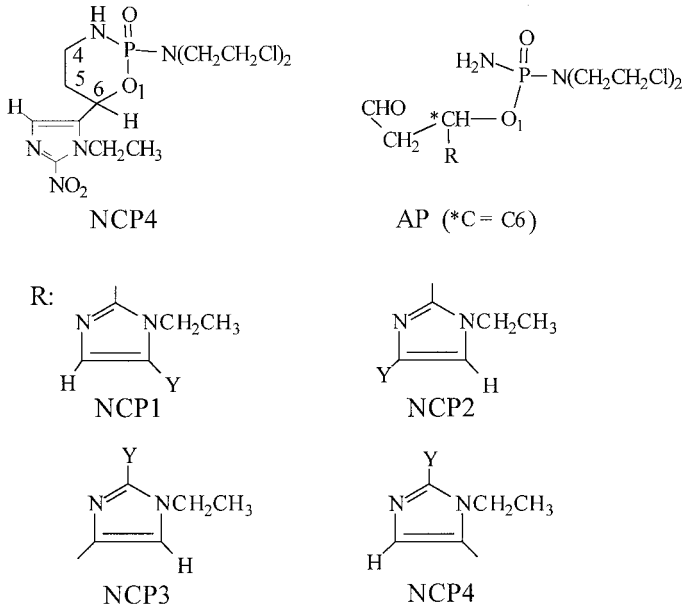

Figure 5. The proposed bioreductive nitroimidazole-based cyclophosphamides, NCP1–NCP4 and the corresponding aldophosphamides. The R group represents the 4 alternative nitroimidazole moieties and the Y group represents the $-NO_2$ group (which can be reduced to $-NO_2^-$, $-NO$, -NHOH or $-NH_2$).

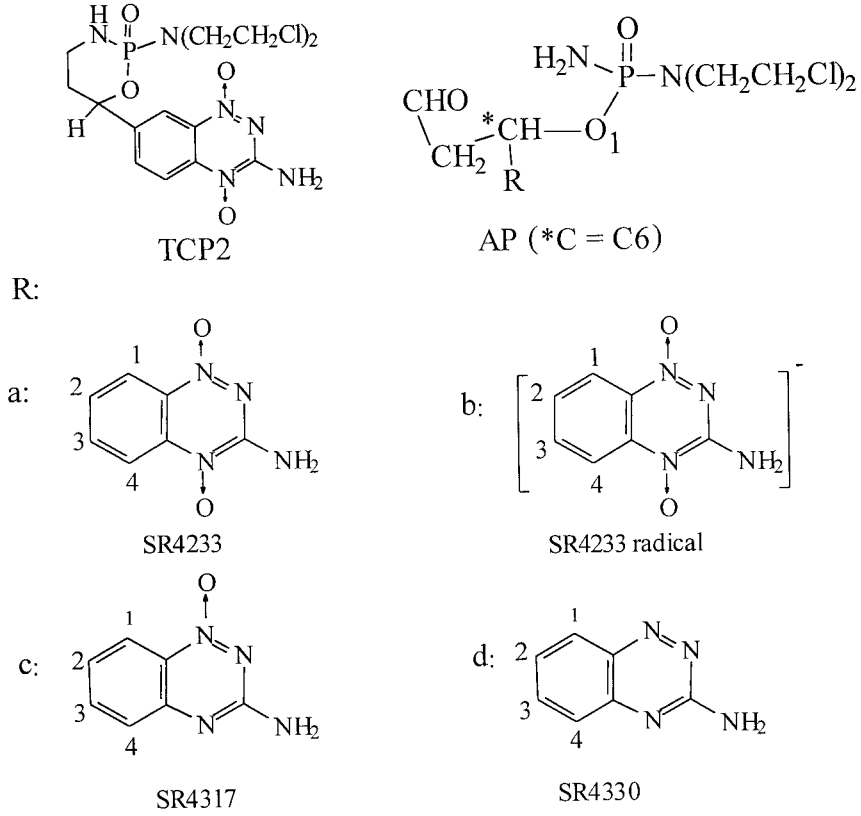

Figure 6. The proposed bioreductive SR4233-based cyclophosphamides, TCP1–TCP4 (SR4233 is also called Tirapazamine). The numbers 1–4 denote the position where the R group (SR4233) is attached to the aldophosphamide (AP); the letters a–d denote the SR4233 moiety in its various states of reduction.

