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Pharmacological target based novel molecules design and validation for Breast and Colorectal cancer using molecular docking studies

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ABSTRACT

Molecular modeling method has been used for modeling a new molecule for Breast and colorectal cancer using Topotecan, a drug that's already designed. This drug is drawn using HYPERCHEM and its R group is modified by replacing different functional groups like OH, CCl_2OH, CF_2OH, $CH_2CH_2CH_3$, CH_2CH_3, CH_3, Cl, F, H, and NH2, etc in its place. Molecules designed as such are optimized using different algorithms and their affinity is checked with the protein. The binding free energy of the protein is calculated by performing docking process. The docking process is done with the help of GOLD software. The molecule with minimum binding energy will have the maximum binding affinity. From the results obtained it's clear that ligand "2(CCl_2OH)"has the maximum binding affinity and this molecule is determined as the best lead molecule targets computationally. The calculated binding affinities between inhibitors 1,2,3,4,5,6, 7,8,9,10 are compared. The calculated binding affinities of the inhibitors indicate that inhibitor "2" (CCl_2OH) would be expected to be better inhibitor than lead inhibitor 1,3,4,5,6,7,8,9 and 10. Inhibitor "2'' predicted to be the most potent inhibitor of TOPOTECAN inhibitor as compared to all the other inhibitors considered in this study. For all the cases the minimization results provided qualitative agreement with experimental results. Therefore, this approach could be very useful for screening a larger set of compounds prior to synthesis accordingly; there is a need for methods that enable rapid assessment of large number of structurally unrelated molecules in a reasonably accurate manner. Energy components calculated by performing molecular mechanics calculations both in explicit solvent and complex states are sufficient to estimate the relative binding free energy differences between inhibitors qualitatively.

Keywords: Breast cancer, Colorectal cancer, Topotecan, CADD, Hyperchem, GOLD software.

INTRODUCTION

Breast Cancer: Breast cancer is cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk[1].

Causes: However, a combination of environmental factors and genetic mutations are thought to be responsible for this cancer. In familial breast cancers, a molecular change in the genes BRCA1 and BRCA2 play a major role in the onset of the disease[2].

Types: Breast cancer is mainly of two types:
- **Ductal carcinoma**- occurs in milk ducts
- **Lobular carcinoma**- occurs in the milk secreting breast lobules[3]

Categorically breast cancer can also be divided into following types:
- **In-situ breast cancer**- cancer cells remains confined within their place of origin and do not attack surrounding breast tissue[4].
- **Invasive or metastatic breast cancer**- cancer cells break free of their place of origin, and spread to different parts of the body[5].

Symptoms:
- Unusual swelling of all or one specific part of the breast
- Continuous skin irritation or dimpling
- Persisting pain in breast[6]
- Persisting nipple pain or inversion of nipple
- Inflammation or thickening of the nipple or breast skin
- An unusual discharge from the nipple other than breast milk
- Lump in the underarm area[7]

Risk factors: The risk factors are as follows –

Geographical: It is much more common in the western world[8].

Gender: Women are a hundred times more likely to have breast cancer as compared to men.

Age: Breast cancer risk increases with age.

Genetic: A family history of breast cancer will increase the risk of developing breast cancer in a woman by three to five times. Recently, a breast cancer gene (BRCA1) has been identified[9].

Hormonal: It appears to be more common in women who did not bear children. It is also less common in women who have their first child at early age.

Life Style:
Fitness levels and life style related factors such as smoking are also some of the most commonly known breast cancer risk factors that can be checked[10].

Breast Cancer Prevention:
- Restricted alcohol consumption
- Maintaining a healthy body weight
- Inclusion of limited fat in diet
- Regular exercise
- Avoiding unnecessary consumption of antibiotics
- Reverting to organic food free of pesticides. Pregnancy and breast feeding have a protective effect in preventing breast cancer[11].

Diagnosis: Diagnosis is done by following methods

1.Mammography
85 to 90% of all breast cancers are detectable by mammography. Approximately 10 to 15 percent of breast cancers are not visible on mammography, but can be felt on physical examination of the breast[12].

2. Ultrasound
3. Biopsy
Breast Cancer Treatment:

- Surgery
- Radiation
- Surgery followed by Radiation
- Chemotherapy
- Combined Therapy
- Adjuvant and Neo-adjuvant Therapy for Breast Cancer
- Hormonal Therapy - Aromatase Inhibitors
- Targeted Therapies
- Complimentary and Holistic Medicines
- Angiogenesis Inhibitors Therapy[13]

Colorectal cancer: Tumors of the colon and rectum are growths arising from the inner wall of the large intestine. Benign tumors of the large intestine are called polyps. Malignant tumors of the large intestine are called cancers. Benign polyps do not invade nearby tissue or spread to other parts of the body. Benign polyps can be easily removed during colonoscopy and are not life-threatening. If benign polyps are not removed from the large intestine, they can become malignant (cancerous) over time. Globally, cancer of the colon and rectum is the third leading cause of cancer in males and the fourth leading cause of cancer in females. The frequency of colorectal cancer varies around the world. It is common in the Western world and is rare in Asia and Africa. In countries where the people have adopted western diets, the incidence of colorectal cancer has been increasing[14].

Causes :
Factors that increase a person's risk of colorectal cancer include
Diet: Diets high in fat are believed to predispose humans to colorectal cancer.
Colon polyps
Ulcerative colitis
Genetics factors[15]

Symptoms: Colon cancer are numerous and nonspecific. They include fatigue, weakness, shortness of breath, change in bowel habits, narrow stools, diarrhea or constipation, red or dark blood in stool, weight loss, abdominal pain, cramps, or bloating. Other conditions such as irritable bowel syndrome (spastic colon), ulcerative colitis, Crohn's disease[16].

Treatment:
Surgery is the most common treatment for colorectal cancer.

Chemotherapy is the use of 5-flourauracil (5-FU). Topotecan is a drug of choice for both Colo-rectal & breast cancer. Common side effects include anemia, loss of energy, easy bruising and a low resistance to infections, hair loss, mouth sores, nausea, vomiting and diarrhea.

Radiation therapy in colorectal cancer has been limited to treating cancer of the rectum[17]. Side effects of radiation treatment include fatigue, temporary or permanent pelvic hair loss and skin irritation in the treated areas.

Other treatments have included the use of localized infusion of chemotherapeutic agents into the liver, the most common site of metastasis. This involves the insertion of a pump into the blood supply of the liver which can deliver high doses of medicine directly to the liver tumor[18].

