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Research Article

VIRTUAL SCREENING AND MOLECULAR DOCKING STUDY OF BLOOM'S SYNDROME PROTEIN (BLM) FOR FINDING POTENTIAL LEAD DRUG CANDIDATE

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Abstract

Increased levels of locus-specific mutations within the BLM result in development of Bloom Syndrome and patients are found to be immune deficient. HRDC domain amino acid Lys1270 is presumably to play role in mediating interactions with DNA. Single point mutation of Lys1270 (K1270V) reduces the potency of Double Holliday junction (DHJ) DNA unwinding so BLM lead to its functional loss. Quadruplex formation have role in immunoglobulin heavy chain switching and inhibiting RecQ helicases activity in-vitro in BLM. Variety of G-Quadruplex ligands are employed by molecular docking for arriving at lead compound identification. The scoring function of docking results describes protein-ligand interaction and it conjointly instructed that docking of ligand at mutational binding site shows some repressing function to make potential lead drug molecule. So as to know the elaborated purposeful functional mechanism of protein and to relate impact of mutation with function and activity; dock screening, hit identification and lead optimization facilitate in design of lead drug compound.

Key words: BLM HRDC domain; DHJ; G-Quadruplex Ligand; RecQ helicase; scoring.

Introduction

BLM is a member of the RecQ family of helicases and is a 1417 amino acid protein, which was mapped to the long arm of chromosome 15 (15q26.1) (Ellis *et al.*, 1995). BLM RecQ helicase thought of as essential enzyme function in DNA recombination and repair. Expression of BLM protein play major role in development of tumors. Apoptotic response is happening in response of BLM proteolysis (Wang and Hu, 2008). Bloom syndrome patients exhibit elevated frequency of chromatid exchanges, chromatid breaks, sister chromatid exchanges (SCE) and increased levels of locus-specific mutations (German, 1995). Bloom syndrome is a rare human recessive disorder associated with growth retardation, high risk of malignancy and immunodeficiency at an early age (German, 1974). Mutation in BLM gene shows 10-fold elevation in SCE frequency (Chaganti *et al.*, 1974). Excessive Homologous recombination's (HR) exhibited by person with Bloom syndrome and have striking genomic instability. New York dermatologist Dr. David Bloom in 1954 was firstly described the condition of bloom syndrome (Bloom, 1954). Five RecQ family helicases termed RecQ1, BLM, WRN, RecQ4 and RecQ5 encodes in human genome. Among these three of paralogues are affected in genetic diseases: BLM, WRN, and RecQ4 in Bloom's syndrome, Werner's syndrome and Rothmans-Thomson syndrome (RTS) respectively (Bachrati and Hickson, 2008). The RNase D C-terminal domain (HRDC, amino acids 1190-1290) has DNA-binding roles and C-terminal region (1291-1417) play roles in protein-protein interaction. HRDC domain C-terminal part resulted in defects in strand annealing, DHJ-binding and dissolution activities indicating the role of the HRDC domain in this process (Wu *et al.*, 2005).

BLM (S1209T) HRDC domain point mutation causes Bloom's syndrome (German *et al.*, 2007) and another HRDC domain (K1270V) single substitution have an effect on DHJ dissolution. BLM role in controlling re-combination by interact with one topoisomerase III isoform, TOPO III (Johnson *et al.*, 2000). BLM is unique among the five human RecQ helicases in that it is able to process a DHJ with Topoisomerase IIIa (Topo IIIa) (Wu *et al.*, 2000). DNA helicases catalyze the unwinding of double-stranded DNA to provide single stranded templates in repair, recombination and transcription (Lohman *et al.*, 1996).

For preventing genome in-stability BLM induce DHJ branch migration as an anti-recombinase (Wu and Hickson, 2003; Karow, 2000). HRDC domain amino acid Lys1270 is possibly to play role in mediating interactions with DNA (Wu *et al.*, 2005). BLM HRDC bound to ssDNA N-terminal helix region and hydrophobic loop of 3_{10}-helix. It is observed that BLM HRDC domain had quite totally

different modes of binding with ssDNA and DHJ. On the basis of these finding, we propose that BLM HRDC involves in protein-DNA interactions. Lys1270 is located opposite site of main ssDNA-binding groove containing hydrophobic loop of 3_{10}-helix. So, Lys1270 is not involved in ssDNA binding but single point mutation of Lys1270 (K1270V) reduce the efficiency of DHJ DNA unwinding suggested that it have function in DHJ dissolution (Wu *et al.*, 2010).

