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ORIGINAL PAPER

Some physicochemical properties of the antitumor drug thiotepa and its metabolite tepa as obtained by density functional theory (DFT) calculations

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Abstract Density functional theory (DFT) using the B3LYP functional was applied to elucidate the molecular properties of the antitumor drug thiotepa and its main metabolite tepa. Aqueous solvent effects were introduced using the conductor-like polarizable continuum model (CPCM). The protocol for calculating the pK_a values obtained with different cavity models was tested on a series of aziridine and phosphoramide compounds. An efficient computational scheme has been identified that uses the CPCM model of solvation with a universal force field (UFF) cavity. The method has been used to evaluate the basicities of thiotepa and its metabolite. Our calculations show that the basicities of the aziridine moiety of thiotepa and tepa are dramatically reduced compared to free aziridine, indicating that highly acidic media are needed to produce substantial yields of the N-protonated form of the drug. Finally, the mechanisms of reaction of the drug and its metabolite are discussed based on our theoretical results. The calculations reproduce the experimental trends very satisfactorily.

Keywords ThioTEPA · TEPA · Density functional theory · Solvent effect · Absolute pK_a

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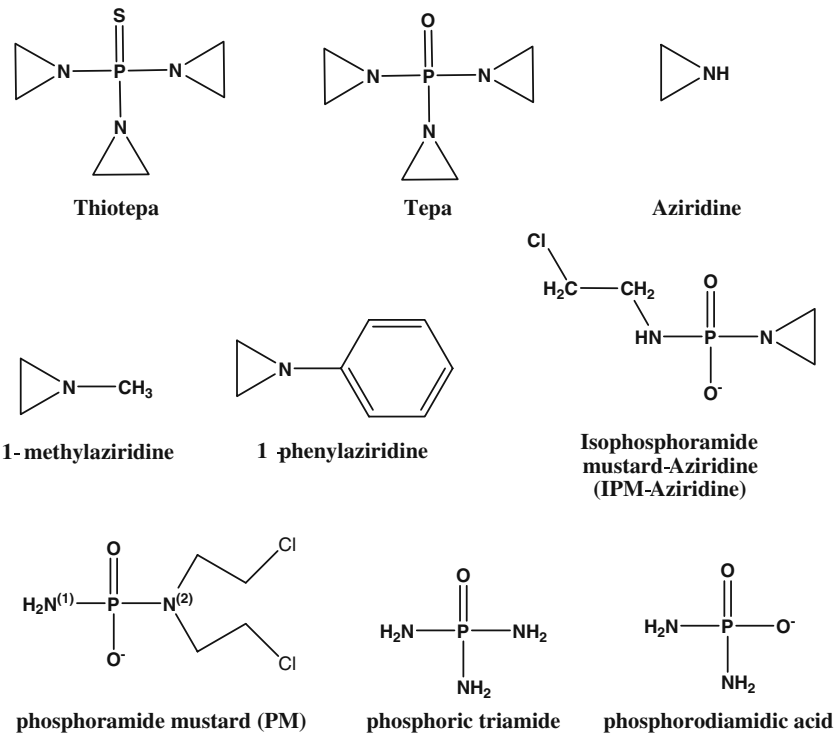
Introduction

The alkylating agent *N*,*N*′,*N*″-triethylenethiophosphoramide (thiotepa) presented in Scheme 1 has been applied in cancer therapy for 60 years [1–4]. Because of its broad spectrum of antitumor activity, thiotepa has recently been used in high-dose combination regimens for breast cancer, ovarian cancer, and other solid tumors [1–4]. The interaction of thiotepa with DNA has been studied by several biochemical and pharmachemical methods, but the results are as yet inconclusive [1–4]. Metabolic studies of thiotepa resulted in the identification of its oxo analog (tepa) as the major metabolite of thiotepa (see Scheme 1). Tepa is formed after the oxidative desulfuration of thiotepa in the liver, catalyzed by cytochrome P450 [1–4]. Thiotepa is a phosphoramide containing three aziridinyl functionalities that react with nucleophiles present in DNA. In vivo and in vitro studies show that alkylation of DNA by thiotepa can follow two pathways, as described in Scheme 2, but it remains unclear which pathway represents the precise mechanism of action [1–4]. Thiotepa is a polyfunctional alkylating agent and is capable of forming crosslinks with DNA molecules according to pathway 1. In pathway 2, thiotepa acts as a cell-penetrating carrier for aziridine, which is released intracellularly after hydrolysis. The released aziridine can react with DNA, resulting in the formation of a stable guanine adduct in the DNA chain [5, 6]. The reaction of tepa, a metabolite of thiotepa, with DNA is believed to follow pathway 2 [1].