Plan of Work:

- Energy Calculations of Ligand in Air by Single Point, Geometry Optimisation, Molecular Dynamics, Monte Carlo
- Energy Calculations of Ligand with different replaced groups
- Energy Calculations of Ligands (Solvent Intra)
- Energy Calculations of Ligands (Protein Intra)

- Docking
- Free Energy Calculations for more effective drug
- Protein Analysis by different Databases

MATERIALS AND METHODS

Open Eye scientific software: Open Eye Scientific Software develops large-scale modeling applications and toolkits. Primarily geared towards drug discovery and design, areas of application include structure generation, docking, shape comparison, electrostatics, chemical informatics and visualization. The software is designed for scientific rigor, as well as speed, scalability and platform independence. Ligand –solvent interactions (Inter-solvent)(solvent PB).For optimization of small molecules in solution, the electrostatic part of molecule-solvent interactions will be calculated using Poisson-Boltzmann model of Open Eye scientific software.

Gold Genetic Optimisation for Ligand Docking: GOLD is a program for calculating the docking modes of small molecules into protein binding sites. The product of collaboration between the University of Sheffield, GlaxoSmithKline plc and CCDC. GOLD is very highly regarded within the molecular modeling community for its accuracy and reliability. Ligand –protein interactions (Inter-protein) (Docking) for Docking of small molecules into the protein active site, the VDW, Hydrogen bonds and hydrophobic energies of ligand-protein interactions will be calculated using GA of Gold software[19].

Hyperchem: Hyperchem is a versatile molecular modeler and editor and a powerful computational package. It offers many types of molecular and quantum mechanics calculations. For optimization of small molecules in solution and protein complex the intra molecular energies of ligand-solvent and ligand protein will be calculated using molecular mechanics calculations of Hyperchem software[20].Computational assessment of the binding affinity of enzyme inhibitors prior to synthesis is an important component of computer-aided drug design paradigms. In this study, the molecular mechanics (MM) method is used for the estimation of relative binding affinities of inhibitors to an enzyme. Qualitative predictions of relative binding affinities of Beta Secretase inhibitors using MM method are discussed[21]. The results indicate that the MM based method is useful in the qualitative estimation of relative binding affinities of enzyme inhibitors prior to synthesis. CADD approach has contributed to the successful discovery of numerous novel enzyme inhibitors including inhibitors of thymidylatesynthase, HIV-1 Protease (Reddyand Appelt, 2001) and purine nucleoside phosphorylase (Montgomery et al., 1993) inhibitors[22]. In each case CADD was used to predict the binding affinity of an inhibitor designed from a lead compound prior to synthesis. Earlier, Jorgensen et al. (2000), reported results using the free energy perturbation calculations in an iterative structure-based design program to accurately predict relative binding affinities of COX-1, COX-2 and SRC SH2 domain and linear interaction energy results for thrombin and HIV –RT. This work focuses on lead inhibitor optimization strategies using molecular mechanics method by predicting relative binding affinities of galantamine inhibitors[23].

RESULTS

List of Inhibitors Developed

Sl.no	Ligand	Molecule
1	Ligand -1	R1=OH (STD)
2	Ligand -2	R1=CCl_2OH
3	Ligand -3	R1=CF_2OH
4	Ligand -4	R1=$CH_2CH_2CH_3$
5	Ligand -5	R1=CH_2CH_3
6	Ligand -6	R1=CH_3
7	Ligand -7	R1=Cl
8	Ligand -8	R1=F
9	Ligand -9	R1=H
10	Ligand -10	R1=NH_2

Table 1: Solvent (Intra)

Molecule	Solvent Intra Energy (X2)
R1=OH (STD)	28.91
R1=CCl_2OH	41.51
R1=CF_2OH	35.77
R1=$CH_2CH_2CH_3$	38.62
R1=CH_2CH_3	36.43
R1=CH_3	35.47
R1=Cl	36.94
R1=F	32.55
R1=H	37.04
R1=NH_2	36.75

Table 2: Energy Of Ligand In Air (X1)

Molecule	Energy In Air (X1)
R1=OH (STD)	-129.34
R1=CCl_2OH	-74.01
R1=CF_2OH	-143.73
R1=$CH_2CH_2CH_3$	-94.63
R1=CH_2CH_3	-111.63
R1=CH_3	-134.03
R1=Cl	-93.69
R1=F	-161.05
R1=H	-82.09
R1=NH_2	-163.08

Table 3: Protein

Molecule	Protein Intra Energy In Air (Y1)
R1=OH (STD)	33.09
R1=CCl_2OH	42.49
R1=CF_2OH	37.56
R1=$CH_2CH_2CH_3$	32.12
R1=CH_2CH_3	35.64
R1=CH_3	30.55
R1=Cl	33.13
R1=F	33.98
R1=H	34.64
R1=NH_2	33.56

Table 4:- Docking (Inter)

Molecule	Docking(Y2)
R1=OH (STD)	-26.95
R1=CCl_2OH	-29.28
R1=CF_2OH	-55.9
R1=$CH_2CH_2CH_3$	-34.74
R1=CH_2CH_3	-34.85
R1=CH_3	-30.88
R1=Cl	-23.23
R1=F	-27.66
R1=H	-26.83
R1=NH_2	-27.01

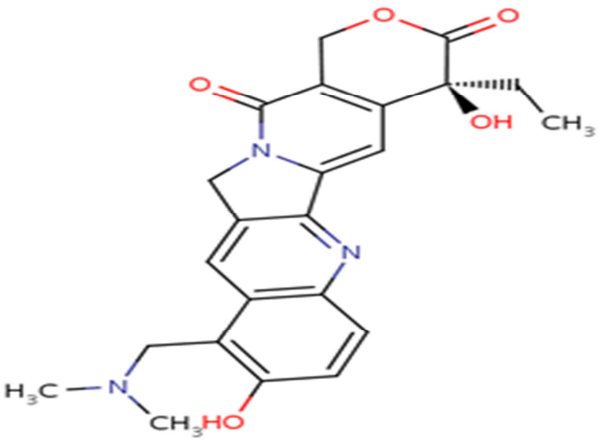
Molecule	Solvent (X=X1+X2)	Protien (Y=Y1+Y2)
R1=OH (STD)	-100.43	6.14
R1=CCl_2OH	-32.5	13.21
R1=CF_2OH	-107.96	-18.34
R1=$CH_2CH_2CH_3$	-56.01	-2.62
R1=CH_2CH_3	-75.2	0.79
R1=CH_3	-98.56	-0.33
R1=Cl	-56.75	9.9
R1=F	-128.5	6.32
R1=H	-45.05	7.81
R1=NH_2	-126.33	6.55

