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A facile *in silico* drug design strategy based on reference listed drugs and computational modeling of novel anticancer therapeutics

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ABSTRACT: Heterocyclic compounds present unique structural and physicochemical diversity. Especially, the aromatic heterocycliccompounds like indole exhibit anti-cancer properties by facilitating cancerous cell death. Drug discovery and development process relyto a large extent on the *in silico* identification of the putative targets and rational design of potentially therapeutic ligands. We propose a facile strategy of *in silico* drug designing hereby curating libraries having a functional group modification of the indole heterocyclic compounds with 2-chloro-N-(2-chloroethyl)-N-methylethanamine. Subsequently, we compare the designed drugs with the existing alkylating Reference Listed Drugs (RLDs) of the USFDA. We computationally model the indole ring as a basic scaffold and induce 2-chloro-N-(2-chloroethyl)-N-methylethanamine substitution on the C-3 of indole with experimentally available target DNA receptor to design an extensive library of 200 molecules. This was followed by extensive ligand-DNA docking studies to predict putative targets andADMET prediction of an optimized ligand. Our simple *in silico* strategy reveals that the designed compounds such as AGSPBM134, AGSPBM133, AGSPBM131, AGSPBM130, AGSPBM132 and AGSPBM019 exhibitstructural similarity towards the RLD as shown by ECFP-6 fingerprints. We show that they pass all the similarity criteria of the physicochemical parameters with no violation of the Lipinski's rule of five. We positthat these agents can potentially be a good choice for further synthesis in the development of novel anti-cancer agents.

KEYWORDS: Indole; drug designing; molecular docking; computer-aided drug designing; ADMET.

1. INTRODUCTION

Cancer remains the leading cause of premature death across the globe. In the US, it is the second most common cause of mortality amongst individuals. As per the 2019 report fromthe American Cancer Society, roughly 1,762,450 new cancer cases and 606,880 deaths due to cancerare expected in the coming years in the US alone [1]. By the year 2030, it is projected that there will be ~26 million new cancer cases and ~17 million cancer deaths per year [2]. International agency for research on cancer in 2018 (GLOBOCAN 2018) reported 2,129,118, 210,537 and 1,157,294 cases in the USA, Turkey, and India respectively, with breast cancer being the predominant cancer type [3]. More than hundred distinct cancer kinds are currently identified: the carcinomas contribute ~90%, Leukemias and Lymphomas occur in ~8% of cases, while Sarcomas appear rarely [4]. Early diagnosis is a crucial factor in improving the outcomes of cancer patients [5]. Anticancer drugs therapy is generally classified into chemotherapy, hormonal therapy, and immunotherapy. Alkylating agents are drugs that are the oldest form of chemotherapy and were the first compounds to be identified asbeneficial in the treatment of cancer [6]. These anticancer agents hold an important position in the World Health Organization's list of Essential Medicines for the treatment of cancer [7]. They are cytotoxic in nature and is known to actby threedifferent mechanisms:(1)Theycan form covalent cross-bridges between nucleotides in DNA. Cross-linking helps resists DNA synthesis by inhibiting DNA strand separation for transcription. The alkylating agents bind preferentially at the N-7 position of guanine producing inter-strand with two different molecules while forming adducts and inhibiting DNA synthesis;(2)Alkylating drugs may also induce mutations due to

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mispairing of the nucleotides;(3) Alkyl group of drugs may also attach with DNA bases facilitate DNA fragmentation[8].

The 2-chloro-N-(2-chloroethyl)-N-methylethanamine is fatty nitrogen mustard first approved by USFDA and is now rarely used as an alkylating agent because of its poor selectivity.This led to the development of aromatic alkylating agents [9]. Chlorambucil, Cyclophosphamide, Melphalan HCl, Ifosfamide and Bendamustine HCl are few examples of USFDA approved nitrogen mustard derivatives having the 2-chloro-N-(2-chloroethyl)-N-methylethanamine side chain [10-11]. On the other hand, heterocyclic compounds have versatile, but unique physicochemical properties, and are the active component of widely marketed anticancer drugs [12]. Among the current USFDA approved drugs, many heterocyclic anticancer drugs are available, including doxorubicin (Adriamycin®)[13], daunorubicin (Cerubidine®)[14], 5-fluorouracil (Efudix®)[15], *etc.*Few natural product alkylating agents like vinblastine and vincristine are also commonly used for treatment [16]. Recently, heterocyclic compounds having indole moiety have been shown to exhibitpromising anti-cancer potential [17]. Also, indole is a common pharmacophore for a large number of naturally occurring biomolecular compounds [18]. Naturally occurring Vinca alkaloids present indole moietyhaving anticancer activity.Several *in silico* drug design techniques including molecule docking by Glide[19], fingerprint analysis by Canvas[20], Cresset TorchLite for physicochemical descriptors[21], ADMET by QikProp [22] and Multivariate analysis [23] are used effectively to design and develop the *in silico* drug development successfully. Earlier studies show that the combination of nitrogen mustard with other natural scaffolds produces more effective anti-cancer agents with low side-effects. A novel nitrogen mustard-based hybrid – evodiamine have been recently reported to have anti-proliferative activity [24]. An alkylating agent was used in conjugation with natural alkaloid to give special chemical scaffold for enhancing activity and reducing the side effect of nitrogen mustards. Also, steroid-linked nitrogen mustard with an affinity for its receptor was successfully synthesized and can lead to highly selective and less toxic antineoplastic therapeutics [25]. Chlorambucil is a nitrogen mustard derivative combined with estradiol to get an estradiol-chlorambucil hybrid for the treatment of breast cancer [26]. Some acridine-linked aniline mustards synthesized successfully for anticancer activity on the basis of DNA cross-linking mechanism[27].

