Theoretical Researches on the Molecular Structures of DNA Crosslinks Induced by Chloroethylnitrosoureas and QSAR Analysis

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Abstract: - Chloroethylnitrosoureas (CENUs) are important clinical antitumor agents. Their cytotoxicity is associated with the generation of DNA interstrand crosslinks. In this work, QM/MM computations are carried out to investigate DNA crosslinks by CENUs with ONIOM hybrid method. The crosslinked DNA are subdivided into three layers, each of which are described at B3LYP/6-311+G(d,p), AM1 and UFF level of theory respectively. The result shows that the deformation of DNA with dG(N1)-dC(N3) crosslink is much less than the other crosslinks, which indicate that the most favorable crosslink is between the N1 atom of guanine and the N3 atom of the complementary cytosine. The quantitative structure-activity relationship (QSAR) of CENUs is studied by *ab initio* computations at MP2/6-311G(d,p) level of theory. 37 kinds of CENUs with experimental anticancer therapeutic index (TI) are selected as models. Their activation energies of formation of the electrophilic centers on the α- and the β-carbon, and the octanol-water partition coefficient, are selected as structural parameters. Through numerical fitting of the computational data with the experimental anticancer therapeutic index, a formula is established to predict the anticancer activity of CENUs. The correct discrimination ratio between the computing and the experimental anticancer activity comes up to 94.6% through a five degrees classification.

Key-Words: - Chloroethylnitrosoureas, DNA crosslinks, QSAR, Anticancer activity, *ab initio*, DFT, ONIOM

1 Introduction

Chloroethylnitrosoureas (CENUs), including 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3- methylcyclohexyl-1-nitrosourea (meCCNU) and chlorozotocin, are significant anticancer agents in the clinical treatment of human malignancies, including Hodgkin's disease, Burkitt's lymphoma and cerebral neoplasm [1]. Several lines of evidences indicate that the cytotoxic activity of CENUs is related to the formation of DNA interstrand crosslinks [2,3]. The formation of the crosslinks with an ethidene between complementary base pair is supposed to be the critical step for the anticancer activity of CENUs. Tong and coworkers [4] isolated the crosslinked dinucleoside from calf thymus DNA, which was exposed to CENU. Bodell et al [5] observed that a dramatic increase in the formation of crosslinks when Tris buffer was used in the reaction of DNA and CENUs. Fischhaber [6] determined the molecular structure of the interstrand crosslinked DNA, 1-(N3-deoxycytidyl)-2-(N1-deoxyguanosinyl)ethane, by comparison of its mass spectrum, high pressure liquid chromatography (HPLC) retention time, and UV spectrum to an authentic standard prepared by chemical synthesis. However, because of the complexity of the biochemical reactions of CENUs with the cellular macromolecules, the pathways leading to the formation of the critical lesion have not yet been described clearly. It is essential to reveal the details of the type and the nature of the DNA interstrand crosslinks by CENUs to attempt to account for their anticancer mechanism.

Recently, it has been reported that some antinioplastic agents have caused secondary cancers in patients or are likely to be carcinogenic to humans [7]. Although sufficient efforts have been devoted on the quantitative structure-activity relationship (QSAR) of CENUs to enhance their antitumor activity [8], the risk of the secondary cancers induced by CENUs has not been attached much importance while maximizing their anticancer effect.

In the present work, ONIOM (Own N-layered Integrated molecular Orbital and molecular Mechanics) hybrid computations are carried out to investigate the molecular structures of the interstrand crosslinks of DNA. *Ab initio* calculations are carried out to investigate the QSAR of CENUs, in order to shed lights on the development of more effective

nitrosourea anticancer agents with less carcinogenic side effect.

