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Docking analysis of aryl derivatives of diepoxide alkylating agents

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Abstract: In this research, molecular structural manipulation of treosulfan alkylating agent and resultant changes in binding is studied to assist in designing derivatives of treosulfan for synthesis. Molecular docking has been conducted on simulated heterocyclic polyaromatic alkylating diepoxide derivatives of treosulfan with DNA nucleobases of dodecamer duplex of sequences d(CGCGAATTCGCG) and d(CGCGAATTCGCG) using Autodock vina package. Two series of simulated diepoxide molecules were designed with increasing aryl ring chain in linear and fused aryl way between the two epoxide reactive rings. Relationship between increasing no. of aryl rings (both linear and fused) between epoxide moieties on the binding energy values was evaluated. We also identified that designed molecules bind specifically to Guanine and Cytosine (GC) base pairs on DNA. Mode of interaction and resultant behavior as an alkylating agent or as minor groove binder was also found to be dependent up on the no. of aryl rings and their connectivity in the molecule. Both linearly bonded and fused aryl rings in higher number, between the epoxide rings, gave the strongest binding with the binding energy up to -8.1 and -8.7 Kcal/mol, respectively. These relationships can immensely help in designing and synthesis of derivatives of treosulfan like diepoxide based alkylating agents.

Keywords: Epoxy compounds, alkylating agents, DNA nucleobases, molecular docking simulation.

INTRODUCTION

Epoxide rings are used as an alkylating agent for DNA crosslinking in the treosulfan molecule (Structure 1 in fig. 1) (Singh *et al.*, 2018). Alkylating agents is one major class of chemotherapy drug molecules. They react with nucleophilic sites on with the DNA molecule, bonding alkyl group to them. This prevents the uncoiling and replication DNA strands, as a result the cancer cell dies and does not replicate (Lind, 2020). Examples of antineoplastic alkylating agents are N-mustard, nitrosourea, epoxide, alkyl sulfonate and Pt coordinate complexes (Sheikh *et al.*, 2018). Such molecules have Lewis acidic sites which, due to their inherent polarity, are attacked by basic groups of nucleobases, thus alkylating them and crosslinking the two DNA strands together. (Cao *et al.*, 2015; Zhang *et al.*, 2019; Hou, *et al.*, 2017; Pilon-Thomas *et al.*, 2016). DNA interacting molecule can bind to double stranded DNA through groove binding, intercalation (Gilad and Senderowitz, 2014) between nucleobases and covalent bonding to the nucleobases (Sheikh *et al.*, 2018). There is a possibility that other than nucleobases, molecule may bind to the sugar-phosphate strand but in this research, we are focusing on interaction with nucleobases. Potential nucleophilic sites in DNA nucleobases are N-1 and N-3 of Adenine (A), the N-3 of cytosine (C) and the O-6 of Guanine (G). The most common Lewis basic site in the DNA nucleobases is the sp^2 hybridized N-7 atom of the purine ring in the Guanine base.

In this research, we found the binding energy resulting from interaction of DNA nucleobases with simulated designed polyaromatic diepoxide derivatives of treosulfan molecule. Stronger binding energy implies higher chances of favorable SN2 reaction with the LUMO orbital of the C-O bond on the diepoxide ring molecule in proximity of the HOMO of the Lewis basic site on DNA nucleobase to create an overlap (fig. 1(B)). Different chain length of phenyl rings, linear (1-6) and fused (7-10) in between the two heterocyclic reactive diepoxide rings are considered to check the impact of the increasing aryl chain length on the binding energy (fig. 1(B)). Mode of interactions dependence upon no. of aryl ring and their connectivity in the molecule is also evaluated.

Diepoxide molecule can work as an alkylating agent. Because of the inherent ring strain of epoxide ring (Wade and Simek, 2017), it is supposed to give rise to the higher potential energy (P.E.) or overall instability in the molecule. Epoxide has significant amount of ring strain because of combination of angle, torsional and steric strain and deviation from optimal bond length as shown in fig. 1(A). This instability referred to as ring strain enhances the reactivity of epoxide rings which can lead to ring opening reaction by Lewis basic sites on nucleobases with less activation energy and higher rate of reaction as shown in fig. 1(B).