In the current study, we employ the indole heterocyclic moiety as a potential pharmacophore after extensively perusingall heterocyclicanticancer compounds [28].We design a library of the hybrid indole pharmacophore with 2-chloro-N-(2-chloroethyl)-N-methylethanamine derivatives as potentially promising anti-cancer agents for therapeutic intervention. We further subjectthe designed molecules to *in silico* computational studies with the USFDA approved alkylating agents to find the sameness characterization.DNA is the crucial target for treatment in multiple pathophysiological conditions [29]. DNA-Intercalators [30] are used as anticancer agents. DNA acts as a target for varied heterocyclic compounds other than indole which were reported previously as DNA groove binders and alkylating agents [31-33]. The scope of the current work further extends to the computational studies of these designed indole alkylating ligands on DNA as a target, to understand the DNA-intercalation with respect to USFDA Reference Listed drugs.

2. RESULTS AND DISCUSSION

In the present study,200 compounds molecular library designed with constant bis(2-chloroethyl)amine functional group at the 3rd position of an indole. Other position of substituted with a different functional group. Designed compounds and RLD arranged as per radial plot of physicochemical descriptors and selected for docking study. To understand the structural basis of the designed ligands binding to target each ligand and 7 USFDA approved Reference Listed Drugs (RLDs) were individually docked with the receptors at their active site using Glide program. The target DNA structure obtained from PDB (PDB ID: 1AXL) Solution NMR.The docking results revealed that all the designed compounds were energetically favorable in terms of Glide dock score (see Table 1). The results were described in the terms of Docking score, Glide evdw, Glide ecoul Glide Energy, Glide Emodel. The Glide docking score of the designed compounds was in the range from -8.93 to -7.971, where designed compound AGSPBM131 showed the lowest binding energy with -8.93 compared with Bendamustine RLD -6.232.

All the designed molecules and RLDs were docked in the DNA active site (see Figure 1). The designed indole derivatives showed a considerable affinity to the binding site. The similarity of designed indole derivatives was calculated with respect to the RLDs by using ECFP-6 fingerprints [34-35] (see Table 2). The generated results were rationally analyzed using Molecular Visualization Tool, Pymol and Discovery Studio [36-37].

Table 1. Docking results of RLD and the designed molecules reveal that the designed indole derivatives compounds show Glide dock scores better than the RLD molecules.

Type	Comp. No.	Docking Score	Glide evdw	Glide ecoul	Glide Energy	Glide Emodel
R	Bendamustine	-6.232	-32.118	-7.219	-39.337	-52.984
R	Melphalan	-5.959	-26.339	-4.811	-31.150	-43.643
R	Chlorambucil	-4.743	-30.510	-0.469	-30.979	-40.905
R	Cyclophosphamide	-4.167	-26.230	-5.41	-31.639	-36.979
R	Mechloarmethamine	-3.720	-18.615	-10.416	-29.0301	-35.319
R	Ifofsamide	-3.555	-20.339	-6.413	-26.752	-30.904
T	AGSPBM131	-8.930	-36.602	-16.342	-52.943	-74.391
T	AGSPBM130	-8.612	-35.264	-21.087	-56.351	-81.715
T	AGSPBM134	-8.542	-37.635	-22.124	-59.759	-86.579
T	AGSPBM133	-8.314	-37.278	-11.891	-49.169	-59.939
T	AGSPBM132	-8.270	-35.673	-11.642	-47.315	-64.894
T	AGSPBM019	-8.232	-28.565	-22.087	-50.652	-63.416
T	AGSPBM054	-8.207	-34.944	-18.763	-53.707	-75.144
T	AGSPBM022	-8.184	-31.447	-16.101	-47.549	-61.046
T	AGSPBM138	-8.117	-33.25	-17.107	-50.357	-67.871
T	AGSPBM055	-7.971	-36.622	-12.589	-49.21	-64.384

During similarity searching, Reference Listed Drugs are compared to designed compounds. Thus, similarity searching can be mimicked by systematically comparing different RLD having the same activity with each other and RLD to the designed library of indole derivatives. ECFP-6 fingerprint similarity score (see Table 2) for bendamustine found 74, for designed AGSPBM134, AGSPBM133 is 67 and for AGSPBM131, AGSPBM130 found 63 respectively. TPSA (Topological Polar Surface Area) givesoverall polar atoms or molecules, primarily oxygen and nitrogen, also including their attached hydrogen atoms. TPSA of designed representative AGSPBM131and reference Bendamustine molecule (see Table 2) found 68.9 and 58.4 are comparable.