Table 6:-Binding Free Energy Changes		
Molecule	**Z-Values (Y-X)**	**E Bind (Z2-Z1)**
R1=OH (STD)	106.57 (Z1)	0
R1=CCl_2OH	45.71 (Z2)	-60.86
R1=CF_2OH	89.62 (Z3)	-16.95
R1=$CH_2CH_2CH_3$	53.39 (Z4)	-53.18
R1=CH_2CH_3	75.79 (Z5)	-30.78
R1=CH_3	98.23 (Z6)	-8.34
R1=Cl	66.65 (Z7)	-39.92
R1=F	134.82 (Z8)	28.25
R1=H	52.86 (Z9)	-53.71
R1=NH_2	132.88 (Z10)	26.31

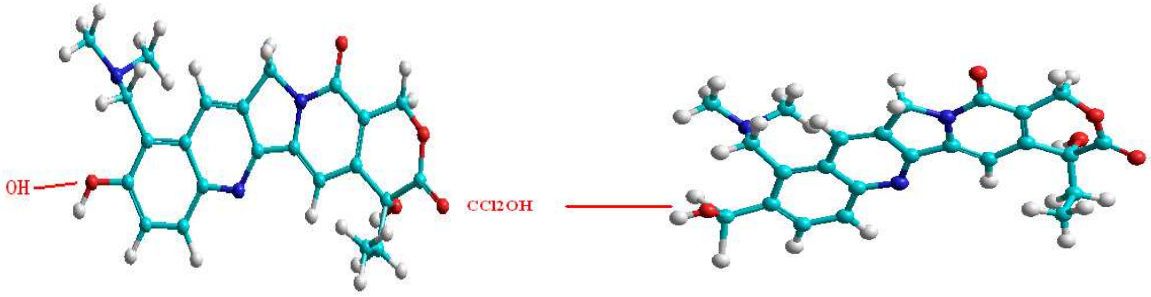
Docking(y2) (or)
Fitness (y2) = S (hb – ext) + 1.3750 × S (vdw - ext) + S (hb - int) + 1.0000 × S (vdw - int)
(Solvent) X = x1 + x2,(Protein) Y = y1 + y2
Binding free enrgy Z = Y - X
E bind = Z2 – Z1

Standard drug name **:** TOPOTECAN
Chemical Formula **:** C23H23N3O5

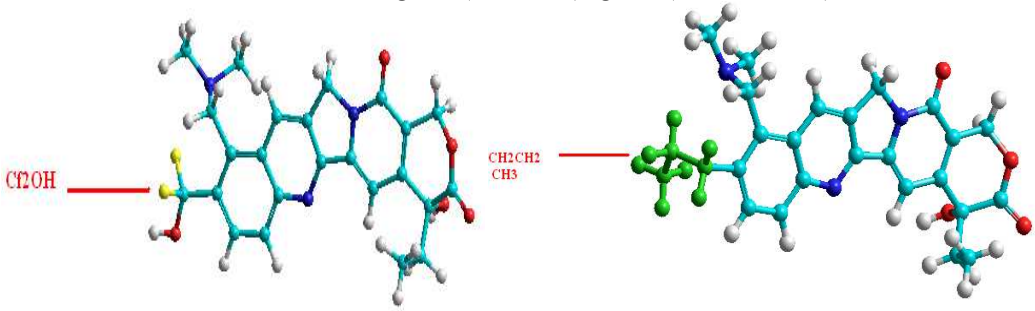
Structure:



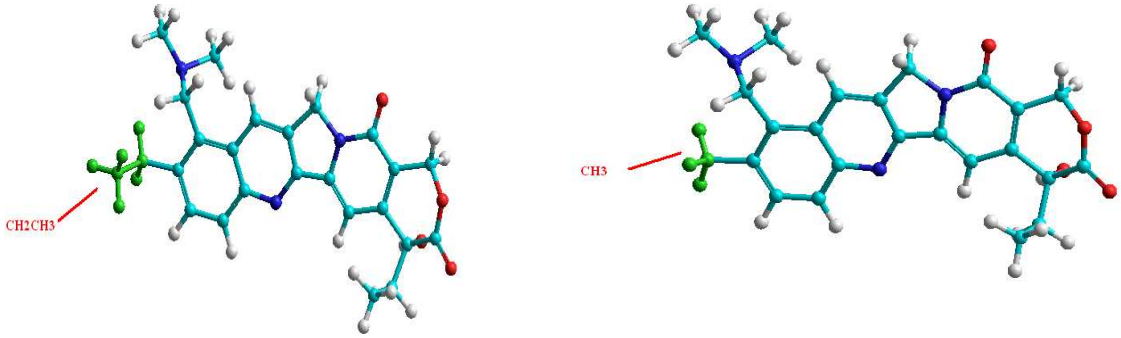
Optimization Of Ligand In Air:
Standard Ligand-1Ligand-2(R1=CCL_2OH)



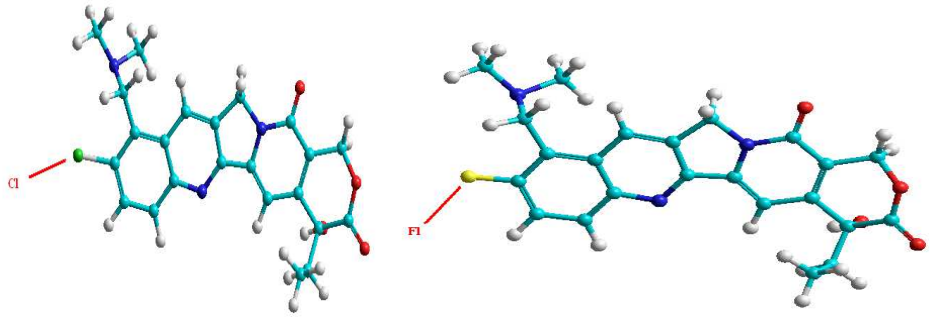
Ligand-3 (R1=CF_2OH)Ligand-4 (R1=$CH_2CH_2CH_3$)



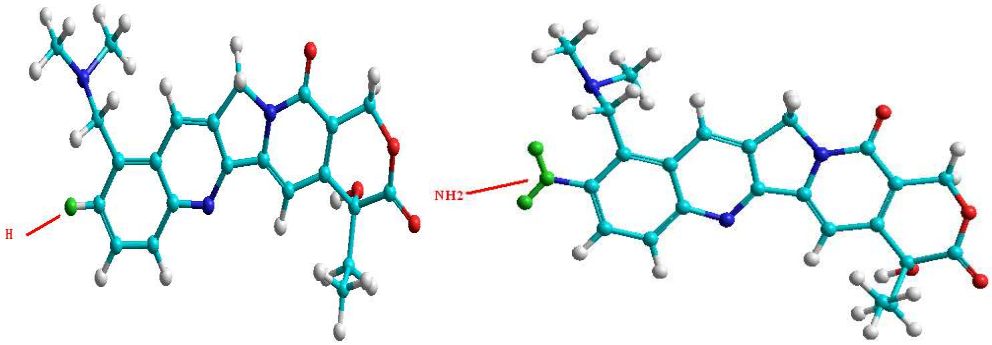
Ligand-5 (R1=CH_2CH_3)Ligand-6 (R1=CH_3)



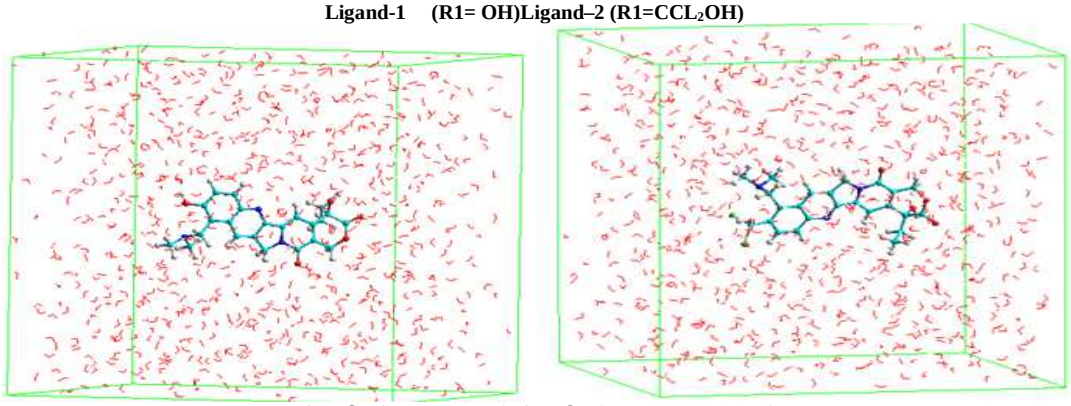
Ligand-7 (R1=Cl)Ligand-8 (R1=F)



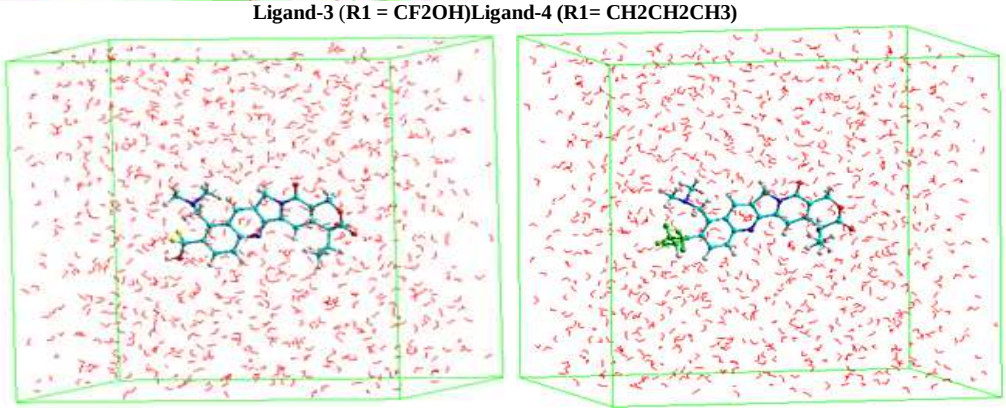
Ligand-9 (R1=H)Ligand-10 (R1=NH_2)



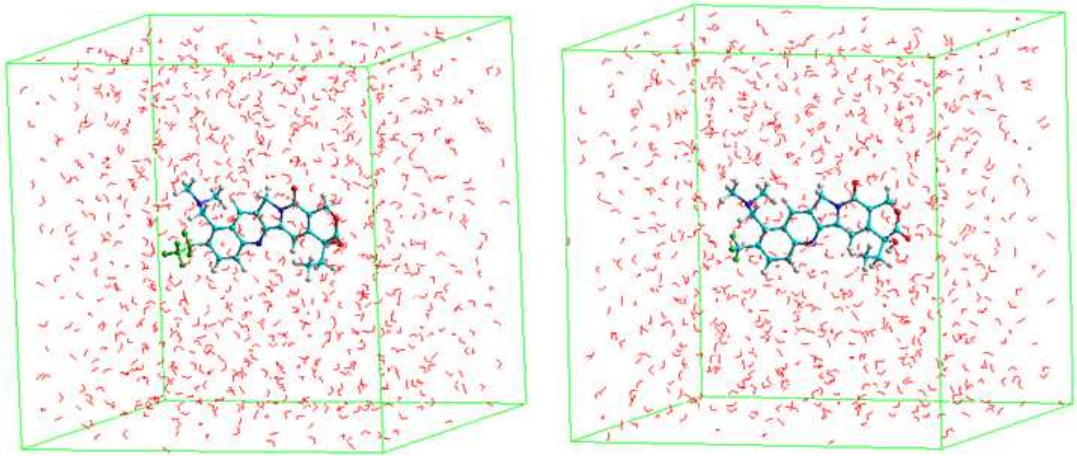
Optimization Of Solvent – Ligand Complex:
Ligand-1 (R1= OH)Ligand–2 (R1=CCL_2OH)



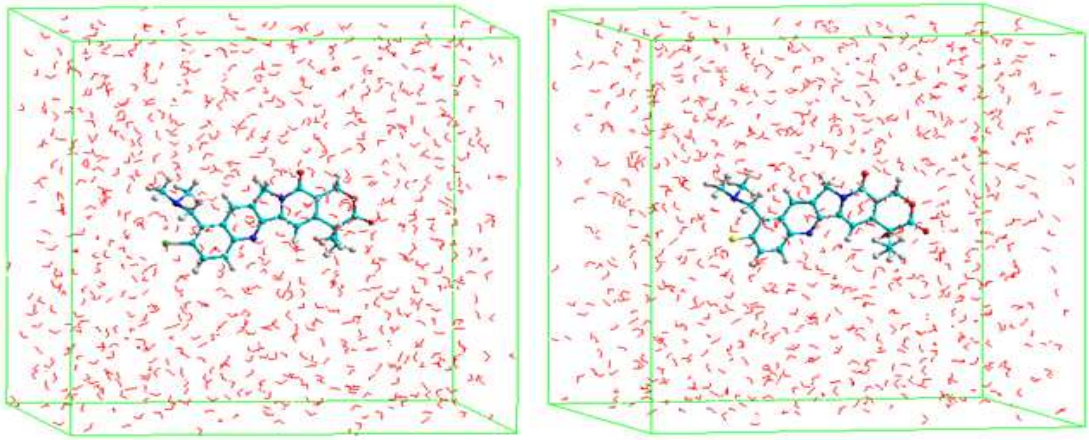
Ligand-3 (R1 = CF2OH)Ligand-4 (R1= CH2CH2CH3)



