INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-4, Oct-Dec-2012 Coden : IJABPT Copyrights@2012

ISSN : 0976-4550

Received: 29th August-2012 *Revised: 31st August -2012* *Accepted: 03rd September-2012*

Research article

HIGH-THROUGHPUT VIRTUAL SCREENING FOR NOVEL DHFR INHIBITORS OF *P. JIROVECI* FROM THE ZINC DATABASE

Jayaprakash Chinnappan[1] and Lakshmi Palanisamy Thanga Velan[1&2*]

1Phytomatics Laboratory, Department of Bioinformatics, Bharathiar University, Coimbatore-641 046
2Phytomatics Laboratory, Center for Bioinformatics, School of Life Sciences, Pondicherry University, Puducherry- 605 014
* Corresponding author:Lakshmi P.T.V., Center for Bioinformatics, School of Life Sciences, Pondicherry University, Kalapet,Puducherry- 605 014, India. Email:lakshmiptv@yahoo.co.in;lakanna@bicpu.edu.in ; office no: 0413 2655947 ; mobile no: 9486383094

ABSTRACT : Many of the opportunistic infections that occur at this late stage can be fatal and since that *Pneumocystis carinii* pneumonia (PCP) is a leading opportunistic infection found among immunocompromised (CD4 cell < 200) patients worldwide. DHFR is responsible for the growth and maturation of sporozoites stage (life cycle) in *Pneumocystis* as reported. Currently, 13 million chemical compounds are available for virtual screening in ZINC database. The biological information of four known drug molecules like TMP/SMX, Dapsone, Atovaquone and Pentamidine were collected from the PubChem compound database. Q-Site Finder online tool was used to determine the active site of DHFR in *P. jiroveci*. LogP values of chemical compounds were identified with the Atom-additive method. Since, existing drugs are synthetic chemicals that give more side effects in *Pneumocystis* affected patients. Polar surface area value of oxamide (86.18) was predicted to be in the ranges of existing drug values. Pentamidine was proved to be a more efficient ligand based on the dock score of -26.3398 still could not be considered as the natural compound oxamide also was highly comparable with the value of -20.3173. The binding affinity of the selected molecule was analyzed through Pose View and LigPlot.

Key words: Opportunistic infection, sporozoites, compounds, active site and oxamide.

Abbreviations : CD4: T-cells, DHFR: Dihydrofolate reductase, THFR: Tetrahydrofolate reductase, TMP/SMX: Trimethoprim/sulfamethoxazole.

INTRODUCTION

People with advanced HIV infection are vulnerable to infections and malignancies that are called opportunistic infections because they take advantage of the opportunity offered by a weakened immune system. A biased list of the world's most common HIV-related opportunistic infections and diseases includes fungal diseases such as PCP, candidiasis, cryptococcosis and penicilliosis along with HIV-associated malignancies such as Kaposi's sarcoma, lymphoma and squamous cell carcinoma. In this paper completely focused on the uncultivable disease of PCP.

Pneumonia is an inflammatory condition of the lung, especially inflammation of the alveoli or when the lungs fill with fluid (Leach RE, 2009). There are many causes of Pneumonia of which infection is the most common and infecting agents could be bacteria, viruses, fungi, or parasites (Pommerville JC, 2010). Many of the opportunistic infections that occur at this late stage can be fatal and since that Pneumocystis carinii pneumonia (PCP) is a leading opportunistic infection found among immunocompromised (CD4 cell < 200) patients worldwide has become the major killer of people with HIV extending its mortality rate to about 10% in the total population. The organism that causes human Pneumocystis pneumonia with Pneumocystis jiroveci.