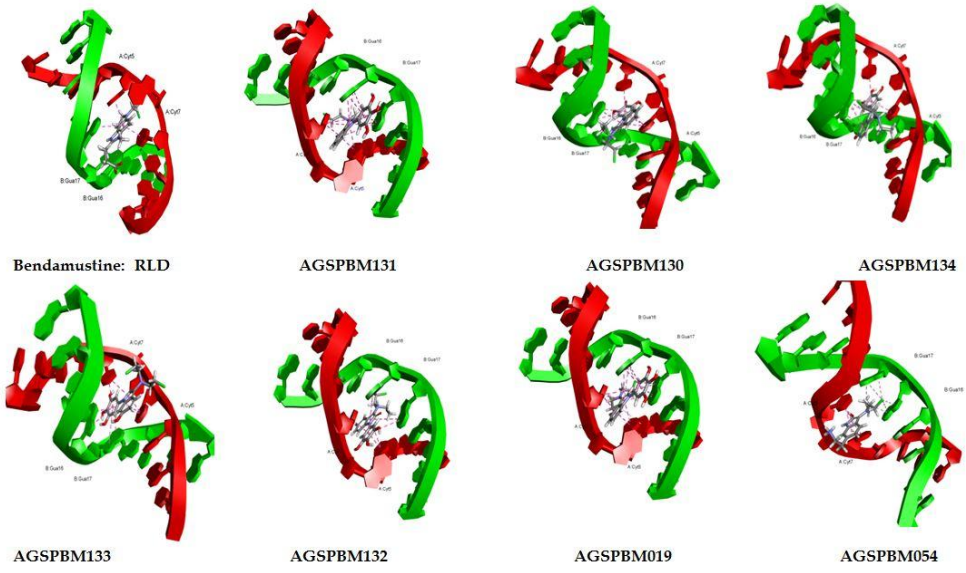
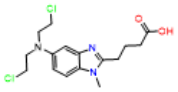
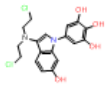
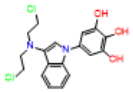
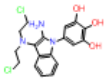
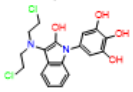
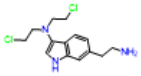
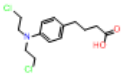
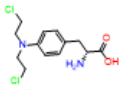
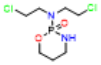
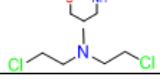


Figure 1. Glide docking representative poses of Bendamustine RLD and designed indole derivatives.

Table 2. Comparison of the Bendamustine and designed molecules by ECFP-6 fingerprint similarity, TPSA, MR and molecular weight.

Name	Type	Structure	Name	Chemical Formula	Mol weight	ECFP-6 fingerprint similarity	TPSA	MR
1	R		Bendamustine	$C_{16}H_{21}Cl_2N_3O_2$	358.26	74	58.4	95.6
2	T		AGSPBM134	$C_{18}H_{18}Cl_2N_2O_4$	397.25	67	89.1	104.8
3	T		AGSPBM133	$C_{18}H_{18}Cl_2N_2O_5$	413.25	67	109.3	106.8
4	T		AGSPBM131	$C_{18}H_{18}Cl_2N_2O_3$	381.25	63	68.9	102.8
5	T		AGSPBM130	$C_{18}H_{19}Cl_2N_3O_3$	396.27	63	94.9	107.2
6	T		AGSPBM132	$C_{18}H_{18}Cl_2N_2O_4$	397.25	63	89.1	104.8
7	T		AGSPBM019	$C_{14}H_{19}Cl_2N_3$	300.23	57	45.1	84.2
8	R		Chlorambucil	$C_{14}H_{19}Cl_2NO_2$	304.21	48	40.5	81.0
9	R		Melphalan	$C_{13}H_{18}Cl_2N_2O_2$	305.20	46	66.6	78.9
10	T		AGSPBM010	$C_{13}H_{16}Cl_2N_2$	271.19	45	8.2	76.6
11	R		Ifosfamide	$C_7H_{15}Cl_2N_2O_2P$	261.09	37	51.4	62.6
12	R		Cyclophosphamide	$C_7H_{15}Cl_2N_2O_2P$	261.09	29	51.4	62.6
13	R		Mechloarmethamine	$C_5H_{11}Cl_2N$	156.05	14	3.2	38.6

After designing the molecules, we have evaluated ADME calculation by using alkylating agents drug as RLD for the assessment of drug-likeness as well as pharmacokinetic properties. A computational study for prediction of ADME properties by using QikProp (Schrödinger, LLC, New York, NY) software. The CNS activity, human oral absorption, Predicted aqueous solubility (QPlogS), Predicted IC50 value for the blockage of HERG K+ channels (QPlogHERG) and Prediction of binding to human serum albumin (QplogKhsa). The comparison of ADME descriptor value presented for Reference and designed molecule (See Table 3). All the value of designed molecules is close to RLD showing druglike properties.