Pathway 1 can be carried out according to two different reaction schemes. In the first mechanism, the ring opening reactions are initiated by protonating the aziridine, which then becomes the primary target of nucleophilic attack by the N7 guanine of DNA. Indeed, the active form of an aziridine derivative drug is the highly electrophilic ethyl-eneimonium or aziridinium ion, which has a positive charge

Scheme 1 Structures of thiotepa, tepa and the compounds included in this study in order to validate the computational scheme of pK_a

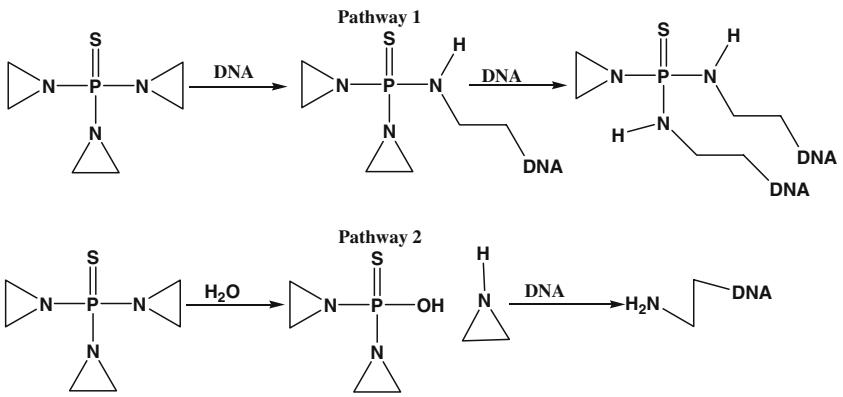


located on nitrogen [7]. The second mechanism is via the direct nucleophilic ring opening of aziridinyl groups. The reactivity of the aziridine ring is dependent on the substituent on the nitrogen atom. The presence of electron-withdrawing substituents activates the ring, which then reacts easily with nucleophiles to produce ring-opened products [8]. Thiotepa and tepa are activated aziridines with –P=S and –P=O as nitrogen substituents, respectively.

Thus, the question is: just how is pathway 1 carried out? To answer this question, we must investigate the acid–base chemistry of thiotepa and tepa and relate this to their pharmacological properties. The pK_a value of a compound is an important property that determines the amounts of the neutral and protonated forms of the compound present in aqueous medium at any given pH value. Determining the pK_a values of thiotepa and its metabolite will allow us to better interpret their mechanisms of reaction with DNA, and will give useful information on the existence of the protonated forms of thiotepa and tepa under physiological conditions. Several experimental studies have described the effect of pH on the stability of the drug and its metabolite tepa [9–11]. Unfortunately, no information is available on the pK_a values of these compounds.

In this work we calculate the absolute pK_a values of the compounds under study. The computational scheme is first validated on the series of aziridine compounds presented in Scheme 1: aziridine, 1-methylaziridine and 1-phenylaziridine, for which experimental pK_a values have been published. Four phosphoramide derivatives are also studied to confirm the computational scheme of pK_a, since they resemble thiotepa and tepa. These derivatives are isophosphoramide mustard aziridine (IPM aziridine), phosphoramide mustard (PM),

Scheme 2 Possible interaction of thiotepa with DNA. Pathway 1: formation of crosslinks between thiotepa and DNA. Pathway 2: thiotepa as a prodrug for aziridine



phosphoric triamide ($PO(NH_2)_3$) and phosphorodiamidic acid ($PO_2(NH_2)_2^-$).

Thiotepa has been the subject of only a few theoretical studies [12, 13]. The aim of the latter work was to clarify the molecular structure of the compound using semiempirical (MNDO) and ab initio (HF) methods. In this paper, we attempt to investigate the molecular physicochemical properties of thiotepa and tepa (proton affinity, solvent effect, pK_a, frontier orbitals, charge distributions, etc.) by means of density functional theory calculations, which are extremely useful for gaining a thorough understanding of the anticancer activities of these compounds.

Methods

The B3LYP [14, 15] density functional method as implemented in the Gaussian 03 package [16] was used throughout the whole study. All structures were fully optimized using the 6-31G(d) basis set. The anionic species used in this study were obtained with the larger 6-31+G(d) basis set. The gas phase Gibbs free energies were obtained from an analytical frequency analysis. The solvation free energies for the protonated and neutral species were obtained theoretically using a continuum solvation method. The polarizable continuum model (PCM) [17] was applied using the conductor-like polarizable continuum variant (CPCM) [18]. CPCM calculations were performed as single points (without optimization) on the gas-phase geometries, since this has been shown to give better results than reoptimization [19]. Water was modeled with a dielectric constant of $\varepsilon = 79.39$. Continuum models require a description of the shape and size of the cavity occupied by solute molecules in the solvent. The Gaussian 03 software suite offers several options for choosing a set of atomic radii. For comparison purposes, we used three of the standard sets of radii: UA0, UAKS and UFF. In Gaussian 03, the default set of radii is UA0. The UA0 cavity is built up by applying the united atom topological model (UATM) to atomic radii of the universal force field (UFF) [20]. The UAKS cavity uses UATM with radii optimized for the PBE0/6-31G(d) level of theory. A set of radii from the UFF was used to produce the UFF cavity. The pK_a calculations were carried out using the following relationship (discussed in detail in [21]):

$$pK_a = \left[G_{gas}(B) - G_{gas}(BH^+) + \Delta G_{solv}(B) - \Delta G_{solv}(BH^+) - 269.0)\right] /1.3644 \quad (1)$$

The charge distributions were characterized with natural population analysis (NPA) [22, 23] and by fitting the molecular electrostatic potential to atomic point charges using the CHELPG [24] method.

Results and discussion

Optimized geometries of thiotepa and tepa

Thiotepa and tepa were fully optimized without any constraints. The optimizations were performed in the gas phase using the B3LYP method in conjunction with the 6-31G(d) basis set. The structure of aziridine was also included for comparison purposes. Geometric optimization in water was also performed for the same systems using CPCM to examine the effect of water on the geometry. The optimized structures of thiotepa and tepa represented in Fig. 1 exhibit a distorted C_3 symmetry, which is in good agreement with the X-ray structure obtained for thiotepa in the solid state [13]. Table 1 compares the important geometric parameters of thiotepa, tepa and aziridine in the gas phase with those calculated in aqueous solution. Data analysis indicates that the structural parameters are slightly affected by the polarity of the solvent. Aqueous solvation reduces the P–N bond length (by ~0.01 Å) for both thiotepa and tepa. There is a concomitant increase in the P=X (X = S, O) bond length (~0.03 Å for thiotepa and ~0.01 Å for tepa), signifying that electron delocalization occurs from the P–N bond to the P=X (X = S, O) bond in the aqueous phase. It is worth noting that, in the structures of thiotepa and tepa, none of the aziridine ring distances and angles are significantly different from those obtained for free aziridine in either the gas or the aqueous phase.

Proton affinity (PA) and solvent effects

The proton affinity (PA) of a molecule is an important gas-phase thermodynamic property that helps us to understand the basicity of the molecule and its susceptibility to electrophilic substitution. The computed proton affinities of the nitrogen sites of aziridine, thiotepa and its oxo-analog were evaluated at the B3LYP6-31G(d) level, and

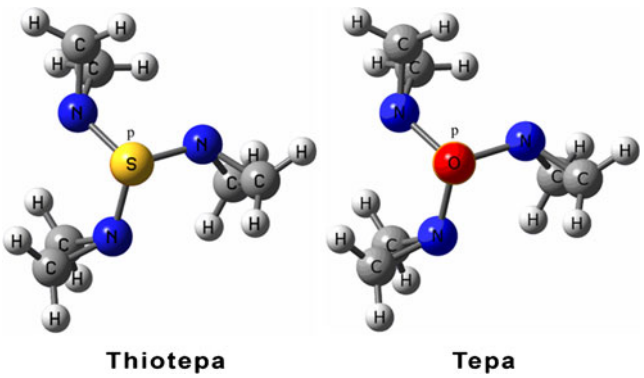


Fig. 1 Optimized structures of thiotepa and tepa obtained at B3LYP/6-31G(d). The P=X (X = S, O) bond is perpendicular to the plane of projection

Table 1 Calculated bond lengths (Å) and bond angles (°) of thiotepa, tepa and aziridine in both the gas phase (first row) and the aqueous phase (second row) at B3LYP/6-31G*

Parameters	Thiotepa	Tepa	Aziridine
P–X[a]	1.947	1.484	–
	1.977	1.496	
P–N	1.708	1.701	–
	1.694	1.691	
N–C	1.464	1.466	1.473
	1.472	1.474	1.478
N–C	1.474	1.471	1.473
	1.480	1.476	1.478
C–C	1.486	1.486	1.485
	1.484	1.484	1.483
C–N–C	60.8	60.8	59.7
	60.3	60.4	60.2
X–P–N	114.1	113.5	–
	112.2	112.3	
P–N–C	121.3	120.2	–
	122.2	121.1	
P–N–C	123.8	124.2	–
	123.8	124.3	

[a] X = S, O

they are listed in Table 2. The experimental value for the proton affinity of aziridine is 216 kcal mol^{-1} [25]. Our theoretical value is in good agreement with (within 4 kcal mol^{-1} of) this experimental value. It is worth noting that our calculations indicate that the proton affinities of the N sites of thiotepa and tepa are very similar. Furthermore, the proton affinities of the N sites of thiotepa and tepa deviate from that of free aziridine by only 7 kcal mol^{-1}. Because the calculated energy difference between the PAs of thiotepa and tepa is very small, it seems reasonable to surmise that the electron-withdrawing effect of P=X (X = S, O) is very small. This conclusion agrees well with that obtained by Igor Novak et al. [13] by means of UV photoelectron spectroscopy (UPS). The electronic structure of thiotepa revealed by UPS was analyzed by comparison with the assigned spectrum of aziridine. This comparison revealed important similarities in the number and types of bands present in different ionization energy regions, suggesting that intramolecular interactions between the P=X (X = S,O) function and the lone pair of electrons on the nitrogen are likely to be weak in all tepa derivatives. We also note that the differences between the proton affinities of the S, O and N sites in both tepa and thiotepa are not very large.

Table 3 lists the total electronic energies of the neutral and protonated forms of aziridine, thiotepa and its metabolite tepa in the gas and aqueous phases. A comparison of the gas-phase energies shows that the N-protonated form of thiotepa is more stable than the S-protonated form. On the other hand, the O-protonated form of tepa appears to be more stable than the N-protonated form. In the aqueous phase, our results indicate that the N-protonated forms of thiotepa and its metabolite tepa are the most stable protonated species. Our theoretical results suggest that, in aqueous solution, the protonation of tepa and thiotepa occurs preferentially at the nitrogen ring atom.

Absolute pK_a value calculation

The biological activity of a drug is usually tested in the aqueous rather than the gas phase, and the protonated form of the molecule may also differ from the form it adopts in the isolated state. The pK_a value of a molecule determines the amounts of neutral and protonated forms of the molecule present in aqueous medium. The scheme used to compute the pK_a was first validated on a series of aziridine compounds, as shown in Scheme 1 with known experimental pK_a values. The DFT-calculated thermal and solvation free energies of the different systems and their protonated forms are depicted in Table 4. pK_a constants

Table 2 Gas-phase proton affinities (PA) calculated at the B3LYP/6-31G(d) level

Compound [a]	Calculated PA (kcal/mol)	Experimental PA (kcal/mol)
Thiotepa $\{NH\}^+$	225.1	–
Thiotepa $\{SH\}^+$	221.2	–
Tepa $\{NH\}^+$	225.2	–
Tepa $\{OH\}^+$	225.7	–
Aziridine	218.9	216[b]