2 Models and computations

2.1 ONIOM model of DNA crosslinks

The structure of B-DNA double helix containing 12 base pairs (5'-A-A-T-T-G-C-T-A-A-C-G-C-3') is established with its 5' and 3' terminals blocked by hydroxyl groups hydroxyl. 22 hydrogen atoms are added to the phosphate groups along the backbones to neutralize the DNA molecule. 5 kinds of DNA interstrand crosslinks, dC(O^2)-dG(N^2), dC(N^4)-dG(O^6), dC(N^3)-dG(N^1), dT(O^4)-dA(N^6) and dT(N^3)-dA(N^1), are designed on the sixth C-G bases pair and the seventh A-T bases pair respectively (see Fig. 1). All crosslinks are situated on the paired negative atoms in the complementary base pairs, because the distance between the paired negative atoms are just matched with the structure of the ethylene generated from CENUs [9]. Fig. 2 shows the ONIOM model of the DNA double helix containing interstrand crosslinks. The DNA molecules are subdivided into three layers, each of which is described at a different level of theory and finally combines to get the predictive result. The Small Model (SM) system is the crosslinked base pair which is the crucial position in the molecule system. It is described by density function theory (DFT) method at B3LYP [10] level with the 6-311+G(d,p) basis set, which is the highest theoretical level in the whole molecular system. The subsequent two layers are treated using progressively cheaper approaches of theory. The Intermediate Model (IM) system contains the upper and the lower base pairs adjacent to the crosslinked base pair, as well as the riboses and the phosphoric acids between them, which are computed at the middle level with AM1 method [11]. The Real (R) system is the full DNA double helix, which is treated at the lowest level with UFF method [12].