Compound AGSPBM131 is very similar to Bendamustine RLD in permeability and enzymatic activity. Both reference and designed molecules show BBB permeability, CYP2C9 inhibitor and CYP3A4 inhibitor activity (See Table 4**).** Compound AGSPBM022 showing 0.36 mg/mL estimated aqueous solubility which is better than Bendamustine 0.09 mg/mL.

The compound AGSPBM131 and AGSPBM130 among all designed molecules show good drug candidate compared to the RLD and follow the criteria for injectable preparation. Hence these compounds may have a good potential for eventual development as the injectable dosage form.

Table 3. ADME properties of RLD and selected the top 10 designed molecules.

Compound	CNS activity[a]	QPlogPo/w	QPlogS	QPlogHERG	QPlogHERG QPPCaco	QplogKhsab[b]	Human oral Absorption[c] (%)
Bendamustine	-1	4.752	-5.236	-3.295	143.035	0.354	93.3
Melphalan	-1	1.021	-2.949	-3.536	24.332	-0.139	57.7
Chlorambucil	-1	4.688	-4.537	-3.033	224.583	0.289	96.5
Cyclophosphamide	0	1.022	-1.368	-3.776	4267.557	-0.931	100.0
Mechloarmethamine	2	2.002	-0.411	-3.830	2377.947	-0.373	100.0
Ifofsamide	0	0.983	-1.368	-3.394	4047.810	-0.926	100.0
AGSPBM131	0	3.543	-5.257	-5.919	96.974	0.512	83.3
AGSPBM130	-1	2.845	-5.089	-6.05	55.137	0.207	74.8
AGSPBM134	-2	2.761	-5.175	-5.799	29.777	0.306	69.5
AGSPBM133	-2	2.594	-5.345	-5.958	16.113	0.225	63.7
AGSPBM132	-1	3.374	-5.43	-6.072	52.568	0.432	77.5
AGSPBM019	2	5.528	-5.472	-6.217	1337.764	1.072	100.0
AGSPBM054	0	1.561	-1.692	-7.211	7.964	0.086	52.2
AGSPBM022	2	2.821	-2.629	-5.981	81.062	0.280	77.6
AGSPBM138	0	2.582	-3.159	-6.12	24.772	0.220	67.0
AGSPBM055	1	2.185	-1.952	-7.141	14.813	0.293	60.7

[a] Predicted central nervous system activity on a − 2 (inactive) to + 2 (active) scale; [b] Prediction of binding to human serum albumin (acceptable range − 1.5 to 1.5); [c] Predicted human oral absorption on 0–100% scale (> 80% is high and < 25% is poor).

Table 4. SwissADME result for solubility, permeability and enzyme activity.

Name	type	ESOL Solubility[a] (mg/ml)	BBB permeant [b]	Pgp substrate[c]	CYP[d]1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Bendamustine	R	0.09	Yes	No	No	Yes	No	Yes	No
AGSPBM131	T	0.01	Yes	No	Yes	Yes	No	Yes	Yes
AGSPBM130	T	0.01	No	No	Yes	No	No	Yes	No
AGSPBM134	T	0.01	No	No	Yes	No	No	Yes	No
AGSPBM133	T	0.01	No	No	Yes	No	No	Yes	No
AGSPBM132	T	0.01	No	No	Yes	No	No	Yes	No
AGSPBM019	T	0.16	Yes	No	Yes	Yes	No	Yes	No
AGSPBM054	T	0.05	Yes	Yes	No	Yes	No	Yes	No
AGSPBM022	T	0.36	Yes	Yes	Yes	No	No	Yes	No
AGSPBM138	T	0.22	Yes	Yes	Yes	No	No	Yes	No
AGSPBM055	T	0.15	Yes	Yes	No	No	No	Yes	No

[a] ESOL: estimating aqueous solubility; [b] BBB: blood-brain barrier; [c] Pgp: P-glycoprotein; [d] CYP: Cytochrome P450.

3. CONCLUSION

In silico modeling can be effectively utilized and explored for drug designing and simulations that may help the medicinal chemists and drug discovery scientists to design drug-like candidates. These candidate ligands identified through *in silico* studies can then be essentially studied for their efficacy using relevant *in vitro* and *in vivo* models.