A number of naturally occurring protein have been identified which selectively bind to G-Quadruplex, these include the helicases implicated in Bloom's and Werner's syndrome. Most of the direct and indirect evidence suggests that G-Quadruplex is also present in eukaryotic cells and tightly regulated to allow DNA replication and cell division (Huppert, 2008). G-Quadruplex ligands inhibit RecQ helicases activity in vitro in WRN and BLM (Li *et al.*, 2001). BLM interact with Topo IIIa and two other proteins with OB-fold domains, RMI1 and RMI2, to form a RTR complex (RecQ/Topo III/RMI) essential to maintenance of genome stability and function in the recombination intermediates (Li *et al.*, 2007; Liu Y and West, 2008). The Topo III/BLM complex functions as a repair complex in response to replication defects (Ababou *et al.*, 2000; Amor, 2006). Some evidence also suggested that Quadruplex formation plays a role in immunoglobulin heavy chain switching.

The G-Quadruplex ligands database (G4LDB, http://www.g4ldb.org) provides a unique collection of G-Quadruplex ligands facilitate the discovery the invention of novel therapeutic and diagnostic agents. G4LDB presently contains more than 800 G-Quadruplex ligands with ~4000 activity records (Qian *et al.*, 2012). Computer aided drug designing is a helpful tool in novel drug discovery and has been used to screen G-Quadruplex ligands (Cuesta *et al.*, 2003; Alcaro *et al.*, 2011). Variety of G-Quadruplex ligands have been discovered using molecular docking (Cosconati *et al.*, 2009; Alcaro *et al.*, 2012; Haider and Neidle, 2010; Redman *et al.*, 2009). Currently, 28 ligand/G-Quadruplex complex structures are included in the RCSB Protein Data Bank.

Materials and Methods

Ligand data collection and preparation in different supported file format by downloading 800 ligand data in mol file format from G4LDB and Convert ligand data format in sdf and mol2 by Open Babel GUI Software. Calculate different descriptor (HBA, HBD, log P, rotatable bond, M.W., and ring count for screening and Ligand Excel sheet data generated by Molsoft ICM-Browser-Pro software.

Virtual Screening

Pharmacokinetic properties absorption, distribution, metabolism, excretion and toxicology (ADMET) filter before activity-based screening has additionally been extremely recommended in virtual screening protocols. It has been means that of conserving time, and money. The following rules are drug-likeness rules provided in PreADMET for discriminating between non-drug and drug like compounds- Lipinski's Rule (No. hydrogen bond donors ≤ 5; No. hydrogen bond acceptor ≤10; Molecular weight ≤ 500 and CLogP ≤ 5) (Lipinski *et al.*, 1997). MDDR-Like Rule (Drug-like- No. Rings ≥ 3, No. Rigid bonds ≥ 18, No. Rotatable bonds ≥ 6; Nondrug-like-No. Rings ≤ 2, No. Rigid bonds ≤ 17, No. Rotatable bonds ≤ 5) (Oprea, 2000).

One proficient approach to drug discovery is virtual screening of molecule database for conducting virtual screening, use G-Quadruplex ligand database containing compounds. These compounds were first screened for drug like properties using Lipinski rule of 5 as filter 800 G-Quadruplex database ligands, remaining 251 compounds then passed the screening through MDDR like rule which filter 110 ligand molecule and then these ligand were subsequently analyzed for binding patterns using docking method by Molegro virtual docker (MVD), which generate 48 poses of ligands. Now these pose are further use for lead compound identification and refinement of different interactions. The flowchart Shown in (Fig. 1) is a schematic illustration of the successive virtual screening method with the number of hits reduced for every screening step.