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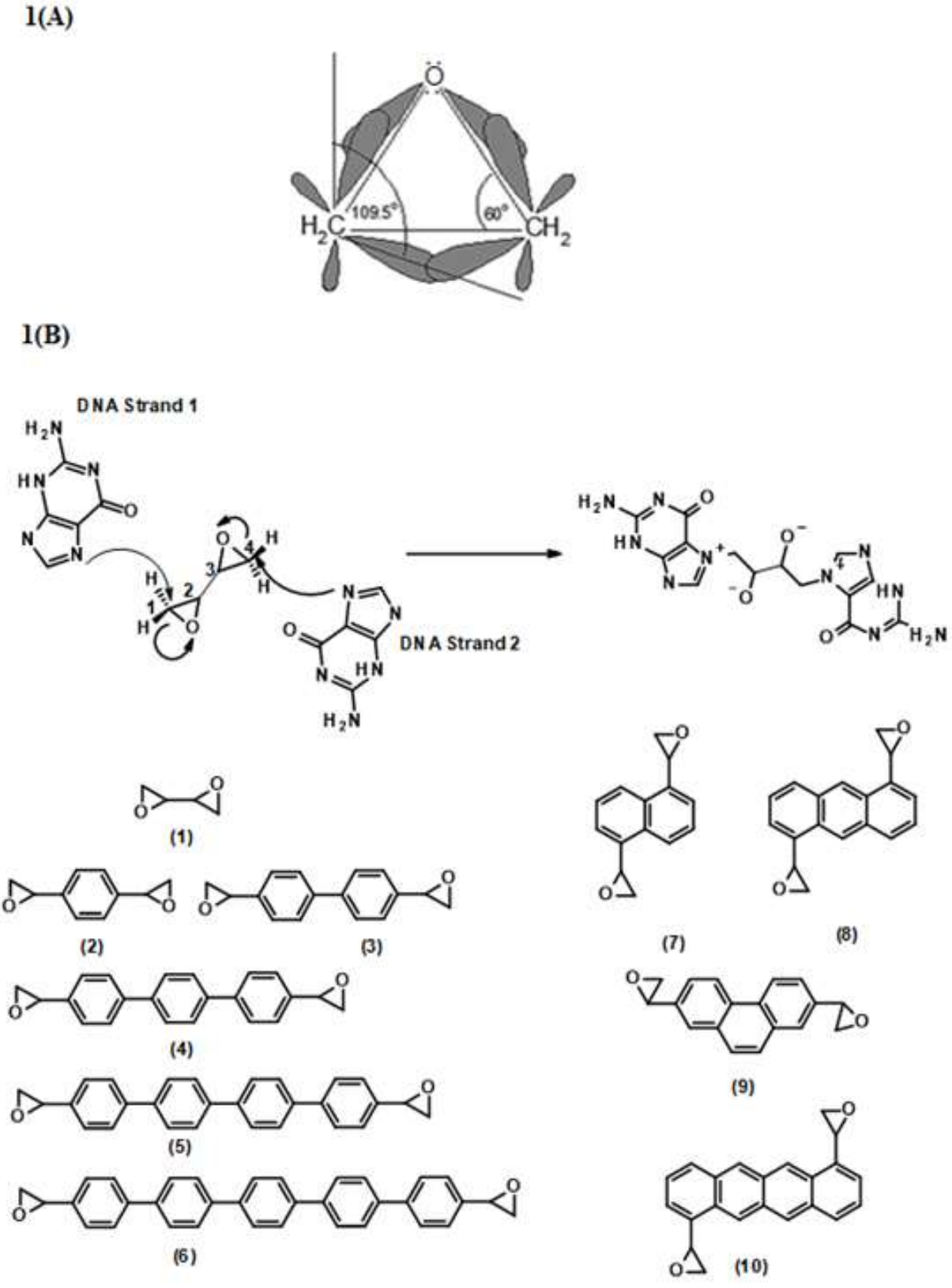

Fig. 1: (A) Angle strain in epoxide caused by less orbital overlap. (B) Alkylation of DNA nucleobase Guanine from both sides by diepoxide molecules. Simulated derivatives are shown as well.