The present research work was planned to design novel moieties derived from the indole scaffold combined with the alkylating agents to aid them as having anti-cancer potential. It was observed that the binding activity in DNA was not affected by the electronic properties. Indole analogs contain functional groups such as -OH and -NH_2 exhibited higher docking score when compared to RLDs. It was observed that the compounds such as AGSPBM131, AGSPBM130, and AGSPBM134 exhibited a Glide XP docking score of -8.930, -8.612 & -8.542, respectively, which is significantly higher than the score (-6.232) for RLD Bendamustine Hydrochloride drug. Furthermore, the ADME parameters were calculated by the QikProp of the complete library. These parameters give the best choice for the preparation of new hybrid indole derivatives as anticancer agents in future with more improved potency. These newly designed indole derivatives can thus act as the starting point for the design and development of a new class of indole-alkylating agent with improved safety, efficacy and bioavailability profile. This can drive the medicinal chemists to explore this pharmacophore for the design of a more potent agent for the treatment of cancer when compared to existing RLDs.

Based on thorough literature survey and to the best of our knowledge, this is the first report of modification on the indole pharmacophore to obtain a novel library of indole-alkylating agents with the fixed 2-chloro-N-(2-chloroethyl)-N-methylethanamine side chain at the C-3 position of the indole nucleus with appropriate functional group modifications at other positions on the pharmacophore.

4. MATERIALS AND METHODS

4.1. Chemistry

Indole nucleus is a naturally occurring popular aromatic heterocycle having very unique properties of binding towards enzymes, proteins, etc., and its binding is reversible in nature. Indole prefers to react mainly through the 3-position by electrophilic substitution. Figure 2A shows 3-substituted scaffold of indole taken for the study is represented and electron distribution compared on Figure 2B, indole and Figure 2C, substituted indole [38-40].

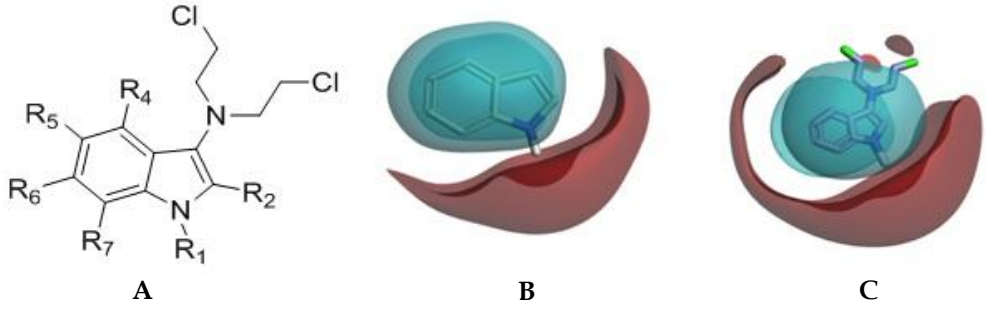


Figure 2. **(A)** Basic scaffold for study. **(B)** Electron distribution on indole **(C)** Electron distribution on 3-substituted indole.

4.2. Designing of library and screening

In drug designing strategies, the molecular features of designed molecules determine their physicochemical properties. The functional groups were selected as per the Structure-Activity Relationship (SAR) of the reported anti-cancer agents available in the market through systematic literature survey [41]. A library of 200 indole compounds was designed and screened using the TorchLite 10.5.0. software [42], which is an easy-to-use interface for a ligand-based application that works on the 3D topology of molecules, sorts and classifies the molecules based on their electrostatic properties and shape similarities. It arranges the designed compounds based on their physicochemical properties using the multi-parametric scoring function to prioritize compounds. The eXtened Electron Distribution (XED) gives the electrostatic interaction pattern shown in Figure 3 of molecules which can be effectively analyzed to compare and contrast reference and designed molecules.

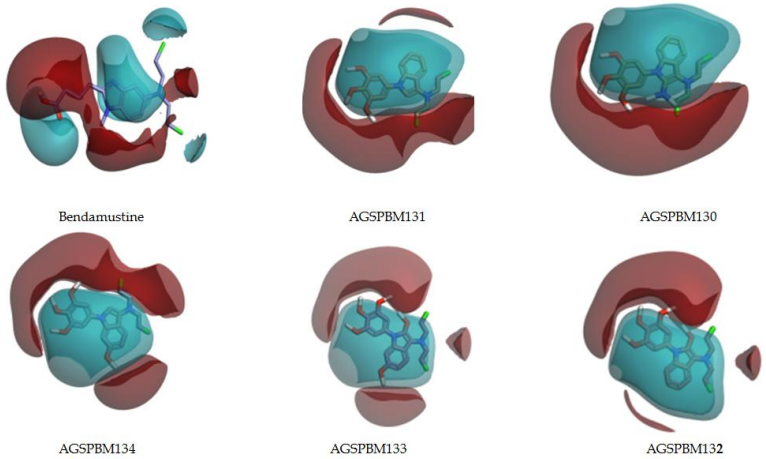


Figure 3. Comparison of eXtened Electron distribution between Bendamustine RLD and designed AGSPBM131, AGSPBM130, AGSPBM134, AGSPBM133, AGSPBM132 molecules.