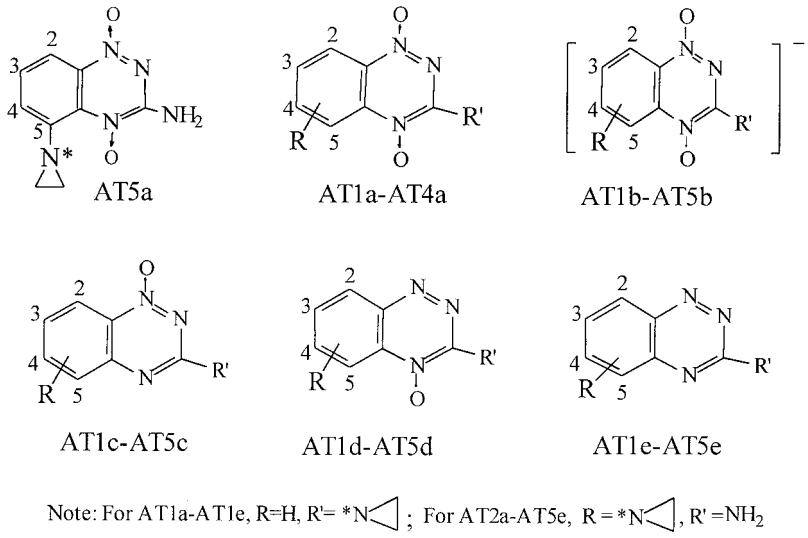

Figure 7. The proposed bioreductive SR4233-based aziridines, AT1–AT5 (SR4233 is also called Tirapazamine). The numbers 1–5 denote the position where the aziridine group is attached to the SR4233; the letters a–e denote the SR4233 moiety in its various states of reduction.

Table 2. The relative solution-phase protonation enthalpies (in kcal mol^{-1}) of CB1954 and its reduced metabolites, $\Delta\Delta H(aq)$

Molecule	$\Delta G_f(A, aq)$	$\Delta G_f(AH^+, aq)$	$\Delta\Delta H(aq)$	Group Y
CB1954	33.7	151.6	0.0	NO_2
CB1954b	−70.0	35.5	−12.4	NO_2^-
CB55c	31.8	149.2	−0.4	NO
CB55d	12.6	122.7	−7.8	NHOH
CB55e	14.5	125.8	−6.5	NH_2
CB56c	35.0	144.9	−8.0	NO
CB56d	8.7	124.8	−1.8	NHOH
CB56e	24.1	129.8	−12.3	NH_2

The calculated solution phase energies are recorded in columns 2 and 3. The $\Delta\Delta H(aq)$ values were calculated relative to the value for CB1954. Here, as in other tables, Y represents the intermediates following reduction of the NO_2 group. The structures are given in Figure 4.

Results and discussion

Model compounds: CB1954 and 3a

The electron density on the nitrogen is the key factor in activation of the mustard. This is most clearly seen in compounds such as $[Co(Meacac)_2dce]^+$ [8–10] where the *Meacac* is a supporting ligand and the mustard *dce* is inactive while coordinated to cobalt (III). This is because the lone pair on the mustard nitrogen is tightly coordinated to the inert cobalt. Cobalt (II) complexes, however, are labile so the mustard nitrogen can diffuse away, exposing the nitrogen mustard lone pair ready for activation. The enthalpy of protonation is also a measure of the availability and hence of changes in the reactivity of the mustard nitrogen lone pair that may result when neighbouring groups are bioreduced. The semiempirical gas-phase data predicts that the most stable closed shell reduced protonation product of CB1954 would be CB55e (Figure 4) – for protonation at the aziridinyl nitrogen, N*. Thus, the relative protonation enthalpy relative to that for CB1954, $\Delta\Delta H(g)$, is −14.9 kcal mol^{-1} (Table 1). The energetics can be interpreted with some confidence since the corresponding non-local density functional results for $\Delta\Delta H(g)$ are in remarkably good agreement with the semiempirical results for most of the compounds. The agreement for protonation of the radical anion is particularly encouraging, since molecular orbital calculations on anions are not necessarily reliable unless diffuse functions are included (for CB1954b, $\Delta\Delta H(g)$ is −92.5 and −96.9 kcal mol^{-1} for AM1 and BLYP, respectively). The greater stability of CB55e over CB56e appears to contradict the experimental results in which CB56e was detected as a urinary metabolite whereas CB55e was not [24]. The solution phase data does however predict CB56e to be the most stable closed shell metabolite, in agreement with experiment, and the protonation enthalpy is most negative as the NO_2 is reduced to NH_2 ($\Delta\Delta H(aq)$ = −12.3 kcal mol^{-1}, Table 2). This suggests that CB1954 does indeed have increased reactivity under hypoxia, in agreement with experiment [24]. The solution-phase calculations therefore provide a good indication of hypoxic selectivity, though we note that