Table 1: Binding energies of designed diepoxide treosulfan derivatives

No. of compound	No. of aryl rings	Binding affinity (kcal/mol)	No. of H bonds	Base Pair
1	0	-3.7	3	DG 10A. (2)DG 16B
2	1	-5.3	2	DG 14 B DG 16 B
3	2	-6.6	1	DG 16 B
4	3	-7.5	-	-
5	4	-8.0	-	-
6	5	-8.1	-	-
7	2	-6.1	3	DG 10A DG 16 B (2)
8	3	-7.5	2	DG 16 B DA 17 B
9	3	-7.2	1	DG 14 B
10	4	-8.7	3	DG 10 A DG 16 B (2)

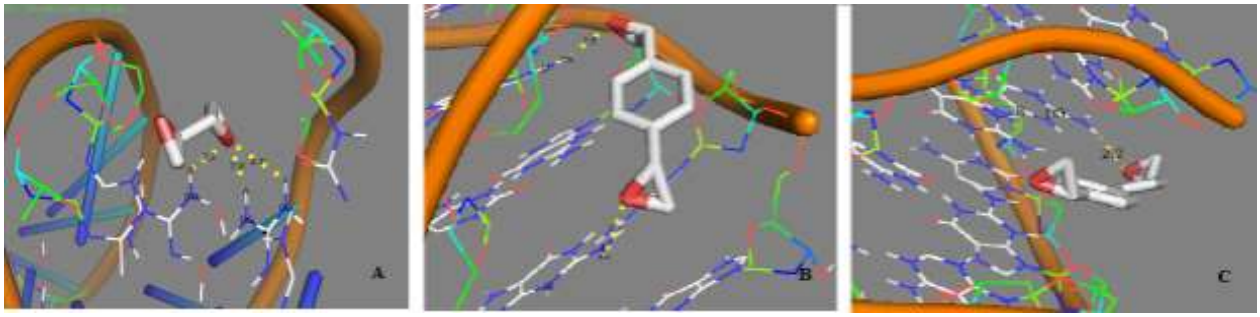

Fig. 2: A= Compound (1), B= Compound (2), C= Compound (3)