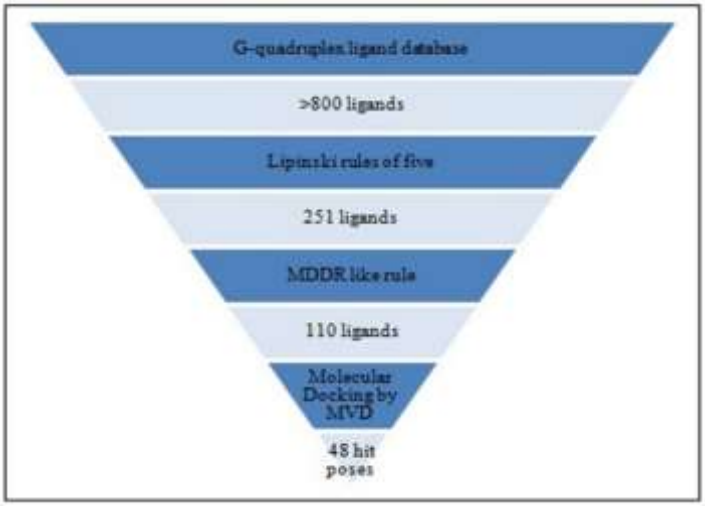


Fig. 1: Steps to followed in virtual screening study of BLM protein.

Docking

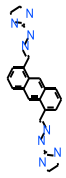
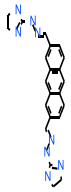
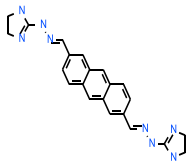
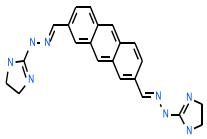
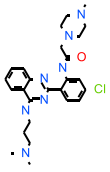
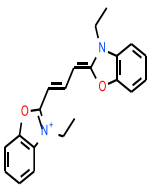
Dock target identification and scoring potential complementary binding sites are widely used in hit identification and lead optimisation. Indeed, there are number of drugs whose development was heavily influenced by structure-based design and screening strategies (Klebe and Rarey, 1996). Hydrogen-bond donors and acceptors, as well as hydrophobic groups are interacting groups at target binding site (Linnainmaa *et al.*, 1988).

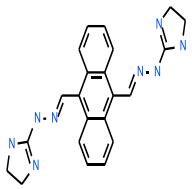
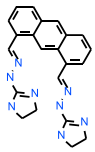
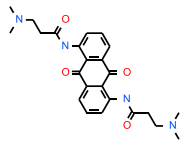
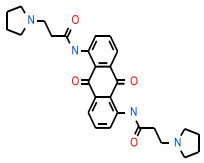
Results and Discussion

Ligand virtual screening

Virtual screening performed on the G-Quadruplex ligand database obtained new inhibitors that have the potential to inhibit BLM. The hit compounds were later on subsequently subjected to molecular docking and evaluated by consensus scoring function that resulted in 48 hit poses by MVD Virtual screening mode option for top-ranked compound finding. Since the set of top-ranked poses found are updated dynamically during the virtual screening run more than the specified percentage of poses will be returned. (Table 1) shows 10 virtual screening ligand data that have hydrogen bonding interaction with targeted binding site

Table 1: Virtual Screening ADME Data for Final 10 Ligand Poses, Which Shows Interaction With Target Site.

Ligand molecule	Name	ADME Weight	ADME H-bond Acceptors	ADME H-bond Donors	ADME log P	ADME Rotatable Bonds	6-Member rings	5-Member rings
	G4L1534	398.520	4	4	0	8	3	2
	G4L1530	398.520	4	4	0	8	3	2
	G4L1537	398.520	4	4	0	8	3	2
	G4L1538	398.520	4	4	0	8	3	2
	G4L6311	496.120	6	2	2.5691	9	4	0
	G4L0178	333.440	2	0	4.3398	6	2	2

	G4L1532	398.520	4	4	0	8	3	2
	G4L1535	398.520	4	4	0	8	3	2
	G4L0031	436.560	6	2	0.2364	8	3	0
	G4L0029	488.640	6	2	0.8876	8	3	2