[a] For each molecule, the site of protonation is given in parentheses

[b] [25]

Table 3 Electronic energies of the neutral and protonated forms of aziridine, thiotepa and its metabolite tepa in the gas and aqueous phases at the B3LYP/6-31G(d) level

Compounds [a]	*E* (gas phase) a.u	*E* (aqueous phase) a.u
Thiotepa	−1139.570604	−1139.58542532
Thiotepa $\{NH\}^+$	−1139.940717	−1140.02277334
Thiotepa $\{SH\}^+$	−1139.931857	−1140.00218265
Tepa	−816.609238	−816.626327138
Tepa $\{NH\}^+$	−816.979807	−817.065480726
Tepa $\{OH\}^+$	−816.981080	−817.052671506

[a] For each molecule, the site of protonation is given in parentheses

Table 4 The thermal and solvation free energies of the different systems and their protonated forms calculated via DFT

Compounds [a]	Thermal free energy	Total solvation free energy		
	G_{gas} (kcal/mol)	ΔG_s (kcal/mol)		
		UA0	UFF	UAKS
Neutral species and their protonated forms calculated at the B3LYP/6-31G(d) level				
Thiotepa	−715011.16	1.08	3.17	−9.83
Thiotepa {NH^+}	−715235.05	−43.05	−38.13	−56.11
Tepa	−512343.72	0.14	1.53	−12.96
Tepa {NH^+}	−512560.49	−45.14	−40.33	−59.83
Aziridine	−84006.68	−0.85	0.45	−7.23
Aziridine-{NH^+}	−84225.71	−63.03	59.57	−70.92
1-Phenylaziridine	−228954.06	1.70	4.85	−4.44
1-Phenylaziridine {NH^+}	−229177.34	−50.95	−44.06	−57.78
1-Methylaziridine	−108660.86	3.72	3.14	−5.06
1-Methylaziridine {NH^+}	−108885.20	−53.52	−52.85	−64.22
$PO(NH_2)_3$	−366750.52	−14.58	−7.71	−11.54
$PO(NH_2)_2(NH_3)^+$	−366963.68	−72.50	−61.79	−66.20
Anionic species and their protonated forms calculated at the B3LYP/6-31+G(d) level				
IPM-aziridine	−765173.64	−55.20	−51.53	−59.58
IPM-aziridine {NH^+}	−765493.71	−18.19	−10.57	−20.81
PM	−1054364.55	−49.80	−44.87	−50.53
PM {$N^{(1)}$-H^+}	−1054673.87	−17.57	−11.40	−17.90
PM {$N^{(2)}$-H^+}	−1054672.63	−16.92	−8.25	−16.60
$PO_2(NH_2)_2^-$	−378915.64	−68.86	−65.60	−68.26
$PO_2(NH_2)(NH_3)$	−379240.73	−23.55	−17.54	−18.92

[a] For each molecule, the site of protonation is given in parentheses

calculated by means of different radii sets are shown in Table 5, together with available experimental data. Analysis of the results reveals that the quality of the calculation depends on the set of radii used.

Table 5 Absolute pK_a values of thiotepa, tepa and some compounds derived from aziridine and phosphoramide calculated using different sets of radii

Compounds	pK_a			
	UA0	UFF	UAKS	Exp
Calculated at the B3LYP/6-31G(d) level				
Thiotepa	0.1	−2.8	1.6	–
Tepa	−5.1	−7.1	−3.9	–
Aziridine	8.9	7.4	10.0	8.0[a]
1-Phenylaziridine	5.1	2.3	5.6	1–2[a]
1-Methylaziridine	9.2	8.3	10.6	7.9[a]
$PO(NH_2)_3$	1.5	−1.3	−0.9	low[c]
Calculated at the B3LYP/6-31+G(d) level				
IPM-aziridine	10.3	7.4	9.0	5.2–5.4[b]
PM {$N^{(1)}$}	5.9	5.0	5.6	4.9[b]
PM {$N^{(2)}$}	4.5	1.8	3.7	<2[b]
$PO_2(NH_2)_2^{\hat{a}''}$	7.9	5.9	4.9	4.9–5.4[c]

[a] [26]; [b] [27]; [c] [29].