Table 3. The relative solution-phase enthalpies, $\Delta\Delta$H(aq) (in kcal mol^{-1}), for protonation at oxygen O* of compound 3a and its reduced species.

Species	ΔG_f(A, aq)	ΔG_f(AH^+, aq)	$\Delta\Delta$H(aq)	Group Y
3a	−165.7	−34.3	0.0	NO_2
3c	−143.6	−15.7	−3.5	NO
3d	−169.6	−41.2	−3.0	NHOH
3e	−161.8	−36.7	−6.3	NH_2

Compound 3a has a 90:1 cytotoxic selectivity towards hypoxic cells [21]. The changes in ΔH(aq), $\Delta\Delta$H(aq), as NO_2 is reduced were calculated relative to the value for compound 3a. The structures are given in Figure 3.

kinetic factors (i.e., steric factors in the active site of the reductase) rather than thermodynamic factors may contribute to the nature of the reduced products.

The results in Table 3 show that the computed solution-phase enthalpies of protonation at O* in compound (3a) (Figure 3) become more negative as the NO_2 group (3a) is reduced to NH_2 (3e, $\Delta\Delta$H(aq) = −6.3 kcal mol^{-1}). Since (3a) is 90-fold more toxic under hypoxic conditions, this again suggests that the method provides a good indication of hypoxic selectivity.

Nitroimidazole CP analogues

The relative *gas-phase* protonation enthalpies (not shown) for the nitroimidazole CP analogues NCP1, NCP2 and NCP4 (Figure 5) become more negative as NO_2 is reduced to NH_2, whereas for NCP3 the most negative value is for NHOH. However, the results of CB1954 suggest that the solution-phase results will be more reliable. Indeed, for the closed shell molecules we find that the solution phase protonation enthalpy is most negative as NO_2 is reduced to NH_2 (Table 4). The most promising compounds, with the most negative relative protonation enthalpies, are NCP3 and NCP4 (Figure 5) since they have the most negative changes in $\Delta\Delta$H(aq) (−6.8 and −6.1 kcal mol^{-1}, respectively).

SR4233 CP analogues

The two-electron reduced product, SR4317 (Figure 6c), is the normal reduction product of SR4233, while the one-electron reduced radical anion (Figure 6b) is the major cytotoxic metabolite [33]. Thus, an initial glance at Table 5 shows that two-electron reduction of the SR4233 to the SR4317 moiety significantly activates the aldophosphamide analogues from compounds TCP1 and TCP2 ($\Delta\Delta$H(aq) = −13.1 and −9.9 kcal mol^{-1}, respectively; Figure 6) whereas the two-electron reduction would not significantly activate the aldophosphamide analogues from TCP3 and TCP4 ($\Delta\Delta$H(aq) = +4.3 and −1.8 kcal mol^{-1}, respectively). Similarly, one-electron reduction of the SR4233 moiety to a radical anion would activate compounds TCP1, TCP3 and TCP4 ($\Delta\Delta$H(aq) = −51.8, −51.1 and −45.1 kcal mol^{-1}, respectively) more than compound TCP2 ($\Delta\Delta$H(aq) = −29.3 kcal mol^{-1}). The reduced enthalpy of protonation of TCP2 relative to TCP1, TCP3 and TCP4 is particularly interesting. Since most oxygenated cells still retain reductive capacity [34], the most appropriate compounds as hypoxia-selective cytotoxins are those where the initially reduced species can be rapidly and reversibly re-oxidised by O_2 in the oxic regions. For this reason, compound TCP2 is likely to be more promising because initially the one-electron reduction product of the SR4233, analogue TCP2b, is less *activating*. This