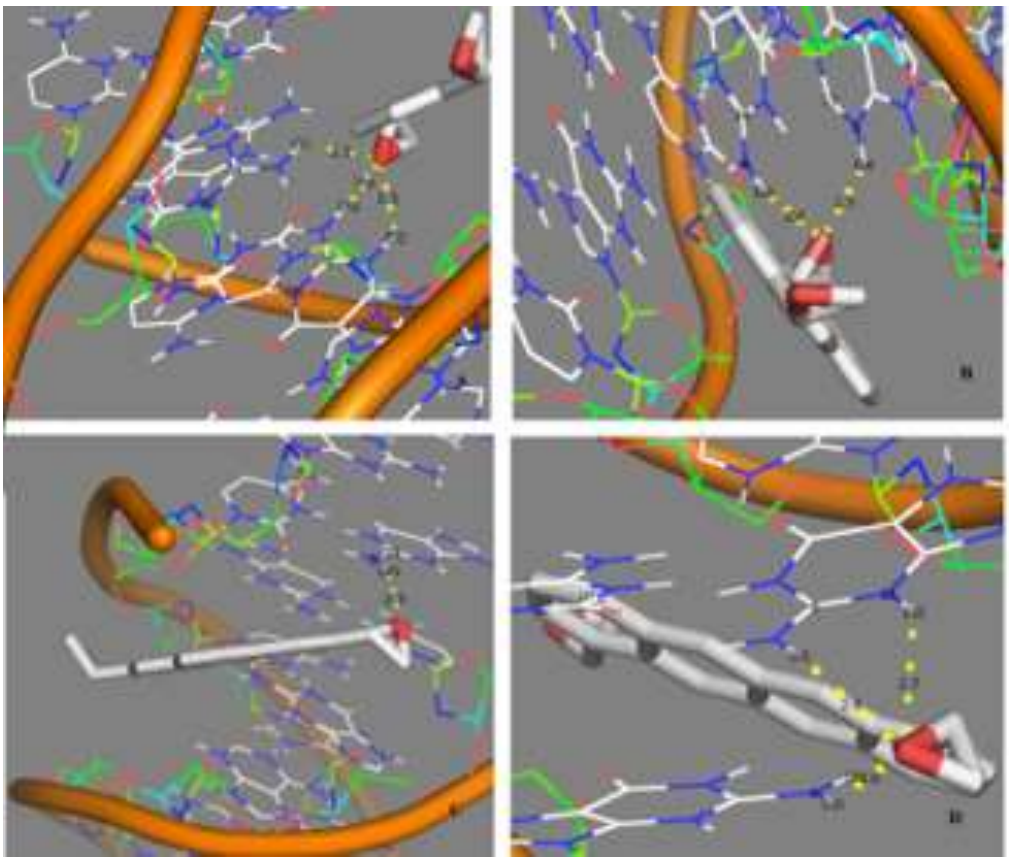

Fig. 3: A= Compound (7), B = Compound (8), C = Compound (9), D = Compound (10)

MATERIALS AND METHODS

For molecular docking study, structure of designed diepoxide molecules (1-10) were drawn on ChemDraw Ultra 2008, then energy minimization of these molecules was carried out by using Avogadro software (Hanwell *et al.*, 2012) with steepest descent algorithm. These energy optimized structures were then changed to .pdb format files by using Open Babel software (O'Boyle, 2011). Using Autodock tools, (Sanner, 1999). pdb structure of (1-6) were converted into .pdpqt format after the addition of H atoms and Kollaman charges which is a requirement of Autodock Vina (Trott and Olson, 2010). For preparation of receptor, dodecamer duplex sequences d(CGCGAATTCGCG) and d(CGCGAATTCGCG was downloaded from Protein Data Bank (Burley *et al.*, 2019) with the code 1BNA in .pdb format. Again, Autodock tools was used to add the H atoms, Kollaman charges while H_2O molecules were removed. This structure was saved as in. pdbqt format. The following parameters were used to perform the docking simulation: size x = 58; size y = 72; size z = 118; center x = 14.693; center y = 21.000; center z = 9.000. Docking analysis was done on the basis of hydrogen bonding, Van der Waals and π-stacking interactions (Anslyn, 2004).

RESULTS

Binding with interstrand base pair

Docking analysis showed that all the docked compounds interacted with active binding region of dodecamer duplex sequences d(CGCGAATTCGCG) and d(CGCGATCGCG). Interactions in docking adducts and the 2D graphical depictions are shown in fig. 2(A). The docked adduct were visualized and analyzed through Py Mol software. The binding energies of all diepoxide (1-10) are listed in table 1. It was found that all simulated diepoxide molecules have high binding affinity for CG base pairs in miner grooves, selectively.

Mode of Interaction

Compound (1) showed (fig. 2A) three hydrogen bonding interactions involving the reactive epoxide ring with the acidic protons of guanine (Dg10) of chain A and (Dg16) of chain B with the bond length of 2.0, 2.7 and 2.2 Å respectively. Compound (2) has two hydrogen bonds involving epoxide reactive ring with the DG14 and DG16 of chain B with the bond length of 2.6 and 2.1 Å respectively and also showed better binding energy than compound (1) (fig. 2B). Compound (3) showed only one hydrogen bond with the DG14 of chain B with the bond length of 2.2 Å (fig. 2C). Compounds (4, 5 and 6) showed no hydrogen bonding but showed good binding interaction as -7.5, -8.0 and -8.1 Kcal/mol respectively because of π-stacking. All of four fused ring diepoxide derivatives (7-10) show increased binding energy with the increase in no. of aryl rings and high binding affinity for CG sequence in miner grooves selectively.