Particularly encouraging is the value obtained for simple aziridine. We calculated a pK_a value of 7.4 with UFF radii for the nitrogen protonation site, which is in very good agreement with the value measured experimentally (8) [26]. Acceptable agreement is also obtained using UA0 radii, while UAKS radii do not reproduce the value accurately. Satisfactory agreement is obtained with UFF radii for 1-methylaziridine. Its experimental pK_a value is 7.9 [26], while our calculation gives a result that is within 0.4 units of the experimental one. If the UA0 and UAKS sets of radii are used, the differences between the calculated and experimental pK_a values are ~1.3 units and ~2.7 units, respectively. For 1-phenylaziridine, an activated aziridine compound, the experimental pK_a value is low. The phenyl is capable of conjugating with the unshared electrons of the nitrogen, significantly reducing the basicity of the nitrogen site of aziridine compared to those of aziridine or methyl aziridine. The value of 2.3 computed using the UFF set of radii captures this effect and agrees well with the experimentally measured value estimated in the interval 1–2 [26]. We note that using UA0 or UAKS causes the pK_a value to be overestimated. We can see that the implicit solvent model using the UFF set of radii is capable of obtaining pK_a values that are within ~1 pK_a unit of the experimental values for aziridine, 1-methylaziridine and 1-phenylaziridine.

Four phosphoramide derivatives are included in this investigation. IPM-aziridine and PM are DNA-alkylating agents that are produced in vivo as metabolites of the widely used anticancer drug cyclophosphoramide [27]. $PO(NH_2)_3$ and $PO_2(NH_2)_2^-$ were also studied for comparison, since they resemble thiotepa and tepa. It has been stated in several studies that phosphoramide exists as a deprotonated species with a negative charge on the oxygen atom at neutral pH [28], as presented in Scheme 1.

In the case of IPM-aziridine, none of the three sets of radii used in this study are able to accurately predict the pK_a value. However, the result obtained with the UFF set of radii is nearest to the experimental value. It should be noted that the experimental pK_a value reported by Millis et al. [27] for this compound was determined at 4 °C. Our theoretical value is assumed to correspond to the value at 25 °C. This great variance in pK_a values may be due to the experimental temperature. Particularly encouraging is the result obtained using the UFF set of radii for phosphoramide mustard (PM) for the two nitrogen sites of protonation. We obtained a pK_a value of 5.0 for the N^1 site and 1.8 for the N^2 site, in very close agreement with the experimental values of 4.9 and <2 respectively [27]. The pK_a value calculated with UA0 for the N^1 site is only slightly erroneous (by an amount not exceeding 1 pK_a unit). For the N^2 site of protonation, UA0 leads to a pK_a value that is too high, by over 2 pK_a units. The values computed using the UAKS set of radii are acceptable. Gamscik et al. [29] described the effect of pH on the NMR spectrum of phosphoric triamide and phosphorodiamidic acid. The authors did not determine the pK_a of phosphoric triamide explicitly; it is possible to conclude from their NMR study that it should be very low. All three sets of radii used in this work confirm this experimental observation. In the case of phosphorodiamidic acid, very good agreements are also obtained with both UFF and UAKS radii. On the other hand, UA0 fails to produce the correct pK_a value, which is greatly overestimated.

All of the above results show that the UFF set of radii yields excellent estimates for the absolute pK_a (max ±1.5 units). These results indicate the quality of the computational method used to calculate the absolute pK_a values of thiotepa and tepa in this study.