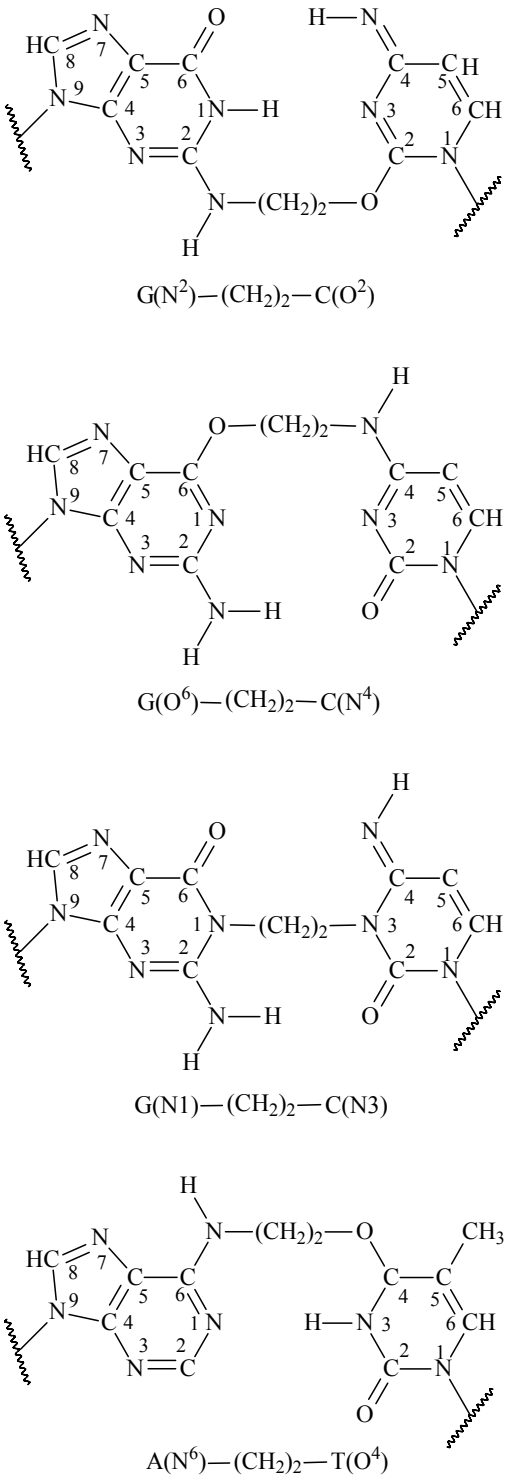


Fig. 1 Molecular structures of the crosslinked base pairs

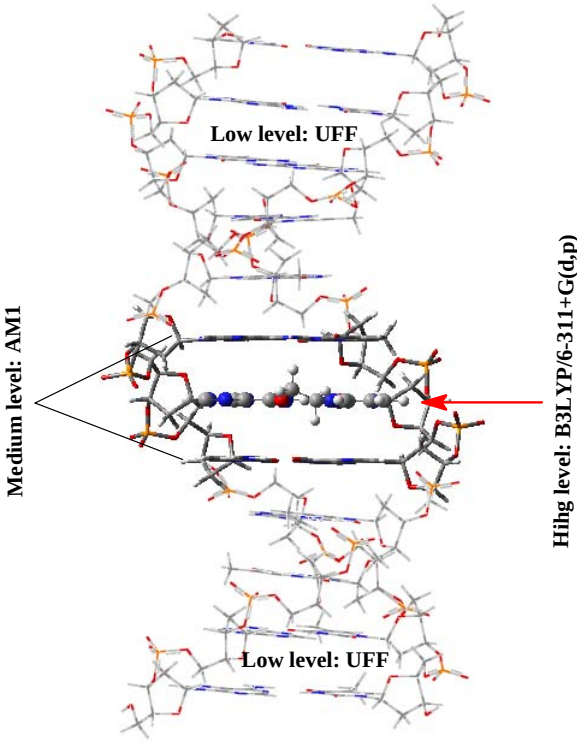


Fig. 2 The three-layered ONIOM model of DNA double helix with interstrand crosslinks

2.2 QSAR model of CENUs

CENUs are highly unstable in aqueous media and undergo decomposition spontaneously under physiological conditions to give rise to alkylating electrophilic agents [13]. These active agents react directly with DNA bases and induce crosslinks on the complementary base pair of DNA, which are supposed to be the key step for the anticancer mechanism of chemotherapeutant [14]. According to Di-region theory [15], the condition for the interstrand crosslinks is that CENUs decompose to generate two electrophilic alkylating centers on the α-carbon and β-carbon of the chloroethyl group (see Fig. 3). In this work, 37 kinds of CENUs with definite experimental anticancer therapeutic index (TI) are selected as models. The mechanisms of the formation of the α- and the β-electrophilic center on the chloroethyl group are studied with *ab initio* computations. An analysis of the relationship between the alkylating activity of these electrophilic centers and their anticancer activity is performed with the data obtained from the *ab initio* computations as structural parameters, including the activation energies of the formation of the α-center (E_a^{α}), the activation energies of the formation of the β-center (E_a^{β}), and the octanol-water partition coefficient (Log*P*). The activation energies are obtained through the geometric optimizations for all molecular structures at MP2/6-311+G(d,p) theoretical level. Numerical fittings are performed between the experimental anticancer TI and the computing structural parameters. Then the anticancer activity is harmonized with the five-degree indexed, i. e. non (-), slight(+), certain (++), fine(+++), and significant(++++) [16]. The computational procedure of the QSAR analysis is shown in Fig. 4.

The DNA structural data are adopted from the standard geometric structure parameters of nucleic acid database of Hypercube Corp. All calculations are performed with GAUSSIAN 03 program package [17].

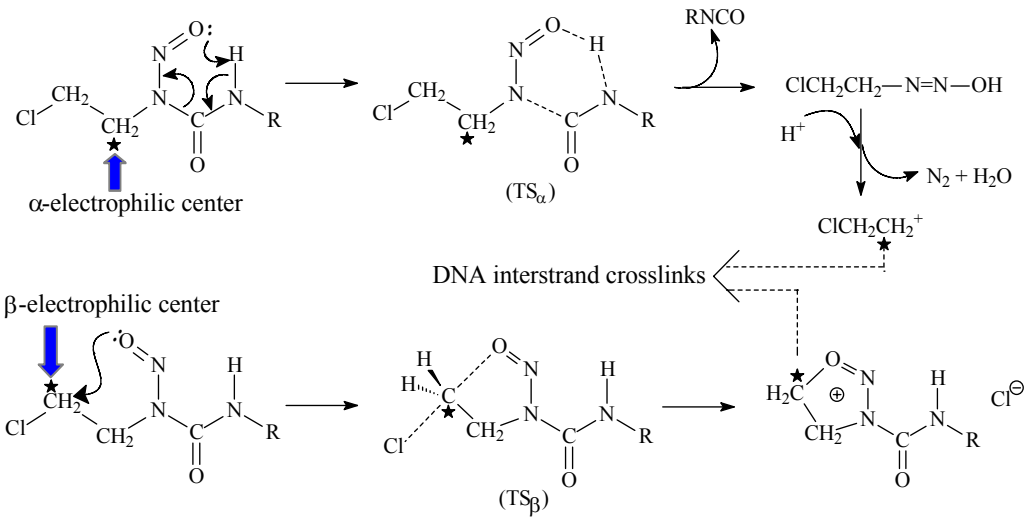


Fig. 3 Mechanism for the formation of the α- and the β-electrophilic centers on CENUs