Field properties give an actual 'personality ' to the molecules in term of the full range of interactions with the target DNA. The protein can interact with a ligand in terms of hydrogen bond (H-bond) donor, H-bond acceptor, hydrophobic, steric and van der Waals (vdW) attractive forces. The explanatory field point comparison of the reference and the designed molecule is shownin Figure 4.

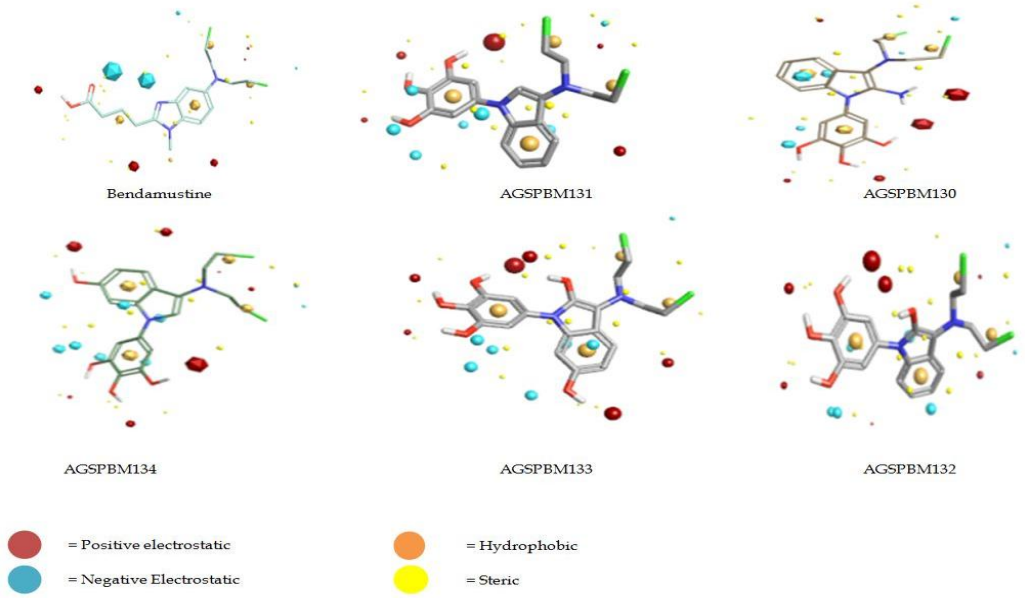


Figure 4. Fields relating to the structure, properties, and activity of reference and test molecules containing H-bond donor, H-bond acceptor, hydrophobic, steric vdW attractive interactions.

4.3. Cresset TorchLite and MVA analysis

The physicochemical parameters established for the designed and reference ligands by Cresset TorchLite. Based on electrostatics, physicochemical descriptors (molecular weights, atoms, 2D similarity, SLogP, TPSA, etc.), the shape of substructures and radial plot molecules were aligned. The default conformation hunt and alignment values were selected and alignment generation was initiated. After alignment, the data generated included calculated physicochemical properties, molecule role. Molecules were arranged based on the radial plot and on the scatter plot shown in Figure 5.