A number of studies have described the effect of pH on the stability of thiotepa and tepa [9–11]. Although the authors did not determine the pK_a values of these compounds explicitly, we can conclude from their studies that the value of pK_a should be low. Our theoretical results obtained with the different sets of radii confirm this trend. This finding shows that thiotepa has very low pK_a, which ensures that it remains unprotonated across a wide pH range. Protonation is improbable in aqueous solution or in plasma at a physiological pH of 7.4. In the case of tepa, the three sets of radii used in this study give large negative values of pK_a. This suggests that the N-protonated form of tepa is not accessible under physiological conditions. Contrary to the results obtained for proton affinity, there are consistent changes in the predicted pK_a values of the N sites of thiotepa and tepa compared to that of free aziridine. Our calculations illustrate that the nucleophilicities of the aziridine groups in thiotepa and tepa are much lower than that of free aziridine (pK_a=8). This result indicates that, in the aqueous phase, the lone pair of electrons on the nitrogen of the aziridine moiety of either thiotepa or tepa is heavily involved in resonance with the P=S and P=O groups, respectively. This observation agrees perfectly with the results obtained for the geometrical parameters of the two structures in the aqueous phase. The solvent field appears to be an important factor for understanding the alkylation mechanism for the parent drug and its metabolite. In summary, the acid-assisted activation process cannot be the main mechanism in DNA alkylation by thiotepa and its metabolite under physiological conditions. We can conclude from these results that the alkylation of DNA (pathway 1, see Scheme 2) by thiotepa occurs through the direct nucleophilic ring opening of the aziridinyl group.

Several experimental studies have suggested that thiotepa and tepa function as prodrugs for aziridine (pathway 2, see Scheme 2) [1–4]. In this way, thiotepa and its metabolite tepa act as cell-penetrating carriers for aziridine, which is released extracellularly after hydrolysis. Experimental observations and our calculations show that free aziridine is a weak base (pK_a (exp)=8.0, pK_a (calc)=7.4). It can exist in a protonated form at biological pH (~7.4), resulting in highly electrophilic ethyleimonium ions that have a positive charge located on the nitrogen.

LUMOs and charge distributions

LUMOs (lowest unoccupied molecular orbitals) based on B3LYP/6-31G(d) calculations were generated as a means for predicting the pathways by which thiotepa and tepa react with DNA. LUMOs delineate the areas on a molecule that are most electron deficient, and hence subject to nucleophilic attack. Figure 2 illustrates the LUMOs of thiotepa and tepa. Obviously, in both compounds, the main lobes of the LUMO are found along the P–N bond as well as on the carbon atoms of the aziridine moiety. Note that the aziridine carbons that are at the β position in relation to the phosphorus atom also make significant contributions. The localization of the LUMO indicates that a nucleophilic attack on phosphorus (a hydrolysis reaction) or on an aziridine carbon (aziridine ring opening by nucleophilic DNA bases) should be feasible for both molecules.