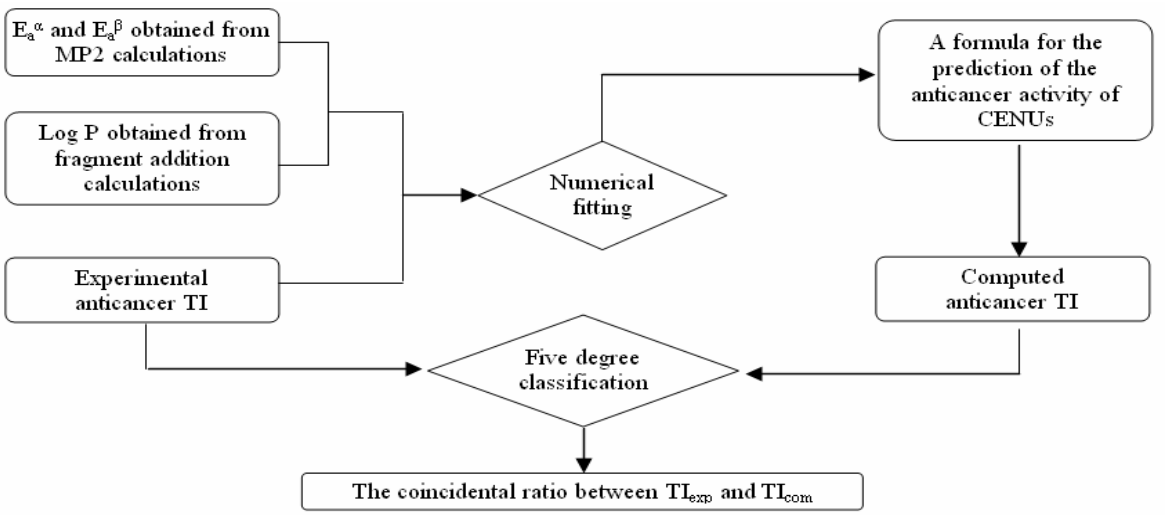


Fig. 4 The computational procedure of the QSAR analysis of CENUs

3 Results and discussion

3.1 Molecular structures of the crosslinked DNA double helixes

The optimized molecular structures of the crosslinked DNA are shown in Fig. 5. The differences in the structures of the DNA double helix can be distinguished when the interstrand crosslink occurs on the different positions of the base pairs. In the crosslinked deoxyguanosine (dG) and deoxycytidine (dC), including the dG(O6)-dC(N4) crosslink in the major groove, the dG(N2)-dC(O2) crosslink in the minor groove, and the dC(N3)-dG(N1) crosslink in the middle of the base pair, the structural deformation of the G-C base pair and the double helix are very slight. However, when the crosslink occurs on the deoxythymidine (dT) and deoxyadenosine (dA), the double helix of DNA is destroyed. In dT(O4)-dA(N6), the A-T base pair obviously deviates from the initial plane in normal DNA structures. The optimization of the geometric structure of dT(N3)-dA(N1) is failed, so the stationary structure of it cannot be obtained.