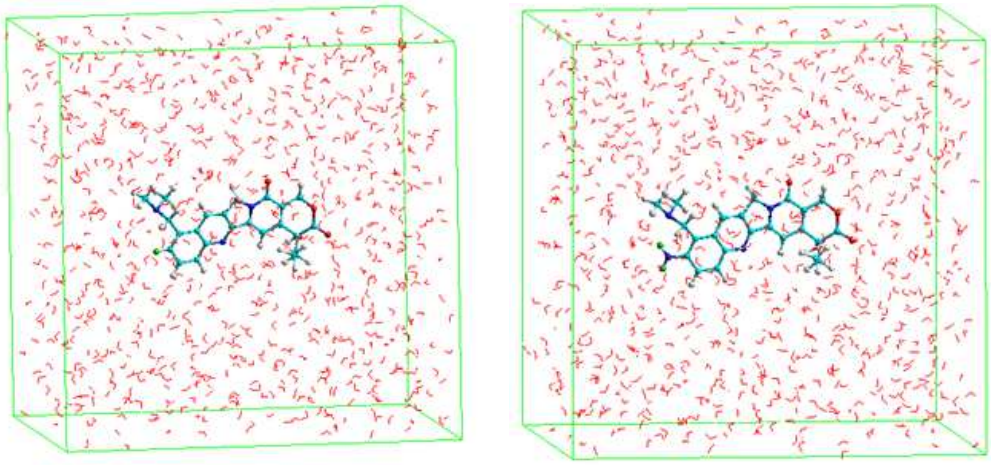
Ligand-5 (R1= CH_2CH_3)Ligand-6(R1= CH_3)



Ligand-7 (R1= CL)Ligand-8(R1=F)

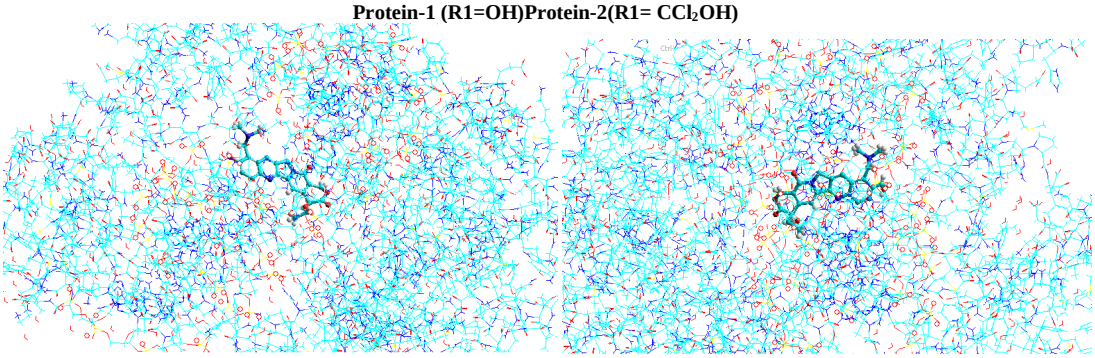


Ligand-9(R1=H)Ligand-10(R1=NH_2)

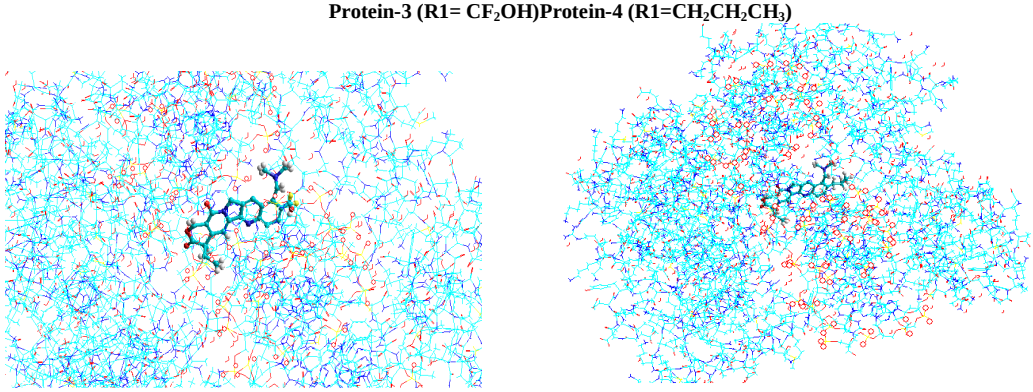


Optimization Of Protein – Ligand Complex:

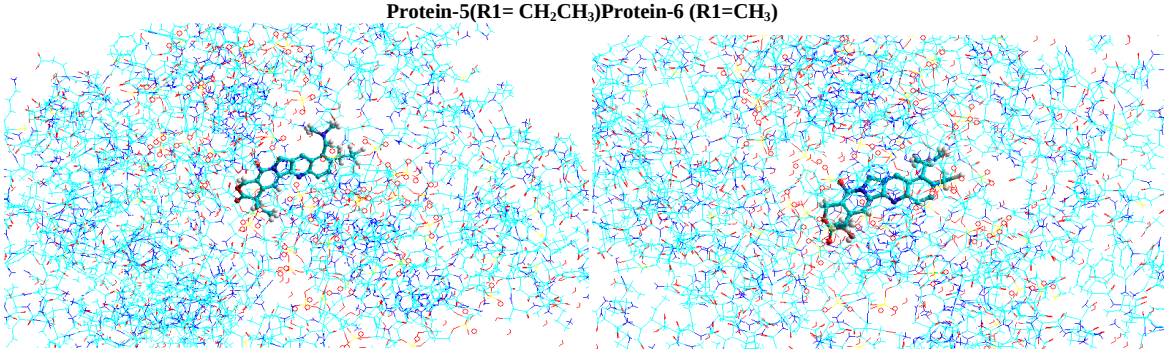
Protein-1 (R1=OH)Protein-2(R1= CCl_2OH)



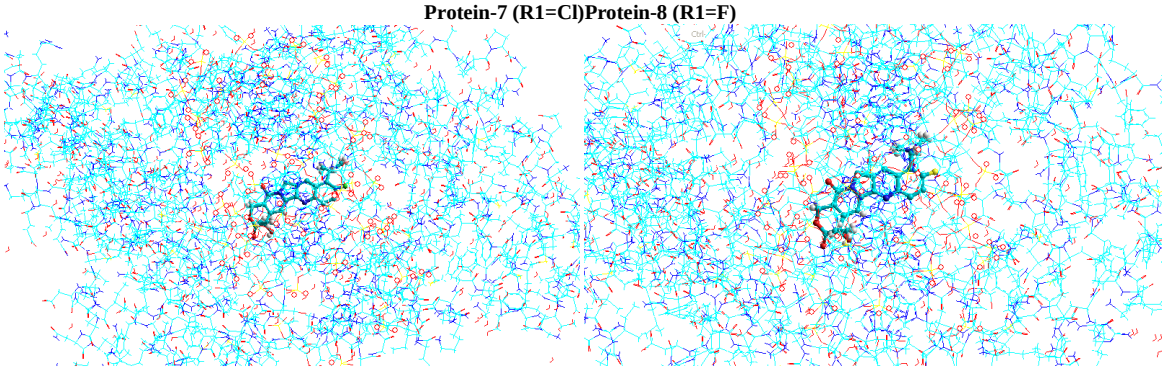
Protein-3 (R1= CF_2OH)Protein-4 (R1=$CH_2CH_2CH_3$)