The disease PCP is relatively rare in people with normal immune systems, but common among people with weakened immune systems, such as premature or severely malnourished children, the elderly, and especially persons living with HIV/AIDS, in whom it is most commonly observed (Ryan KJ, Ray CG, 2004). PCP can also develop in patients who are taking immunosuppressive medications. It almost always affects the lungs and also grows in other parts of the body such as the lymph nodes, bone marrow, spleen, liver and occasionally the eye. The most common signs and symptoms include fever, dry cough, fast heartbeat with trouble in breathing and occasionally pain or tightness in the chest that leads to weight loss, malaise and diarrhea.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

The maturation cycle of *P. jiroveci* has two major forms such as trophozoite and cyst (sporozoite). Dihydrofolate reductase (DHFR), an enzyme encoded by DHFR gene, constitutes 798 bp of DNA helps in the reduction of dihydrofolic acid to tetrahydrofolic acid and is also required for the growth and maturation of sporozoites in *Pneumocystis* as reported (Agarwal R,2005). Hence, targeting DHFR could provide a major scope in the control of its abundant growth and thereby control of the disease, has provided scope for investigating the mechanism of developing resistant to the available drugs viz., TMP/SMX, Dapsone, Atovaquone and Pentamidine that are also responsible for inducing many side effects. Subsequently, the novel plant compound identification is essential to suppress the function of DHFR in *P. jiroveci.*

Thus, a new lead molecule called the Oxamide an alkaloid was identified by using the virtual screening method. Since the small molecule proved very effective under the *in silico* docking interaction was further attempted to envisage research on it. Moreover, this particular compound was also of natural (plant based) in origin, was surfed on the literary information to look for the availability of the plant system. Thus, was revealed to be present in the seeds of *Peganum harmala.*

Methodology

Selection and preparation of lead molecules

The compound library was obtained from the ZINC database. The ZINC database is a collection of 13 million chemical compounds ready for virtual screening from different vendors. The multi-conformational molecular database used in this study was generated from the ZINC online repository (http://zinc.docking.org/) and is explained as a flowchart (Fig 1). Lead-Like Molecules (3073) were downloaded with sequence data file (SDF) format and screened against the targeted DHFR protein. The structures and biological activity of four known drug compounds (TMP/SMX, Dapsone, Atovaquone and Pentamidine) were collected from the PubChem compound database and considered in this work.

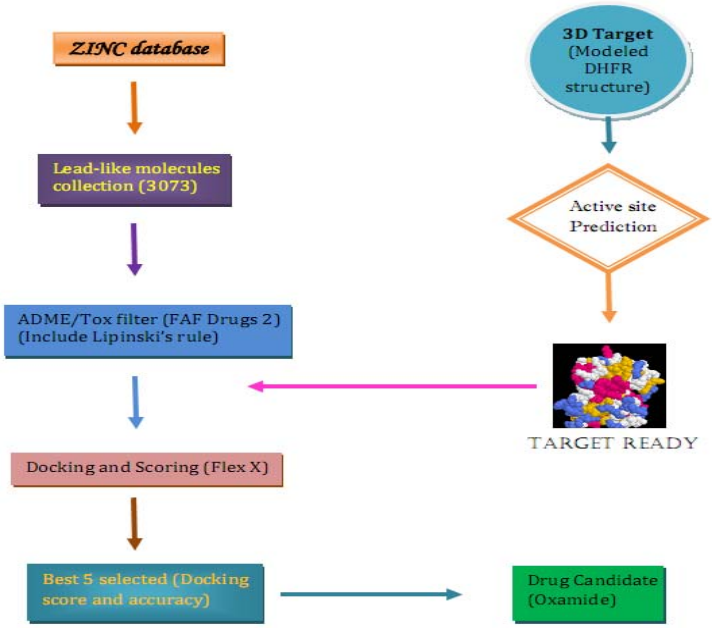


Fig. 1 Flow chart of the absolute *in silico* work of protein preparation and compounds selection

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

All the 3073 molecules were used as input for docking into the protein active site and prioritized (ranked) using a series of scoring functions for protein-ligand interactions. ADME-Toxicity was analyzed and notified upon these input parameter data's were LogP (-2 to 5), Molecular weight (150 to 500), tPSA (20 to 150), