In silico study of novel Dihydropyrimidines against Anti Cancer, Anti Tuberculosis, Anti HIV and Anti Malarial activity.

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Abstract- Our efforts were focused on the introduction of chemical diversity in the molecular frame work in order to evaluating pharmacologically interesting compounds of widely different composition. Virtual screening of the chemical compounds includes filtration of the toxic compounds and docking of the compounds for anti HIV, anti tubercular, anti cancer and anti malarial study. By virtual screening we would be able to find two lead molecules ligand **ID 77** (N,4-bis(4-chlorophenyl)-6-(propan-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxa-mide) and **87** (N,5-bis(4-chlorophenyl)-7-(propan-2-yl)-2,3-dihydro-5H-[1,3]thiazolo[3,2a]pyrimidine-6-carboxamide). Both the ligands showed broad activity against anti-HIV, anti-tuberculosis, anti-malaria and anti-cancer receptors 1IKV, 1QS4, 1G3U, 1ZXL and 2QQJ respectively.

Keywords- DHPM- Dihydropyrimidines, ADME, Molecular docking, Gold score, Lead molecules, Virtual Screening (VS)

1 INTRODUCTION

Testing large numbers of compounds to see if they produce an appropriate biochemical or cellular effect is usually one of the first steps in the drug-discovery pathway, and ways of making this screening faster, more effective and less expensive are thrust area for any pharmaceutical company. A positive response in a first round of screening in a biochemical assay identifies the primary 'hit' compounds. These molecules then go into more screens to see if they have physicochemical and pharmacological properties that are not too incompatible with making a drug — if it passes this filter, a hit becomes a 'lead'. The study of the interaction between chemical compounds and biological targets has dominated modern drug discovery research. Therefore, finding drug candidates by screening large numbers of chemicals against the new targets in a quick and economical fashion has become one of the most challenging tasks for today's drug discovery process.

Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms [1], [2]. 4-Aryl-1, 4-dihydropyridines of the nifedipine type (DHPs, e.g nifedipine) are the most studied class of organic calcium channel modulators.

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More than 30 years after the introduction of nifedipine many DHP analogs have now been synthesized and numerous second-generation commercial products have appeared on the market (e.g.nitrendipine, nicardipine and amlodipine).

Over the last decade, the revolutions of combinatorial chemistry and high-throughput screening (HTS) have undoubtedly changed the way drug discovery is practiced today. Two areas of current activity in VS and computational chemistry methodology address the problem of drug attrition: (i) the development of computational tools and models for *in silico* prediction of absorption, distribution, metabolism, elimination and toxicity (ADMET) properties (reviewed in [3]); and (ii) the development of better computer-aided methods for enrichment of bioactive molecules in a compound collection [3],[4] Therefore, identification and development of potential ligands specifically for a protein target forms the primary goal in drug discovery process **[5], [6]**.

In silico approaches are being successfully applied in the anti tuberculosis[7] anti malarial[8] anti HIV[9],[10] and anti bacterial drug discovery at various stages starting from design; modeling; simulated docking and virtual screening of potential lead compounds; and lead validation and optimization using structure-activity and structure-function relationships. Virtual (database) screening (VS) of molecules promises to accelerate the discovery of new drugs **[11],[12]** and reduce costs by identifying molecules with high probabilities of binding to a target receptor. The large amount of available protein X-ray crystal structures, together with the development of more effective homology modeling

techniques, has led recently to a steep increase in docking-based VS studies. This approach needs computational fitting of molecules into a receptor active site using advanced algorithms, followed by the scoring and ranking of these molecules to identify potential leads. Genetic Optimization for Ligand Docking (GOLD) is a program for docking flexible ligands into protein binding sites **[13],[14].** It was originally written by Jones at the University Of Sheffield, England. Since its release in 1998, it has been distributed, maintained, and improved by the Cambridge Crystallographic Data Centre (CCDC). A docking program requires two basic abilities: a method of scoring any trial ligand pose and a search algorithm for finding the pose with the best score [15],[16],[17],[18].

2. Experimental Section

STEP-1

In first step small library for *in silico* study of **217** Dihydropyrimidines derivatives were created with **ISIS Draw3.2/ chem sketch 11.0.**

STEP - 2

In second step in silico QSAR studies were carried out for drug likeness carcinogenicity and toxicity study using **Sarchitech Miner software**. Out of total **217** compounds **43** were pass the Lipinski rule of five for druglikness. **80** compounds were found to be carcinogenic and **94** compound were mutagenic in nature. From the obtained result filtering of the library for drug like compound led the 43 selected compounds for further study.

STEP - 3

In the third step selected hit from second step were further analyzed for their Anti HIV, Anti tuberculosis, Anti Malarial and Anti cancer activity by molecular docking with **GOLD software**. Virtual screening of the docking solution was performed on the basis of the dock score. This led the **231** top ranking solutions.