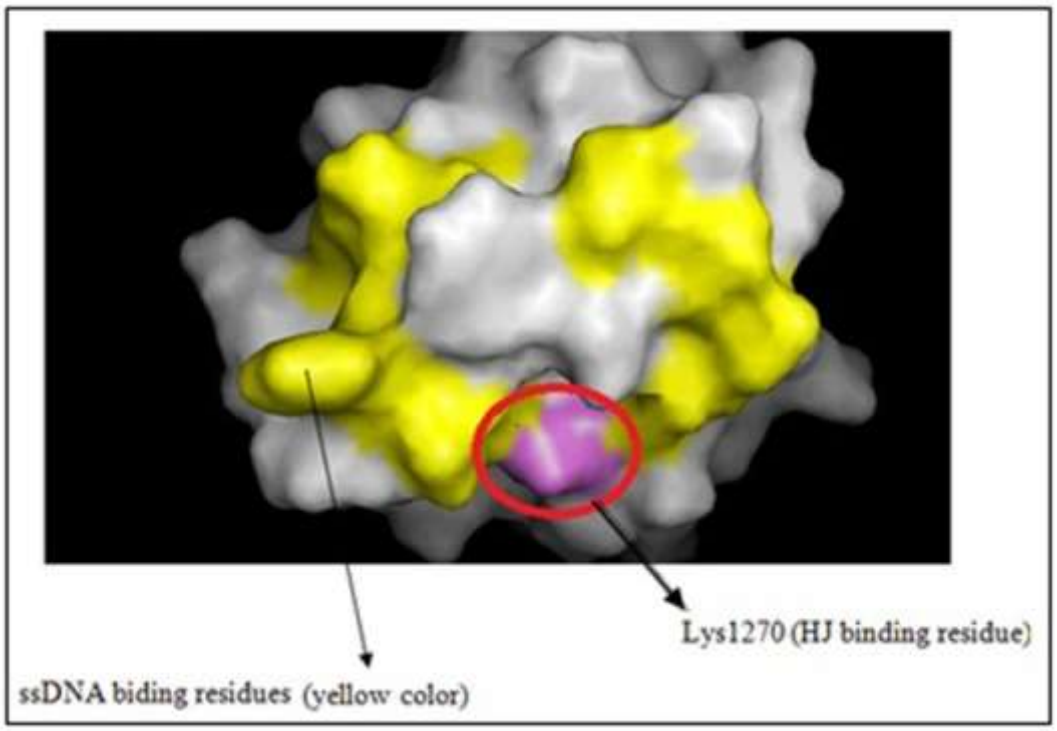


Fig 2: Target binding site on 2RRD protein shows position of Lys1270 and ssDNA biding residues in yellow colour.

Docking

Docking of ligand performed at Lys76 residue in 2RRD BLM HRDC, with position of centre coordinate X -13.22, Y-6.25, Z-8.25. The docking method involves the prediction of ligand poses and their conformation within a targeted binding site as shown in (Fig. 2 and 3) and there docking output data are shown in (Table 2).

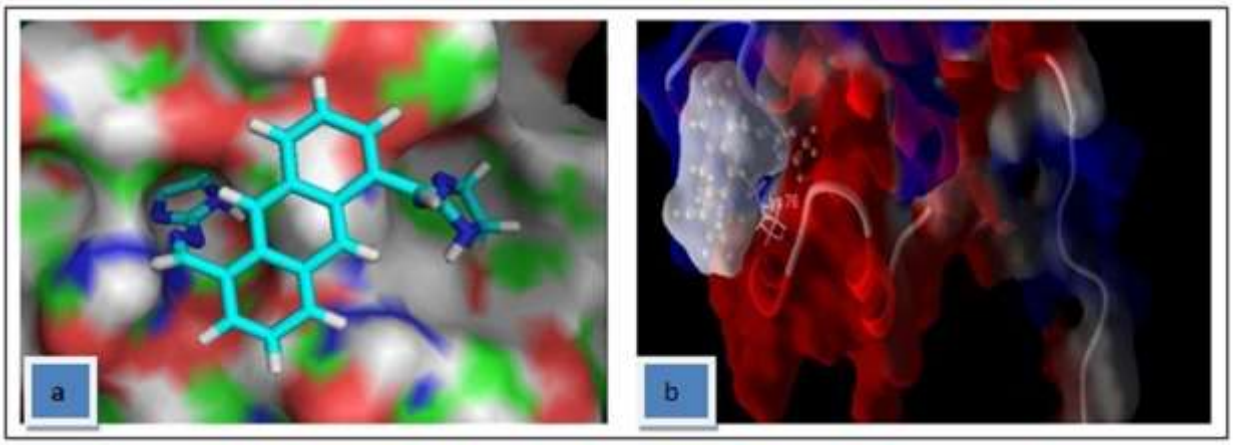


Fig. 3: G4L1534 ligand binding to the Lys 76 residue of 2RRD rendered by PyMol software (Delano Scientific, San Carlos, CA, 2002). (a) Docking complex G4L1534 represented by stick model. The target receptor residues are colour according to surface properties. (b) Human BLM HRDC K1270 (Lys 76 in 2RRD) Holiday junction binding residues for homologous recombination docked by ligand surface view.