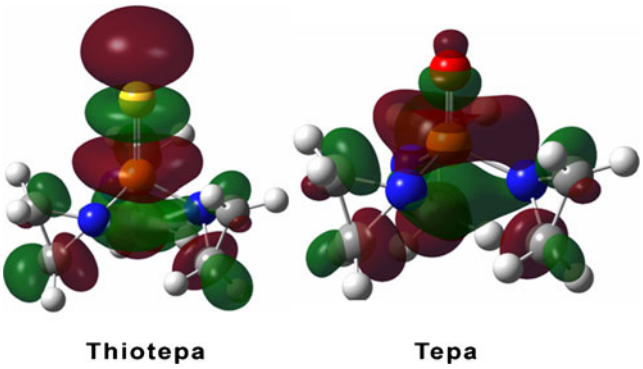


Fig. 2 Illustration of the LUMOs of thiotepa and its metabolite tepa

To clarify the mode of action of the drug and its metabolite tepa, we computed the charges on selected atoms. Because all of the schemes used to assign atomic charges are somewhat arbitrary, we decided to use two different methods, natural population analysis (NPA), which yields information on the electron density in the proximity of each atom, and the CHELPG scheme, which yields information on the electrostatic potential. Table 6 compares the results of NPA and CHELPG for the gas phase with those calculated for aqueous solution. It is worth noting that the main effect of the solvent on the charge distribution is to exert a stress on the charge separation between the different types of atoms. The carbons of the aziridine rings in the compounds studied here are found to have nearly identical NPA atomic charges, while the results obtained from CHELPG show that the aziridine carbons in the β position (compared to the phosphorus atom) have positive values. Moreover, CHELPG indicates that the charges on the β carbons of thiotepa appear larger positive than those found in tepa, making them capable of strong electrostatic interactions, resulting in a high probability of chemical bonding with a nucleophilic agent. These results suggest that ring-opening reactions of the aziridine moiety performed by nucleophilic attack are favored in thiotepa relative to tepa. Interestingly, NPA indicates that the charge on phosphorus is more positive in tepa than in thiotepa; it has a very low electron density around it. CHELPG also indicates that the charge is significantly more positive in tepa, reflecting the cationic electrostatic potential. Our results show that the P–N bond is more strongly polarized (as P^{+}–N^{-}) in tepa than thiotepa.

Table 6 Atomic charges on selected atoms calculated at the B3LYP/6-31G(d) level using natural population analysis (NPA), and charges fitted to the electrostatic potential according to the CHELPG scheme

Species	Atom type	NPA		CHelpG	
		Vacuum	Water	Vacuum	Water
Thiotepa	P	1.878	1.912	0.544	0.659
	S	−0.573	−0.662	−0.387	−0.515
	N	−0.854	−0.865	−0.263	−0.306
	C	−0.252	−0.261	−0.245	−0.244
	C	−0.268	−0.262	0.079	0.085
Tepa	P	2.439	2.455	1.192	1.262
	O	−1.064	−1.120	−0.632	−0.721
	N	−0.863	−0.878	−0.400	−0.448
	C	−0.254	−0.264	−0.171	−0.177
	C	−0.271	−0.265	−0.0183	−0.0046

This result strongly suggests that, in tepa, nucleophilic attack at the phosphorus atom would prevail thermodynamically over aziridine ring opening. We can therefore conclude that the hydrolysis reaction is more favorable in tepa than in thiotepa. This observation is consistent with experimental studies indicating that no crosslinking is observed during the incubation of tepa with cellular DNA [1]. As described in the "Introduction," the reaction of tepa with DNA is believed to follow pathway 2 [1]. For thiotepa, the results of CHELPG support both the hydrolysis reaction and direct nucleophilic aziridine ring opening. This finding agrees well with experimental observations. It should be noted that CHELPG gives a very good description of charge interactions in other species and is a useful tool for examining the interactions between molecules. According to the LUMO and the CHELPG charge results, regioselectivity should play an important role in aziridine ring opening reactions of thiotepa, since it is obvious that the reactivities of the two carbons in each aziridine moiety will differ.

Conclusions

In this work, density functional theory was used in combination with the dielectric continuum model of solvation to investigate some physicochemical properties of the antitumor drug thiotepa and its oxo-analog metabolite. The scheme for computing the absolute pK_a using different sets of radii was validated on a series of aziridine and phosphoramide compounds. In this work, we succeeded in calculating accurate absolute pK_a values using a UFF cavity. The results described in this paper indicate that the implicit solvent model (CPCM) is able to predict satisfactory results; however, appropriate parameters that best describe the solute cavity should be selected.

Our calculations show that thiotepa and tepa are extremely acidic (CPCM using the UFF set of radii predicts pK_a of −2.8 and −7.1, respectively), and should not be susceptible to protonation to form the highly reactive aziridinum ion at physiological pH.

According to all of the results, the solvent field should be considered a significant factor in understanding the mechanism for the alkylation of the drug and its metabolite.

Analyses of LUMOs and CHELPG charges led to a similar conclusion. They provided information on the reactive sites of thiotepa and tepa and consolidated the experimental results, which indicate the importance of phosphorus and carbon atoms in the antitumor activities of these compounds. For thiotepa, our calculations supported both a hydrolysis reaction and a direct nucleophilic ring opening of an aziridinyl group, whereas a fast hydrolysis reaction was predicted for tepa. In addition, it became apparent that the reactivities of the two carbons in each aziridine moiety differ, thus making the direct ring opening reaction of the drug regioselective.

Finally, theoretical studies of the detailed mechanism of the hydrolysis of the drug and its metabolite are in progress.

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