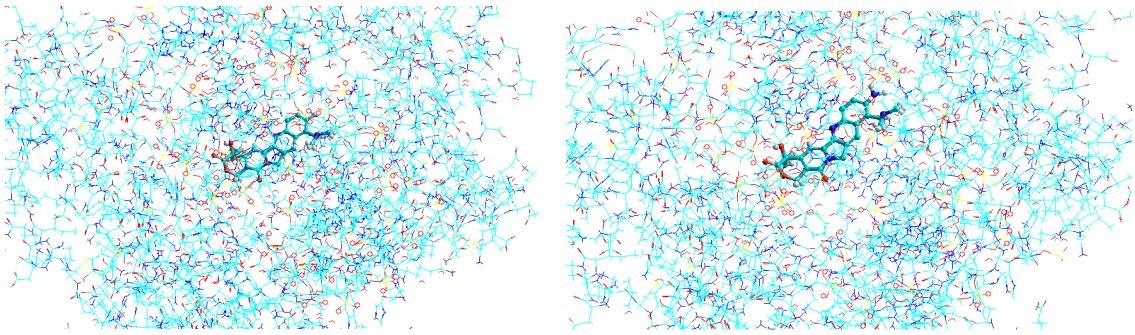
Protein-5(R1= CH_2CH_3)Protein-6 (R1=CH_3)



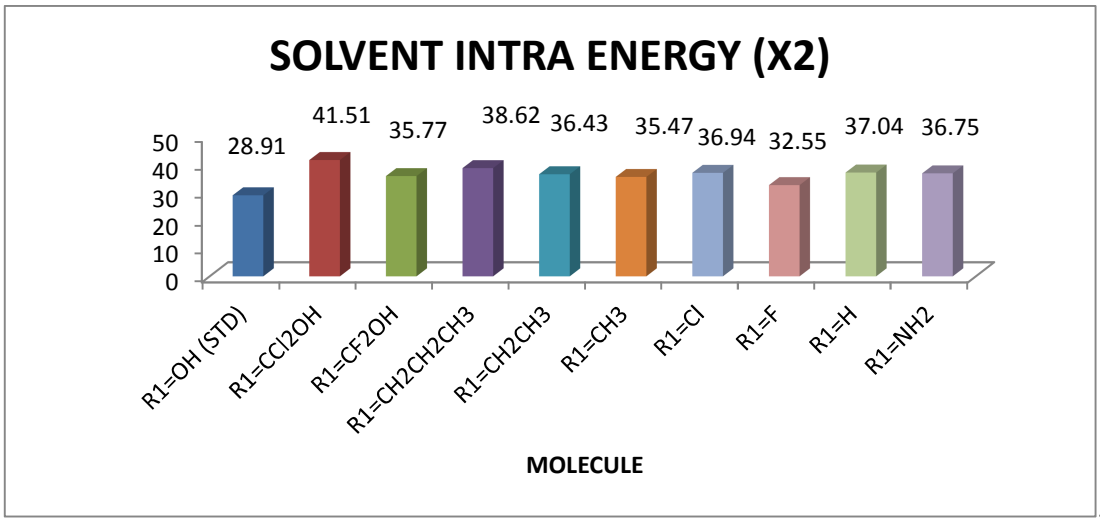
Protein-7 (R1=Cl)Protein-8 (R1=F)