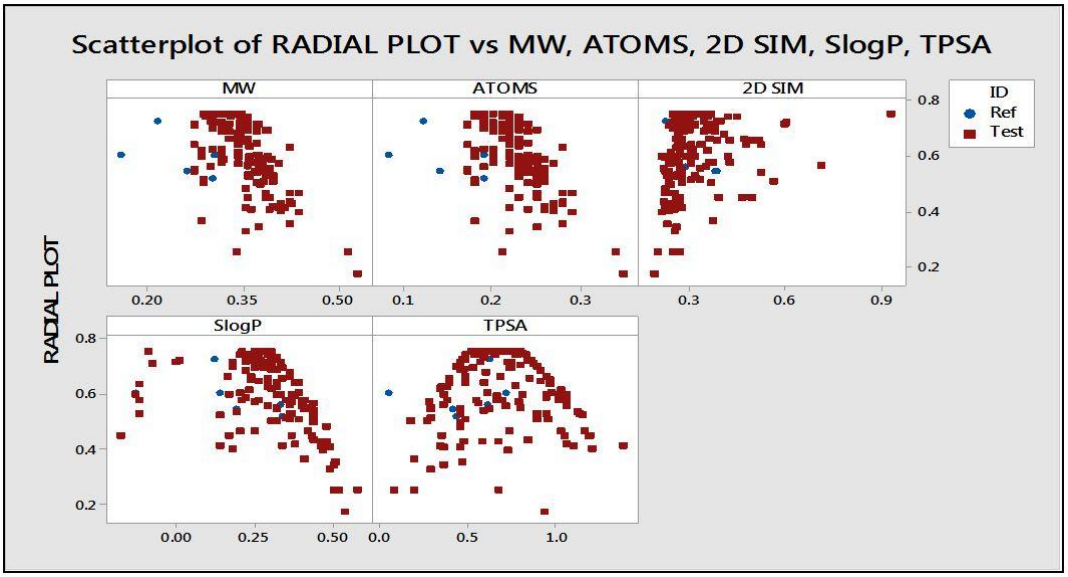


Figure 5. The scattered plot of the radial plot (representative value of molecule) vs. descriptor for 6 references and 200 designed molecules to choose the maximum population close to the exact reference listed drug.

The designed ligands were aligned and compared with reference molecules. Multivariate analysis performed for radial plot descriptors of reference and designed molecules and shown in Figure 6.

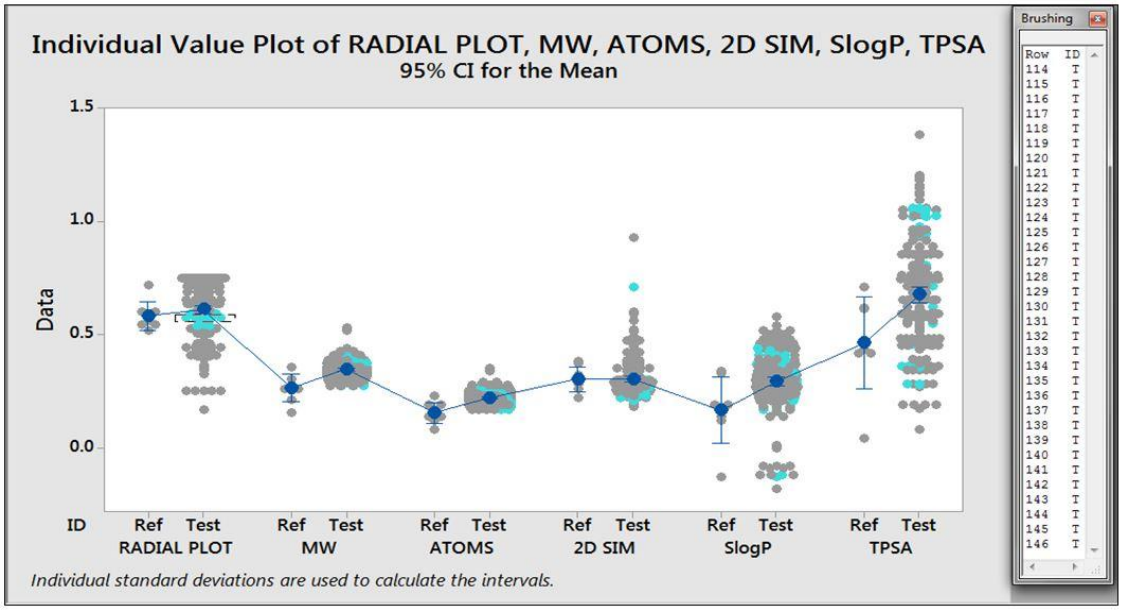


Figure 6. The standard deviation of individual molecules plotted against descriptor like Radial plot, Molecular weight, Atoms, 2D similarity, sLogP, TPSA (Topological polar surface area) with 95 % CI of the mean.

4.4. Reference drugs

Bendamustine, Carmustine, Chlorambucil, Cyclophosphamide, Ifosfamide, Mechlorethamine, and Melphalan are the USFDA approved nitrogen mustard drugs that were selected as the reference ligands for evaluating the activity of the designed 3 substituted indole alkylating agents [43].

4.5. Ligand preparation

The 2D structures of the designed Indole alkylating agent derivatives were drawn by using ChemDraw Ultra 12.0 (PerkinElmer Informatics) and stored in a library in sdf structure format. All the 2D sdf structures were converted to the 3D structure by using the 3D optimization tool i.e., Ligprep of the Schrodinger suit. The drawn ligands were optimized for geometry by using the OPLS-2005 (Optimized Potentials for Liquid Simulations) force field with the Steepest Descent method followed by the truncated Newton Conjugate gradient protocol. All structures with Default settings were processed for proper chirality and low energy in 3D form. Furthermore, the extra precision (XP) was done for the processed ligands by using the Glide module of the Schrödinger suite [44].

4.6. Protein selection/preparation

In structural biology, biomolecular docking (DNA-Ligand docking) is becoming a popular approach to understand the biomolecular interactions responsible for affinity to the target. In this study, we selected the DNA target (PDB ID: 1AXL) from the Protein Data Bank [45]. The solution NMR structure of DNA was extracted from the protein data bank(https://www.rcsb.org/structure/1axl). The selected target was the solution conformation of the (-)-trans-anti-[BP] DG adduct opposite to a deletion site in the DNA duplex D(CCATC-[BP]G-CTACC)D(GGTAG—GATGG, which was considered for docking study. The 1AXL DNA structure is as shown in Figure 7.