Chain length

Table 1 lists impact of increase in no. of aromatic rings and binding energy for both linear (1-6) and fused polyaromatic (7-10) designed molecules.

DISCUSSION

Binding with interstrand base pair

Covalent binding can occur in three ways i.e. monoalkylation, intra and interstrand cross-linking. Before interstrand crosslinking, alkylating agent molecules must bind to nucleobases on two strands in a crosslinking way. It was found that all simulated diepoxide (1-10) molecules have high binding affinity for CG base pairs in miner grooves, while molecule (1, 7 and 10) bind between the two strands A and B in a crosslinking way, as mentioned in table 1. Also, five rings linearly and four rings fused between the epoxide rings gave the strongest binding with the binding energy up to -8.1 and -8.7 Kcal/mol, respectively. High binding interaction implies proximity of Lewis base and Lewis acid and hence facile SN2 reaction for alkylation and subsequent crosslinking of the DNA.

Mode of Interaction

Mode of interaction and resultant reactivity of the designed derivatives was found to be dependent up on the no. of aryl rings in the molecule. Compound (1), (2) and (3) showed three, two and single hydrogen bond between epoxide rings O atom and H of nucleobases. Compounds (4, 5 and 6) showed no hydrogen bonding, this is due to the fact that increase in the size of the molecule causes the epoxide ring to be away from hydrogens of the nucleobases. But at the same time, these three compounds (4, 5 and 6) showed good binding interaction as -7.5, -8.0 and -8.1 Kcal/mol respectively because of π-stacking. This implies that while epoxide rings do not interact because of increase in length of the molecule, the main mode of interaction becomes π-stacking and (4-6) act as excrement miner grove binder. Hence, it was established that up to three linearly bonded aryl rings, the primary mode of interaction is H-bonding between nucleobases and epoxide and these molecules can act as an alkylating agent. Above this value π-stacking becomes the primary mode of interaction and gives no interaction between epoxide ring and nucleobases hence the molecule becomes minor groove binder. In case of compounds (7-10), the fused ring diepoxide derivatives, it was found out that all four molecules showed the trend of increased binding energy with the increase in no. of aryl rings. But in fused ring system, the increasing binding interaction is the result of both hydrogen bonding involving O atom in epoxide ring and π stacking interactions of π system of fused aryl system as shown in compound 7-10 in fig. 3. All four of these molecules have high binding affinity for CG sequence in miner grooves selectively. Hence, fused

polyaromatic series binds through H-bonds and π-stacking same time which implies that enhanced interaction through higher no. of fused aryl rings still sustains the molecular reactivity involving epoxide rings. (10) gave the highest binding energy among the fused ring (7-10) series.

Chain length

From table 1, linear relationship between increase in no. of aromatic rings and binding energy was also established in both linear (1-6) and fused polyaromatic (7-10) molecules of designed molecules. Linearity sustained up to five rings in linear and four in fused way.

CONCLUSION

This research is related to molecular designing of fused aryl ring derivatives of antineoplastic treosulfan drug. We simulated two series of polyaromatic diepoxide derivatives of treosulfan alkylating agent in which aromatic rings are located between the two reactive epoxide rings and are connected either linearly or in a fused way. This research provided retrosynthetic target molecules of fused polyaromatic diepoxide derivatives of treosulfan drug molecule which can prove to be a viable chemotherapeutic molecule. We found that reactivity of designed molecules depends upon inter and intrastrand binding, mode of interaction, chain length, and ring connectivity factors. We established that inter and intrastrand binding, mode of interaction, chain length, ring connectivity dictates the reactivity of the designed molecules. Simulated results resulted into one molecule with four fused rings, with high binding energy, interstrand crosslinking and mode of interaction that puts molecule into a position where Lewis acidic groups are in proximity to nucleobases for crosslinking. Hence an improved treosulfan derivative for retrosynthesis is provided.

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