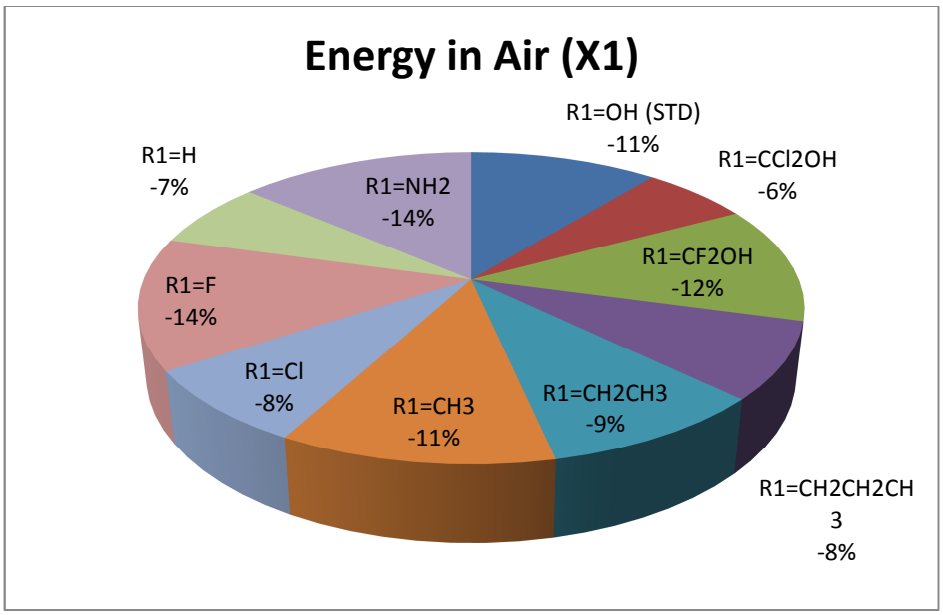


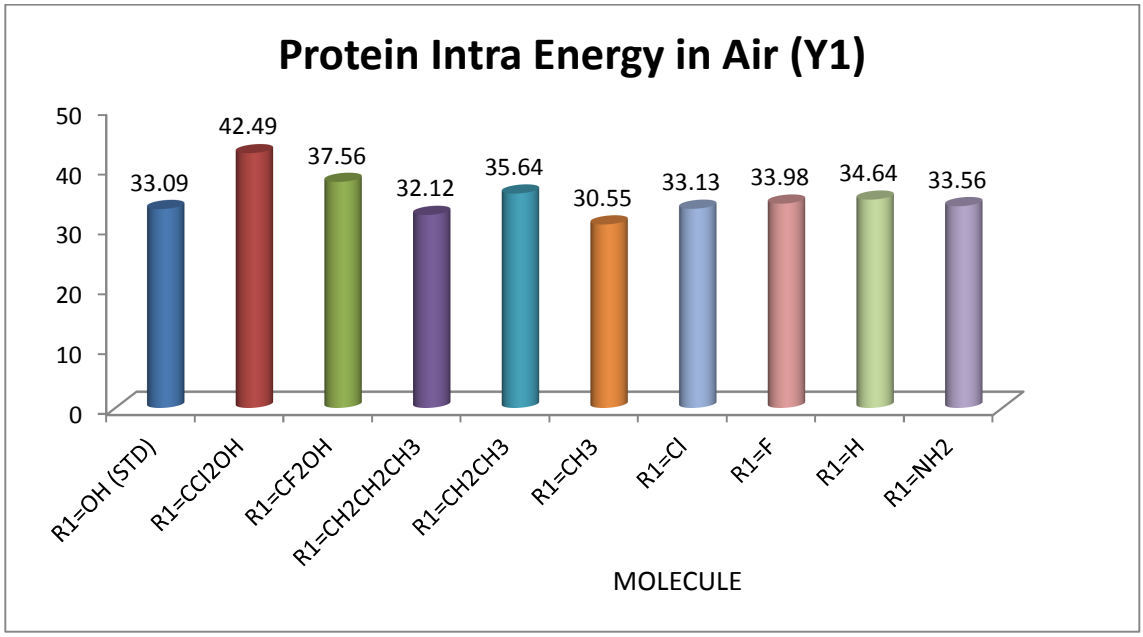
Protein-9 (R1=H)Protein-10 (R1=NH_2)

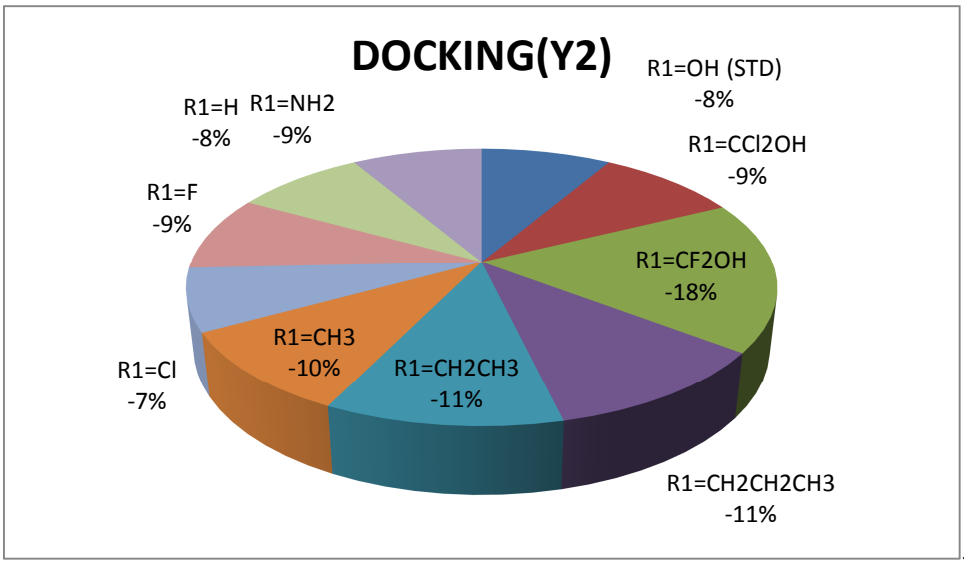


Statistical Interpretation






Protein Intra Energy in Air (Y1)
50
40
30
20
10
0
33.09
42.49
37.56
32.12
35.64
30.55
33.13
33.98
34.64
33.56
R1=OH (STD)
R1=CCl2OH
R1=CF2OH
R1=CH2CH2CH3
R1=CH2CH3
R1=CH3
R1=Cl
R1=F
R1=H
R1=NH2
MOLECULE


DOCKING(Y2)
R1=OH (STD) -8%
R1=CCl2OH -9%
R1=CF2OH -18%
R1=CH2CH2CH3 -11%
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R1=CH3 -10%
R1=Cl -7%
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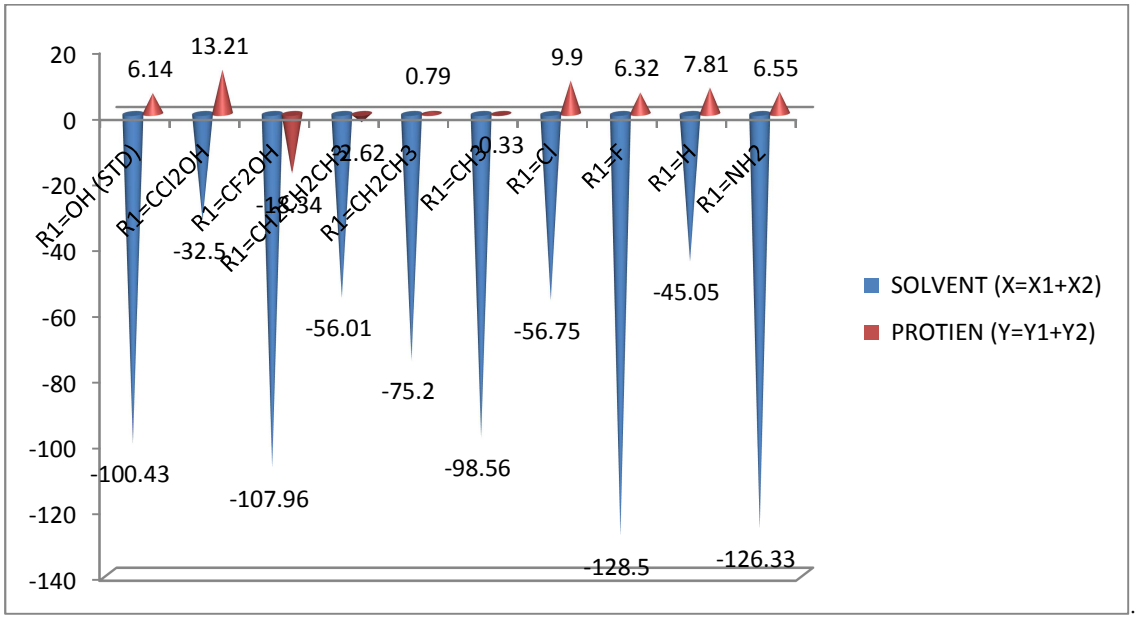
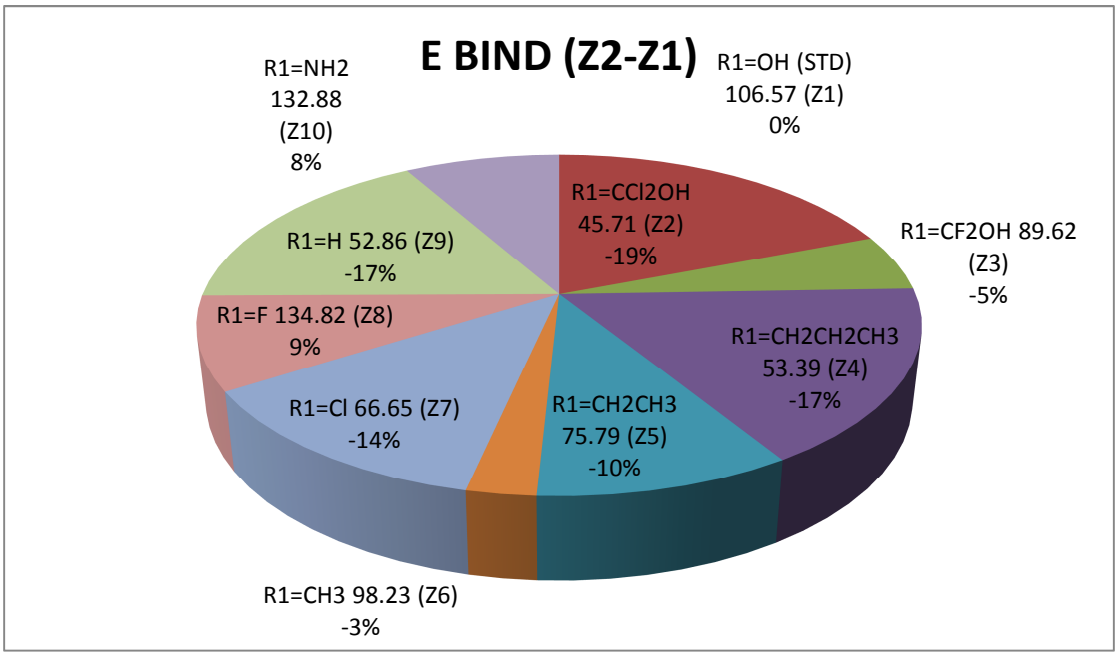
R1=H -8%
R1=NH2 -9%