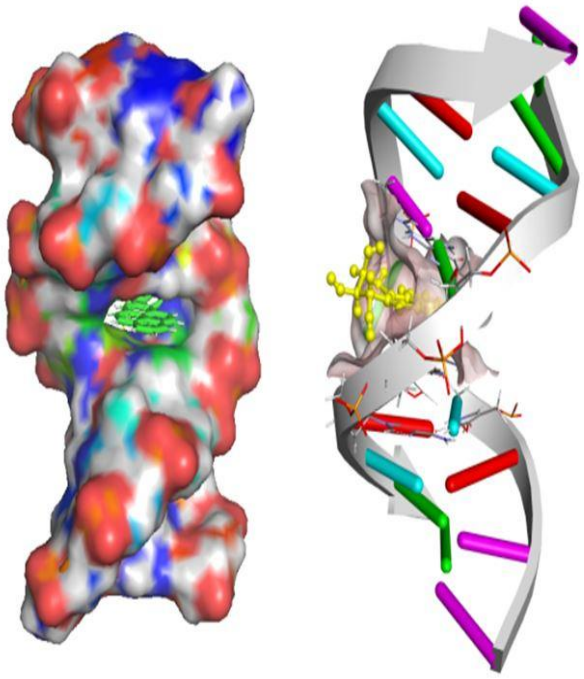


Figure 7. 1AXL (-)-trans-anti-[BP]DG adduct DNA as a target.

4.7. Grid generation at the active site

In structure-based drug design, the identification of the active site is a crucial step. The receptor grid generation was used for the grid generation in the Glide software. After identification of the active site region, the grid box was prepared in such a way that it enclosed the entire active site. In this process, all the other options were kept as default [46].

Subsequently, a multivariate analysis was performed on the compounds according to their similarity to the reference molecules. It was observed that the majority of the studied ligands exhibited a closeness towards the Bendamustine molecule [47].

4.8. Molecular docking

After energy minimization was performed using the Schrödinger Glide searches for possible conformation of the ligand in the active-site region of the receptor, using a set of filters [48]. Using extra precision (XP) glide methodology, the candidate ligands were semi-quantitatively ranked according to their capability to bind to a particular conformation of the protein receptor[49]. By using the default settings, the designed ligand was docked with the 1AXL protein using the XP mode in the Schrödinger's glide software. The hydrophobic and H-bondinteractionsof the best-docked complex were identified using the Pymol and BIOVIA discovery studio.

4.9. Molecular mechanics/Generalized born surface area (MM/GBSA)

The MM/GBSA is force-field based method that computes the free energy of binding based on the Generalized Born continuum solvent model[50-51], as opposed to also modelling free energies of single entities [52].The ligand strain energies and ligand binding of the docked receptor-ligand complexes were calculated according to the molecular mechanics combined with the Generalized Born Model and Solvent Accessibility method (MM/GBSA), using Prime module of the Schrödinger software. The binding free energy (ΔG_{bind}) was calculated using the following Equation 1 [53].

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{SOL} + \Delta G_{SA} \quad \text{(Eq. 1)}$$

Where, ΔE_{MM} is the difference in energies between the protein-ligand complex and the sum of the energies of the unliganded protein and free ligand, using the OPLS force field [54-55]. ΔG_{SOL} is the difference in the GBSA solvation energy of the protein–inhibitor complex and the sum of the solvation energies for the

unliganded protein and ligand. ΔG_{SA} is the difference in surface area energies for the complex structure and the sum of the surface area energies for the non-liganded protein and ligand.

4.10. *In silico* ADMET prediction

After designing and studying the physicochemical properties with reference to the RLD drugs, it was mandatory to match the pharmacokinetics properties i.e. absorption in the body, distribution into the different compartments, metabolism by organs, and elimination through the body [56]. It was necessary to perform a computational study to predict the ADME parameters of the designed molecules to prioritize the molecules for synthesis [57]. Hence ADME study is an essential step for checking the drug-likeness. ADME study of selected whole docked library was carried out using the QikProp (Schrödinger, LLC, New York, NY) [58]. In the present study, properties like molecular weight, predicted central nervous system activity, octanol/water partition coefficient, aqueous solubility, IC-50 value for blockage of HERG K^+ channels, cell permeability, binding of a drug to human serum albumin, number of hydrogen bond donors and acceptors were calculated for each compound and the drug-likeness was evaluated[59]. Moreover, the properties of the ligand with respect to toxicity and carcinogenicity were analyzed online using the ADMET SAR and Swiss ADME [60-61].

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