Data sources

3D structures of compounds were generated by using ISIS Draw3.2 and hydrogen was added in all the ligand structure. In silico QSAR studies were carried out for drug likeness and toxicity study using Sarchitech Minor software. The 3D structures were downloaded from the Protein Data Bank (PDB) water molecules were removed and hydrogen were added.

Docking with GOLD was done by method as described by G. Jones, P. Willett, R.C. Glen, A. R. L. Leach and R. Taylor **[13],[14]** with default parameter with GOLD and Chem. score.

The maximum score was recorded for molecules **77 (*N*,4-bis(4-chlorophenyl)-6-(propan-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide)** and **87 (*N*,5-bis(4-chlorophenyl)-7-(propan-2-yl)-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxamide**). Both the 142 ligand showed broad activity against anti HIV, anti tuberculosis, anti malaria and anti cancer receptors 1IKV, 1QS4, 1G3U, 1ZXL and 2QQJ receptively. Among them **87** showed highest dock score (30.0934) against anti cancer receptor neutrophilin 2 (2QQJ). Whereas ligand **77** showed highest dock score 26.6993 against anti HIV receptor, HIV reverse Transcriptase (1IKV).

Work Flow for molecular docking:

In the final step out of the 231 top ranking solutions top 23 (10% of total docked result) were further analyzed. The docking studies were carried out using following targets

1 Anti HIV receptor: HIV-1 Reverse Transcriptase **(1IKV)**,

2 Anti HIV receptor: HIV-1 integrase **(1QS4)**

3 Anti HIV receptor: HIV-1 PROTEASE **(1AID)**

4 Anti tuberculosis receptor: Thymidylate Kinase **(1G3U)**.

5 Anti Malarial receptor: Enoyl ACP Reductase **(1ZXL)**.

6 Anti cancer receptor: Neuropilin-2 (2QQJ)

3. Results and Discussion

Out of the five receptor included in study highest score was observed against anti cancer receptor (2QQJ) Neutrophilin-2. Compound **(83, 76, 99, 84, 106, 86, 77, 79, 89, 87, 82, 88, 113)** were found to have the docking score in the range of **37.17774 to 23.2643**. Anti malarial docking for compound 79, 77 and 95 were reported. Compound 77, 99, 89, 97, 88, 87 were found to have docking score in range of 26.8399 to 23.658 for Anti HIV receptor 1IKV. Only compound 87 were found to have the 24.1667 docking score for Anti tuberculosis receptor 1G3U. **(Table 1)**

Virtual screening result indicates that compound have the high docking score for anticancer receptor while the least was recorded for the anti tuberculosis receptor. Comparative analysis demonstrates the broad spectrum docking score of compound **87 (*N*,5-bis(4-chlorophenyl)-7-(propan-2-yl)-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-6-carboxamide** for anti cancer, anti HIV and anti tuberculosis receptor. Similarly compound **77 (*N*,4-bis(4-chlorophenyl)-6-(propan-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide)** was also found to get good docking score for anti cancer, anti malaria and anti HIV receptor. Thus lead compound **77** and **87** should be further explored for anti cancer, anti malaria and anti HIV and Anti tuberculosis drug discovery process.

All the 217 molecules used in this study from them 43 compounds were followed Lipinski rule of 5 rest all the compounds were not followed Lipinski rule of 5 or either mutagenic or carcinogenic nature. So they all filtered during Sarchitech filtration. 15 Lead molecules 76,77,79,82,83, 84,86,87,88,89, 95,97,99,106,113 (Table 1) were selected as the lead molecules obtained by dock score of selected lead molecules against successful target for,

- Anti HIV receptor:
  HIV-1 Reverse Transcriptase (**1IKV)**, HIV-1 integrase **(1QS4)**, HIV-1 PROTEASE **(1AID)**
- Anti tuberculosis receptor:
  Thymidylate Kinase **(1G3U)**,
- Anti Malarial receptor:
  Enoyl ACP Reductase **(1ZXL)**.
- Anti cancer receptor
  Neuropilin-2 **(2QQJ)**