Table 4. The solution-phase protonation enthalpies, ΔH(aq) (in kcal mol^{-1}), for protonation at O* of the nitroimidazole aldophosphamide analogues

Molecule	ΔG_f(A, aq)	ΔG_f(AH^+, aq)	$\Delta\Delta$H(aq)	Group Y
NCP1a	−186.0	−70.8	0.0	NO_2
NCP1b	−260.6	−173.8	−28.3	NO_2^-
NCP1c	−160.7	−40.5	13.0	NO
NCP1d	−191.0	−75.1	0.7	NHOH
NCP1e	−183.0	−68.2	−0.4	NH_2
NCP2a	−192.3	−74.8	0.0	NO_2
NCP2b	−258.8	−178.9	−37.7	NO_2^-
NCP2c	−171.2	−49.3	4.4	NO
NCP2d	−192.8	−77.1	−1.8	NHOH
NCP2e	−185.1	−70.9	−3.3	NH_2
NCP3a	−184.5	−70.1	0.0	NO_2
NCP3b	−250.7	−163.9	−27.5	NO_2^-
NCP3c	−163.7	−45.8	3.6	NO
NCP3d	−189.3	−79.5	−4.6	NHOH
NCP3e	−180.8	−73.3	−6.8	NH_2
NCP4a	−105.6	−62.2	0.0	NO_2
NCP4b	−255.6	−159.2	−27.1	NO_2^-
NCP4c	−164.4	−39.8	1.2	NO
NCP4d	−190.9	−52.8	14.7	NHOH
NCP4e	−185.8	−68.5	−6.1	NH_2

The calculations were performed on the AP analogues but the compounds are named after the parent; the letters a–e denote the progressive reduction of the Y group from -NO_2 to -NH_2. The changes in ΔH(aq) $\Delta\Delta$H(aq), as NO_2 is reduced were calculated relative to the value for the compound where Y = NO_2. The structures are given in Figure 5.

Table 5. The relative solution-phase protonation enthalpies of the SR4233 aldophosphamide analogues, $\Delta\Delta$H(aq) (in kcal mol^{-1})

Molecule	ΔG_f(A, aq)	ΔG_f(AH$^+$, aq)	$\Delta\Delta$H(aq)	Group Y
TCP1a	−125.5	17.5	0.0	SR4233
TCP1b	−247.8	−156.7	−51.8	SR4233
TCP1c	−142.0	−12.2	−13.1	SR4317
TCP1d	−156.5	−14.2	−0.5	SR4330
TCP2a	−132.7	−5.0	0.0	SR4233
TCP2b	−230.9	−132.5	−29.3	SR4233$^{\cdot-}$
TCP2c	−141.3	−23.4	−9.9	SR4317
TCP2d	−157.7	−33.4	−3.4	SR4330
TCP3a	−132.3	−3.6	0.0	SR4233
TCP3b	−238.7	−161.0	−51.1	SR4233$^{\cdot-}$
TCP3c	−143.9	−10.7	4.3	SR4317
TCP3d	−156.8	−33.6	−5.7	SR4330
TCP4a	−131.5	−1.8	0.0	SR4233
TCP4b	−241.8	−157.1	−45.1	SR4233$^{\cdot-}$
TCP4c	−142.0	−14.1	−1.8	SR4317
TCP4d	−156.3	−25.9	0.7	SR4330

Y represents the reduced intermediates of SR4233. (The calculations were performed on the AP analogues but the compounds are named after the parent; the letters a–d denote the progressive reduction while the numbers 1–4 denote the site of attachment of the AP to SR4233.) Among the four series TCP1A–TCP1D, TCP2a–TCP2d, TCP3a–TCP3d and TCP4a–TCP4d, the changes in ΔH(aq), $\Delta\Delta$H(aq), as SR4233 is reduced were calculated relative to the values of the species with Y = SR4233. The structures are given in Figure 6.

shifts the probability toward re-oxidation in the presence of O_2 rather than toward β-elimination and so offers protection towards oxic cells.