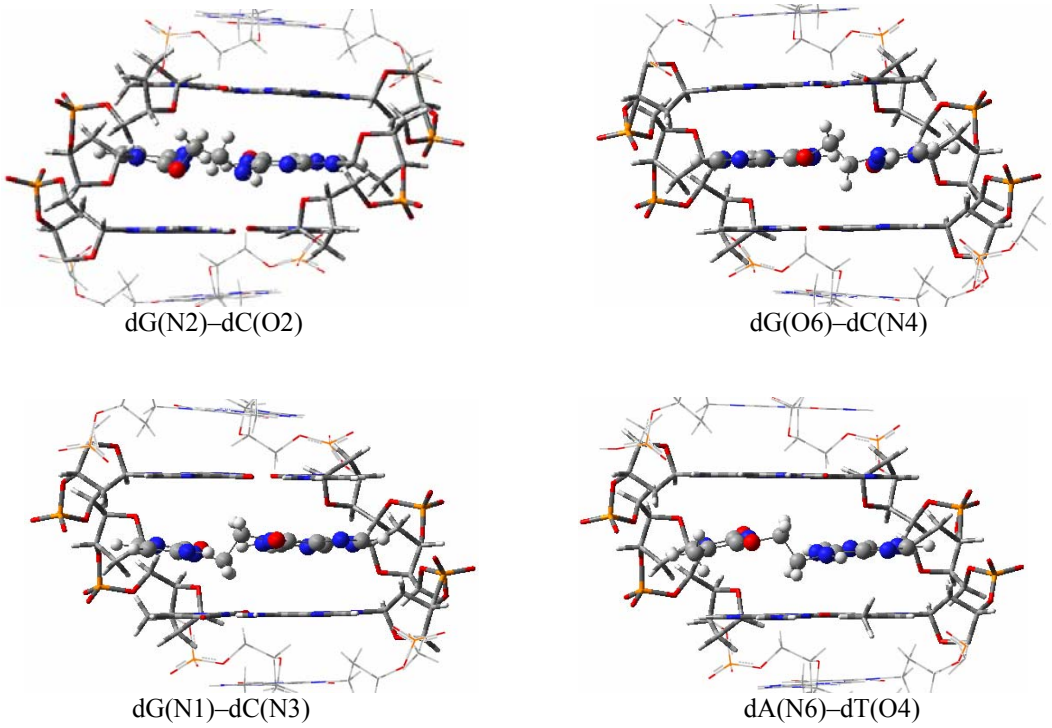


Fig. 5 Structures of the crosslinked DNA obtained from ONIOM computations

The main geometric parameters and total electronic energies are listed in Table 1. In the three G-C crosslinks, the distance between the two paired electronegative atoms are 0.29~0.33 nm. This distance indicates that the hydrogen bonds can be effectively formed between the crosslinked G-C base pair. The crosslinked guanine and cytosine is still coplanar approximately. Their tilt angles from the plane of normal base pair are 5.7° in average. Owing to these characters, the DNA double helix can remain their initial secondary structures roughly. Although the deformation of the DNA is not obvious for all of the three G-C crosslinks, the total electronic energies of them obtained from geometric structural optimization are very different. It can be seen from Table 1 that the total electronic energy of dC(N3)-dG(N1) is obviously lower than that of dC(O2)-dG(N2) and dC(N4)-dG(O6). The relative energy of the dC(N3)-dG(N1) crosslink is remarkably lower than that of dC(O2)-dG(N2) and dC(N4)-dG(O6) by 136.50 kJ·mol-1 and 114.13 kJ·mol-1 respectively. This result indicates that the dC(N3)-dG(N1) crosslink is more stable than the other crosslinks, so the N3 atom of cytosine and the N1 atom of guanine are supposed to be the most favorable positions for the DNA crosslink induced by CENUs. This theoretical result coincides with the phenomena observed in HPLC-MS spectroanalysis [18] and the supposed mechanism of DNA crosslink by CENUs [19], which presumed that CENUs decomposed to generate the initial alkylating product O6-(2-chloroethyl)-deoxyguanosine firstly, and then through intramolecular cyclization N1,O6-ethano-deoxyguanosine is formed followed by the nucleophilic attack on the N3-position of the complementary cytosine (see Fig.6).

In the optimized structure of A-T crosslink, the distance of the paired electronegative atoms, the O4 atom in thymine and the N6 atom in adenine, is 0.34 nm and 0.37 nm respectively, which are longer than the distance of the hydrogen bonds in normal A-T base pairs. This structure results in that the hydrogen bonds are difficult to be formed between the crosslinked dA and dT. Furthermore, the crosslinked adenine and thymidine are obviously dislocated and deviated from the initial plane of normal base pair. The tilt angles of them are 15.6° and 20.3° respectively. Because the double helix of DNA deforms apparently, the DNA segments with A-T crosslinks are supposed to be easily identified by the repairase. Consequently, the DNA repairing process is possibly initiated by deleting this crosslinked region. This result explains the phenomenon that A-T crosslinks are seldom observed in the experiments of DNA crosslinks.

Table 1 Main geometric structural parameters of the crosslinked complementary base pairs

Crosslinks	Distance (nm)		Tilt angle of the crosslinked bases (°)	Total electronic energy (a.u.)
dG(N^2)-dC(O^2)	O^6-N^4	0.311	G 5.8	
	N^1-N^3	0.313	C 7.2	-1008.85016
	N^2-O^2	0.311		
dG(O^6)-dC(N^4)	O^6-N^4	0.320	G 3.4	
	N^1-N^3	0.339	C 5.5	-1008.85868
	N^2-O^2	0.305		
dG(N^1)-dC(N^3)	O^6-N^4	0.326	G 4.5	
	N^1-N^3	0.329	C 7.6	-1008.90215
	N^2-O^2	0.296		
dA(N^6)-dT(O^4)	N^6-O^4	0.369	A 15.6	-991.04032
	N^1-N^3	0.339	T 20.3	

The tilt angle of the crosslinked bases are compared with the initial planes in normal base pairs.