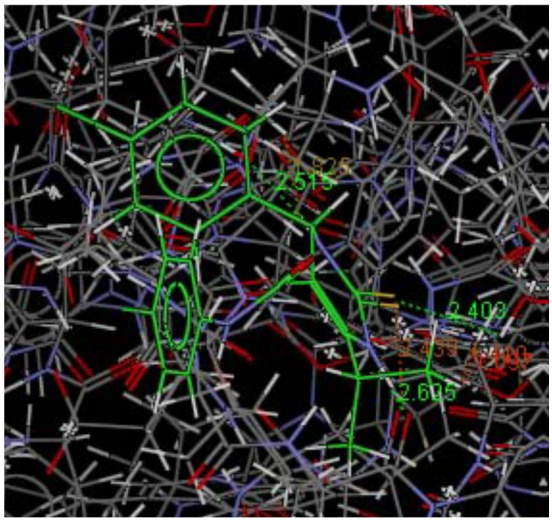

Fig. 1 Dock score of ligand No. 77 against HIV receptor, PDB ID: 1IKV

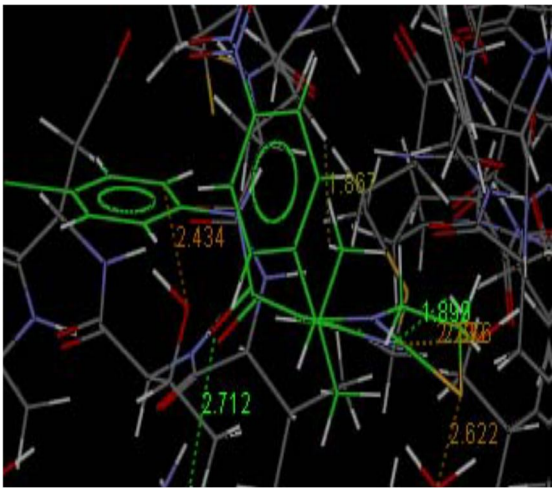

Fig.2 Dock Score of ligand No.87 against Cancer receptor PDB ID : 2QQJ

TABLE 1

SCRUTINIZE TOP 23 LIGANDS-DOCK SCORE

Protein Name	Diseases	Ligand ID	Dock Score
2QQJ Anti cancer receptor Neuropilin-2	Anti Cancer	113	37.1774
		88	32.6148
		82	31.9883
		87	30.0934
		89	29.6809
		79	25.9667
		77	25.2322
		86	24.9796
		106	24.8507
		84	24.5069
		99	23.712
		76	23.5504
		83	23.2643
1ZXL Anti Malarial receptor: Enoyl ACP Reductase	Anti Malarial	79	27.0107
		77	24.6366
		95	23.4661
1IKV Anti HIV receptor: HIV-1 Reverse Transcriptase	Anti HIV	77	26.8399
		99	25.6747
		89	23.8855
		97	23.8849
		88	23.6728
		87	23.658
1G3U Anti tuberculosis receptor: Thymidylate Kinase	Anti T.B	87	24.1667

TABLE 2

SUMMARY OF DOCKING

No	Step	Molecules	Receptor	No of docking pose	Dock score range
1	**Library creation**	Library of total 217 Table No 1	Anti HIV receptor: HIV-1 Reverse Transcriptase **(1IKV)**.	N/A	N/A
2	**QSAR study:** Removal of ligand not following Lipinski rule of five and having mutagenicity based on QSAR study	Selected 42 Molecules Table No 2 & Table No 3	HIV-1 integrase (1QS4) HIV-1 PROTEASE (1AID)	N/A	N/A
3	**Molecular docking:** Docking of ligand with lignafit	Selected 43 Molecules Table No 2 & 3	Anti tuberculosis receptor: Thymidylate Kinase **(1G3U)**. Anti Malarial receptor: Enoyl ACP Reductase **(1ZXL)**. Anti cancer receptor Neuropilin-2 (2QQJ)	231 top ranking solution	37.1774 to -465.7239

TABLE 3

WORK FLOW FOR MOLECULAR DOCKING

Ligand Library **PDB**

↓↓ ↓↓

Filtering for Lipinski rule of five **Selection of target**

↓↓ ↓↓

Toxicological and mutagenic property filter **processing for Docking**

↓↓ ↓↓

Ligand for docking study

↓↓

Docking using Ligand fit

↓↓

Gold score **Chem Score**

↓↓

Consences scoring

↓↓

Best three Ligand

↓↓

In silico ADME → selected hit for further study

4. Conclusion:

We explored 217 compounds for ***in silico*** study, among them 174 molecules were filtered through **Sarchitect Minor software**, which were not followed the Lipinski rule of 5. So, totally 43 compounds were filtered and were further examined for molecular docking against six different target receptors. Total 231 dock score were obtained from the top 10 % ligand molecules which showed highest dock score against receptive target were selected (**Table 1**). By virtual screening we would able to find two lead molecules ligand **ID 77** (N,4-bis(4-chlorophenyl)-6-(propan-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxa-mide) and 87 (N,5-bis(4-chlorophenyl)-7-(propan-2-yl)-2,3-dihydro-5H-[1,3]thiazolo[3,2a]pyrimidine-6-carboxamide). Both the ligands showed broad activity against anti-HIV, anti-tuberculosis, anti-malaria and anti-cancer receptors 1IKV, 1QS4, 1G3U, 1ZXL and 2QQJ receptively. These two ligands were of DHPM series of compounds, which also showed good antimicrobial activity.

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