DISCUSSION

In this work, the binding modes of the putative/proposed inhibitors were obtained by carefully aligning them with the known crystal structures of inhibitors in the active site of the 1CY6. These inhibitors, which are shown in Fig. were then evaluated by performing minimization calculations both in solvent and in complex using the AMBER (Weiner SJ *et al*, 1984) force field.

The technical details used for estimating relative binding affinities using energy components obtained from minimizations of each inhibitor, both in solvent as well as in complex phases, were explained by four stage protocol as described in the in methodology section.

The minimized structures for all the 10 inhibitors in the complex and solvated states were used for calculating the following energy variables:

$$E_{bind}\ (intra) = E_{com}\ (intra) - E_{sol}\ (intra) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$
$$E_{bind}\ (inter) = E_{com}\ (inter) - E_{sol}\ (inter) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (3)$$

Where, E_{bind} (intra) and E_{bind} (inter) are relative intra and intermolecular binding interaction energies of a ligand, respectively, and where E_{com} (intra), E_{com} (inter), E_{sol} (intra), and E_{sol} (inter) are intra and intermolecular interaction energies of a ligand in the complexed and solvated states, respectively. Relative differences in intra, intermolecular and total binding interaction energies for a pair of ligands L1 and L2 are given by,

$$E_{bind}\ (intra: L1, L2) = E_{bind}\ (intra: L2) - E_{bind}\ (intra: L1) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (4)$$
$$E_{bind}\ (inter: L1, L2) = E_{bind}\ (inter: L2) - E_{bind}\ (inter: L1) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (5)$$
$$E_{bind}\ (tot: L1, L2) = E_{bind}\ (intra: L1 \lozenge L2) + E_{bind}\ (inter: L1 \square L2) \quad \ldots\ldots\ldots\ (6)$$

Where, E_{bind} (tot: L1 L2) is the total relative difference in the binding energies of L1 and L2. Hence, an agreement in the overall trends between the experimental measurements and the energy minimization results were expected. In the Table 2, the relative differences in the binding affinities measured experimentally (E_{bind} (exit)) are compared with the relative binding affinities calculated using minimization methods and for all the cases the minimizations results provided qualitative agreement with experimental results.

The calculated binding affinities between inhibitors 1,2,3,4,5,6,7,8,9,10 were compared. The calculated binding affinities of the inhibitors between indicate that inhibitor 2 **(CCl_2OH)**is expected to be better inhibitors than lead inhibitor 1,3,4,5,6,7,8,9 and 10. Inhibitor 2 is predicted to be the most potent inhibitor to TOPOTECAN inhibitor as compared to all the other inhibitors considered in this study. For all the cases the minimizations results provided qualitative agreement with experimental results. Therefore, this approach could be very useful for screening a larger set of compounds prior to synthesis accordingly; there is a need for methods that enable rapid assessment of large number of structurally unrelated molecules in a reasonably accurate manner. Energy components calculated by performing molecular mechanics calculations both in explicit solvent and complex states are sufficient to estimate the relative binding free energy differences between two inhibitors qualitatively.These qualitative methods will continue to improve and become more accurate as;

1) Force field parameters become more refined,
2) Other variables important for binding such as entropy are included,
3) Methods for estimating relative binding entropy changes improve,
4) Docking and scoring procedures improve, and
5) Average molecular dynamics simulations are used to obtain energy variables.

These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities of enzyme inhibitors for more quantitative analysis of the most promising candidates.

CONCLUSION

Comparisons of the calculated binding affinities for structurally similar Inhibitors to TOPOTECAN indicate that the molecular mechanics methods gave suitable analogues. These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis.

Molecular modeling method has been used for modeling a new molecule for Breast and colorectal Cancer using Topotecan, a drug that's already designed. This drug is drawn using hyperchem, and its R group is modified by replacing different functional groups like OH, CCl_2OH, CF_2OH, $CH_2CH_2CH_3$, CH_2CH_3, CH_3, Cl, F, H, and NH2, etc in its place. The molecules designed as such are optimized using different algorithms and their affinity is checked with the protein. The binding free energy of the protein is calculated by performing docking process. The molecule with minimum binding energy will have the maximum binding affinity. The binding free energy is

calculated by the formula Z = Sum of the energy of optimized ligand devoid of solvation parameters and the energy of the protein-ligand optimization. The binding free energy of the designed molecules is obtained by eliminating the energy of the main molecule i.e. Topotecan. From the results obtained it's clear that ligand 2 has the maximum binding affinity. So this molecule is determined as the best lead molecules targets computationally.The inhibitor 2 with substituent CCl_2OHidentified as the most suitable analogue in the present study that needs to be further evaluated in laboratory.

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