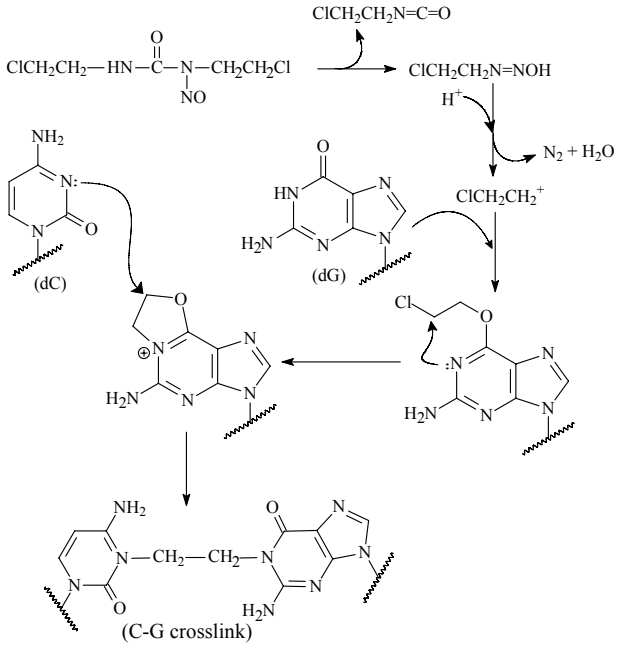


Fig. 6 Mechanism of DNA interstrand crosslinks between N1 of guanine and N3 of complementary cytosine by CENUs

When the interstrand crosslinks occur on the N1 atom of guanine and the N3 atom of cytosine, because the deformation of DNA double helix is much less and the crosslink is more stable, the DNA segment containing this interstrand crosslink is unfeasible to be recognized and repaired by the repairase. Kohn and coworkers compared the DNA crosslink induced by CENUs and nitrogen mustard in

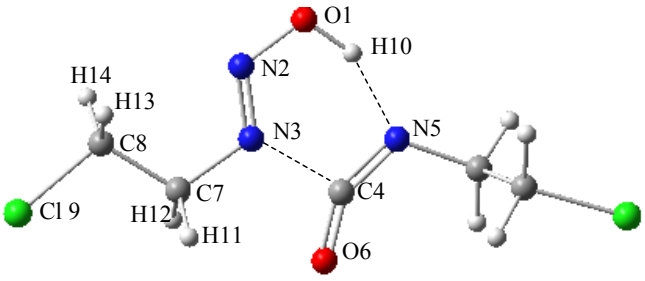
rat's leukaemia L1210 cell [20]. They found that the crosslinks induced by nitrogen mustard can be almost repaired within 24 hours; while the crosslinks induced by CENUs were seldom repaired. Because of this unrepairable interstrand crosslinks, the double strands cannot be opened in the process of DNA replication, which may finally result in the apoptosis of cancer cells. For the other interstrand crosslinks, the crosslinked DNA segment can be deleted by the exonucleases, so this damaged DNA is difficult to be correctly repaired without the complementary base as templates. Finally, these damages may lead to deletion, frameshift or rearrangement of DNA. The incorrect reparations possibly result in the drug resistance of cancer cells, and even the occurrence of secondary tumors [7].

3.2 QSAR analysis of CENUs

The mechanism for the formation of the α- and the β-electrophilic centers on CENUs are studied firstly. The activation energies of the formation of these two electrophilic centers are obtained, and the optimized molecular structures of the transition states (TS) and their main structural parameters are shown in Fig. 7. In the formation of the α-electrophilic center, CENUs undergo decomposition to produce diazohydroxides and isocyanates through a hydrogen shift from the nitrogen atom to the oxygen atom on the nitroso group, which is considered to be the chief decomposing route under physiological conditions [21]. In the formation of the β-electrophilic center, a pentatomic cyclic intermediate is formed under the anchimeric assistance of the nitroso group. The anchimeric assistance proceeds via the β-carbon being attacked by the lone electron pair of the oxygen atom of the nitroso group accompanying the leaving of the chloridion.