With these arguments in mind, Table 6 shows that compound AT5a rather than AT1a–AT4a is likely to have hypoxic selectivity since the relative protonation enthalpy, $\Delta\Delta$H(aq), becomes 4.6 kcal mol^{-1} more negative as the compound is reduced to the stable mono deoxygenated species AT5c. Moreover, the initial one-electron reduction will not activate the nitrogen mustard functionality as the relative protonation enthalpy becomes 7.1 kcal mol^{-1} more positive as the compound undergoes one-electron reduction.

Conclusions

Based on the proposed activation mechanism of cyclophosphamide (Figure 1), CP analogues have been designed to act as hypoxia-activated diffusible alkylating agents. The protonation enthalpy of O_1 in the aldophosphamide analogues has been used as an indicator for the β-elimination reaction (Figure 5, R≠H) and hence as an indicator of hypoxic selectivity of the corresponding cyclophosphamide analogues. A modest-cost computation method for calculating the protonation enthalpy has been established. The gas-phase minimum energy geometries have been obtained using either the AM1 or PM3 semiempirical models by performing a full geometry optimisation at the conformation with lowest energy generated by an artificial intelligence conformational analysis method. By

Table 6. The relative solution-phase protonation enthalpies, $\Delta\Delta$H(aq) (in kcal mol^{-1}), of the mustard nitrogen N* for compounds AT1a, AT2a, AT3a, AT4a, AT5a and their reduced species

Molecule	ΔG_f(A, aq)	ΔG_f(AH$^+$, aq)	$\Delta\Delta$H(aq)
AT1a	136.1	226.3	0.0
AT1b	39.5	128.1	−1.6
AT1c	118.1	264.9	56.6
AT1d	105.7	211.1	14.2
AT1e	129.3	251.3	31.8
AT2a	80.2	186.9	0.0
AT2b	−26.0	84.7	4.0
AT2c	72.4	180.6	1.4
AT2d	128.5	237.3	2.0
AT2e	120.9	230.3	2.7
AT3a	148.4	257.0	0.0
AT3b	34.3	145.5	2.5
AT3c	136.0	245.0	0.4
AT3d	130.0	238.0	−0.6
AT3e	122.6	230.8	−0.4
AT4a	145.5	257.0	0.0
AT4b	34.3	145.8	0.0
AT4c	134.2	244.3	−1.4
AT4d	127.3	239.0	0.2
AT4e	120.7	231.0	−1.2
AT5a	157.0	270.3	0.0
AT5b	43.1	163.4	7.1
AT5c	138.3	246.9	−4.6
AT5d	138.1	260.8	9.4
AT5e	124.5	233.7	−4.0

The letters a–e denote the progressive reduction while the numbers 1–5 denote the site of attachment of the aziridine to SR4233. Among the five series AT1a–AT1e, AT2a–AT2e, AT3a–AT3e, AT4a–AT4e and AT5a–AT5e, the changes in ΔH(aq), $\Delta\Delta$H(aq), as the compounds were reduced were calculated relative to the values for the dioxygenated species. The structures are given in Figure 7.

assuming that geometries of the solutes in solution are the same as the gas-phase optimised geometries, the AMSOL solvation models (AM1-SM2 or PM3-SM3) have been used to evaluate the energetics of the protonation reaction in solution.

The computed results on CB1954 (Table 2) are in agreement with the experimental results. The computed results of compound (3a) (Table 3) are also encouraging since this compound is structurally similar to the CP analogues and our prediction for this compound is in concordance with the experimental result given by Borch et al. Borch showed that compound (3a) has at least 90-fold hypoxic selectivity. These results support the computational methods and the indicators we have used in the evaluation of the hypoxic selectivity of cyclophosphamide analogues. Consequently, compounds TCP1, TCP2, NCP3, NCP4 and AT5a are proposed as ideal candidates for synthesis and biological testing. Compounds TCP2 and AT5a are probably the best ones as they offer the most protection to oxic cells.

In summary, it has been demonstrated that quantum mechanics can play a role in the rational design of bioreductive anti-cancer drugs. Moreover, the work described could be of value in the design of new cyclophosphamide analogues with enhanced efficacy as anticancer agents.

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