Table 2: Docking Output Data Table Showing Different Scoring Functions of Protein-Ligand Interaction, Hydrogen Bond, Electrostatic Interaction and Energy Calculation Data

Ligand	E-Inter (protein-ligand)	E-Intra (tors, ligand atoms)	E-Intra (vdw)	Energy or E-total	Electro	H Bond	Pose Energy	Rerank Score	Steric
G4L1534	-120.66	6.6199	80.2491	-114.04	0	-9.5209	-119.39	-44.153	-111.14
G4L1530	-119.58	3.0462	65.6403	-116.54	0	-10.298	-121.59	-87.762	-109.28
G4L1537	-118.02	2.9508	62.0813	-115.07	0	-8.5365	-116.07	-89.799	-109.48
G4L1538	-113.97	5.3090	63.6355	-108.66	0	-5.8309	-113.93	-84.865	-108.14
G4L6311	-99.645	-1.562	106.597	-101.21	0	-2.6983	-103.18	-34.052	-89.694
G4L0178	-92.421	-0.346	57.549	-92.767	0	-1.4568	-92.242	-71.481	-90.964
G4L1532	-100.01	3.8793	81.8397	-96.133	0	-4.9318	-106.12	-72.555	-95.08
G4L1535	-98.551	-8.336	78.4188	-106.89	0	-6.3936	-115.92	-56.272	-92.158
G4L0031	-108.12	39.891	107.532	-68.230	-6.693	-1.055	-66.877	-68.149	-102.27
G4L0029	-103.27	32.769	101.427	-70.504	0	-3.8161	-71.922	-66.477	-99.457

Ligand energy inspection

Ligand energy inspection final data for target Lys76 (on BLM 2RRD protein) for hydrogen bond and electrostatic interaction analysis shown in (Fig. 4) and their energy score data shown in (Table 3).

Protein-ligand interaction

All hydrogen bond and strong electrostatic interactions between the ligand and the target atoms are visualized as dashed and strong electrostatic interactions are visualized as partial spheres oriented in the direction of the interaction represented in (Fig. 5). Green partial spheres correspond to favourable interactions, while yellow spheres correspond to non favourable interactions.