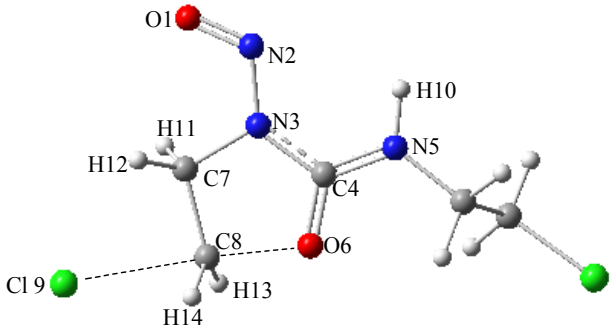
The activation energies (Eaα and Eaβ) obtained from MP2/6-311G(d,p) computations, as well as the LogP obtained from fragment addition calculations of the 37 CENUs are listed in Table 2. Through numerical fitting computation of these structural parameters with experimental anticancer TI (TIexp), the computational anticancer TI (TIcom) of CENUs is formulated as below:

$$TI_{com} = 1.089\times10^{3}\times(\left|E_a^{\alpha}-106.95\right|\cdot E_a^{\beta})^{-1}-1.83\times10^{3}\times(E_a^{\beta})^{-2}-0.12\times Log^2P \quad (1)$$



O1-H10	0.100nm
O1-N2	0.129nm
N2-O3	0.122nm
N3-C4	0.177nm
C4-N5	0.126nm
N5-H10	0.163nm
O1-H10-N5	152.60°
N5-C4-O6	146.77°

(TS_α)



N3-C4	0.138nm
C4-N5	0.131nm
O6-C8	0.179nm
C8-Cl9	0.251nm
O6-C8-Cl9	175.90°
N2-N3-C7-C8	179.23°
N3-C7-C8-O6	0.76°
C7-C8-O6-C4	0.95°
C7-C8-H13-H14	159.46°

(TS_β)

Fig. 7 The optimized molecular structures of the transition states of the formation of α- and β-center

Table 2 Experimental and computed volume for the biological activity of 37 CENUs

No.	$ClCH_2CH_2N(NO)C(=O)NHR$	E_a^{α} (kJ·mol^{-1})	E_a^{β} (kJ·mol^{-1})	Log *P*	TI_{com}	Anticancer activity	
	R					Com.	Exp.
1	H	97.4	153.4	0.39	4.5	–	–
2	$CH_2C(CH_3)_3$	110.6	150.6	2.55	14.0	+	++
3	CH_2CH_2Cl	109.8	156.9	1.44	122.3	++++	++++
4	CH_2CH_2CN	110.9	161.6	0.21	44.9	++	++
5	$CH_2CH_2OSO_2CH_3$	109.9	158.1	-0.30	185.1	+++	++++
6	CH_2CH_2OH	111.8	164.5	0.28	18.2	+	++
7	$(CH_2)_3OH$	111.4	150.8	0.21	35.4	++	++
8	$(CH_2)_4OH$	111.7	150.8	0.70	23.4	+	++
9	$CH_2CH(OH)CH_2OH$	1100	153.2	-0.92	155.1	++++	+++
10	CH_2CH_2COOH	112.2	162.4	0.44	15.6	++	+
11	$CH(CH_3)COOH$	115.9	150.7	0.79	4.5	–	+
12	$CH(CH_2OH)COOH$	96.7	152.5	-0.68	3.7	–	–
13	CH_2CHO	112.5	147.4	0.39	16.8	+	++
14	CH_2CONH_2	113.3	150.1	-0.6	10.3	+	+
15	$CH(CH(CH_3)_2)CONH_2$	116.8	150.6	0.73	3.9	–	–
16	$CH_2COOCH_2CH_3$	110.2	151.8	1.23	91.2	++++	++++
17	$CH_2OCOCH_2CH_3$	110.4	158.4	1.28	53.6	++	+++
18	$CH(CH_3)COOCH_3$	111.1	148.9	1.20	31.0	++	+++
19	NH_2	102.8	152.4	-0.86	36.5	++	++
20	$NHN(CH_3)_2$	110.5	163.7	0.14	60.5	+++	+++
21	$N(CH_3)CHO$	103.7	168.3	-0.83	65.1	+++	+++
22	$N(CH_3)COCH_3$	104.1	163.4	-0.38	169.3	++++	++++
	R=–R'						
	R′						
23	H	104.6	146.8	2.96	104.9	++++	++++
24	CH_3	104.9	148.2	3.38	140.5	++++	++++
25	CH_2CH_3	104.7	148.3	3.87	20.5	+	++
26	$C(CH_3)_3$	105.1	147.6	4.74	16.1	+	+
27	Cl	104.8	151.0	2.94	172.5	++++	++++
28	CH_2Cl	104.7	150.7	2.99	131.1	++++	++++
29	OAc	103.4	150.8	2.43	18.5	+	++
30	CH_2OAc	103.8	149.9	2.23	39.1	++	+++
31	CH_2OCHO	104.0	150.7	2.38	44.5	++	+++
32	COOH	104.4	151.0	2.15	148.1	++++	++++
33	$COOCH_2CH_3$	104.2	150.3	2.92	35.4	++	+++
34	OCH_3	104.0	149.9	2.13	63.7	+++	+++
35	CH_2COOH	100.0	148.5	2.64	1.4	–	+
36	$COOCH_3$	104.2	150.8	2.43	67.3	+++	+++
37	OH	103.4	149.8	1.43	52.5	++	++

1. The experimental data are obtained from reference 22.
2. The TI_{com} is considered to be coincide with the TI_{exp} when there is only one-degree distinction except for the difference of (+) and (-).

Formula (1) demonstrates that the bifunctional alkylating agents on the α- and the β-electrophilic center are necessary for the anticancer activity of CENUs. For the α-electrophilic center, the value of Eaα influences the TIcom remarkably. When the value of Eaα is very low, because the α-center is highly active, CENUs may be decomposed mostly before reaching the target DNA, and cannot cause the interstrand crosslinks of DNA. However, when the value of Eaα is very high, CENUs are unfeasible to generate the chloroethyl cations through decomposition, which weakens the alkylating activity of CENUs and induce the decrease of DNA interstrand crosslinks. So, it is unfavorable for the enhancement of the anticancer activity of CENUs with too high or too low activities on the α-center. For the β-electrophilic center, high value of Eaβ leads to high TIcom, which indicate low activity of the β-center is desirable for the increase of the anticancer activity of CENUs. Because the anchimeric assistance of the nitroso group on the β-carbon is relevant to the carcinogenesis of N-nitroso compounds (NNCs) [9], it can be hypothesized that the anchimeric assistance of the β-electrophilic center may contribute to the carcinogenic side-effect of CENUs. The interstrand crosslinks of DNA is produced through other reaction pathway as shown in Fig.6.

In Formula (1), Log P is a regularization parameter that adjusts the value of TIcom. The physical environment can be considered as a multiple layered system consisted of alternating water layers and lipid layers. Consequently, the speed of the movement of CENUs is influenced by the value of Log P. The movement of CENUs in lipid layers is hampered when they are strongly hydrophilic, and the movement in water layers is hampered when they are lipophilic. So there is a trend toward a higher anticancer activity with the Log P values approaching to zero.

4 Conclusion

The interstrand crosslink between the N1 atom of guanine and the N3 atom of the complementary cytosine through an ethylene is theoretically determined to be the main crosslinking damage in the reactions of DNA and CENUs. The results indicate that DNA segment with this crosslink can remain the normal double helix structure and it is more stable comparing with the other interstrand crosslinks. It is supposed that the dG(N1)-dC(N3) crosslink is difficult to be recognized by the DNA repairase and may be remained in the process of DNA replications, in which case DNA transcription will be hampered and cancer cell will undergo apoptosis finally. For the other crosslinks, the damages may be recognized and repaired incorrectly, which may induce the cancerization of normal cells. Therefore, DNA interstrand crosslinks are involved in not only the anticancer activity of CENUs, but also their carcinogenic side effect.

Both of the TIexp and the TIcom are classified to five degrees as none (-), slight (+), certain (++), fine (+++) and significant (++++). The coincidental ratio between the TIexp and the TIcom comes up to 94.6%. These results show that the activity of the α- and the β-electrophilic centers and their relationship should be considered in the drug design of CENUs as anticancer agents. Proper activity of the α-center on CENUs is essential for the increase of anticancer activity. The carcinogenic side-effect can be prevented by blocking the anchimeric assistance of the nitroso group on the β-center. It is presumed that the anticancer activity of CENUs can be enhanced through controlling the alkylating activity of the α- and the β-electrophilic centers and the lipophilicity by the modification of their substituent groups.

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