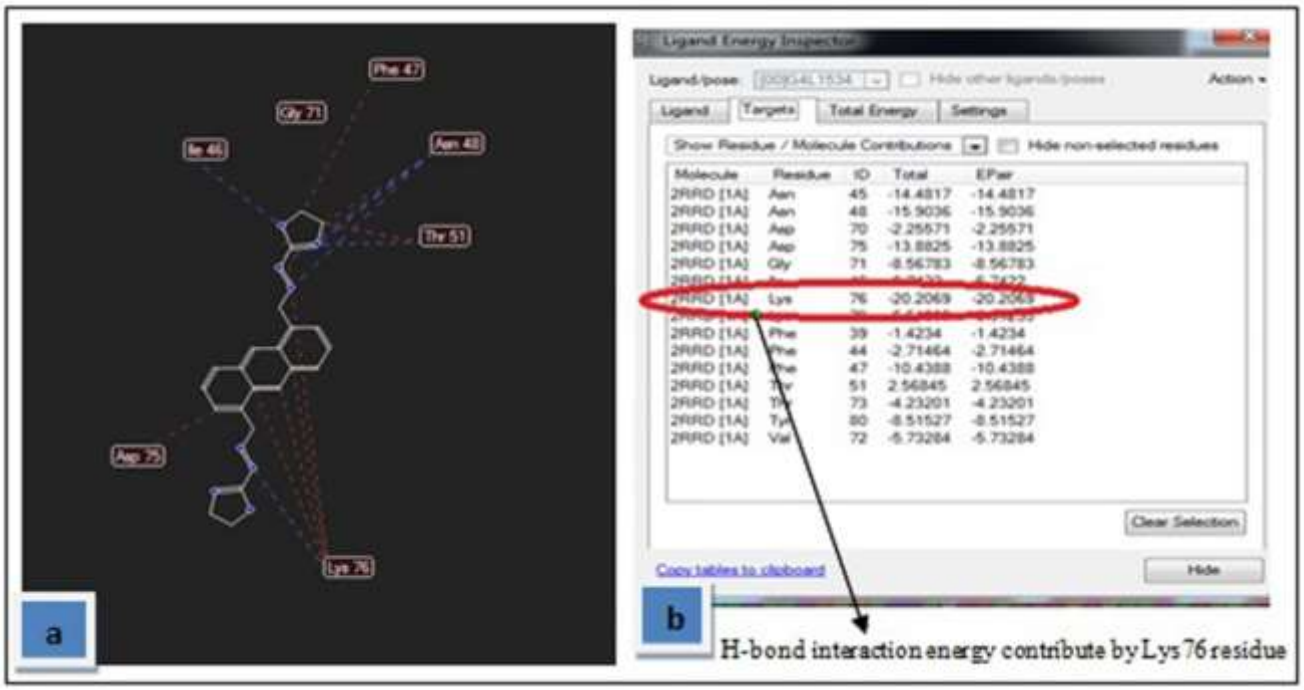


Fig. 4: The docked ligands are shown with their hydrogen bond interactions: **(a)** Lys 76 (dark blue dotted lines), electrostatic, steric interaction shown in red dotted lines and other residues (Asp 75, lle 46, Gly71, phe 47 Asn 48 and Thr 51). **(b)** Hydrogen bonding energy contributed by Lys76 residue between 2RRD receptor and G4L1534 ligand.

Table 3: Ligand Energy Inspection Final Data for Target Lys76 (On BLM 2RRD Protein) for Hydrogen Bond and Electrostatic Interaction Analysis.

Ligand	Total	EPair	EElect
G4L1534	-20.2069	-20.2069	0
G4L1530	-19.2361	-19.2361	0
G4L1537	-18.4112	-18.4112	0
G4L1538	-17.9301	-17.9301	0
G4L6311	-16.3808	-17.105	0.724154
G4L0178	-16.2341	-16.2341	0
G4L1532	-14.0275	-14.0275	0
G4L1535	-13.5519	-13.5519	0
G4L0031	-11.84	-16.4817	4.64168
G4L0029	-11.7377	-11.7377	0

EPair: Hydrogen bonding energy calculation between receptor and ligand atom by pair wise steric method, **EElec:** Pair wise electrostatic interactions.

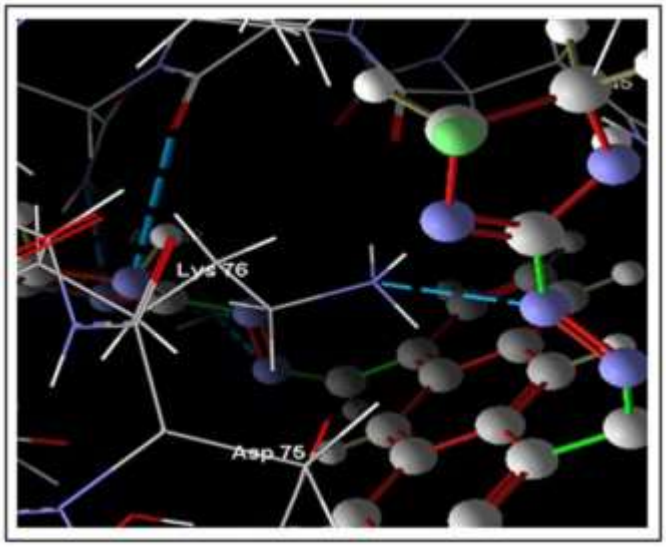


Fig. 5: BLM HRDC domain structure showing hydrogen bond interaction between targeted Lys76 (BLM K1270) residue and docked ligand G4L1534.

Conclusion

The Virtual ligand screening will be helpful to predict new candidate molecules and Ligand finding can give best drug likeness compound. These predicted 10 ligand compounds showed interactions with important residue Lys1270 at the binding site. HRDC domain amino acid Lys1270 is presumably to play role in mediating interactions with DNA. BLM role in controlling re-combination by interact with one topoisomerase III isoform, TOPO III. BLM HRDC domain had quite different modes of binding with ssDNA and HJ. On the premise of these finding, we have a tendency to propose that BLM HRDC involves in protein-DNA interactions. Lys1270 is not concerned in ssDNA binding however single point mutation of Lys1270 (K1270V) reduce the efficiency of HJ DNA unwinding suggested that it have function in HJ dissolution. Different 10 docking pose generated by MVD show interaction with target site, from that G4L1534 show higher hydrogen bond energy -20.2069 with -114.037 E-total energy and another pose G4L1530 shows H-bond energy -19.2361 with -116.535 E-total energy. These protein-ligand interaction study recommend that ligands have known repressive function to make potential lead drug molecule.

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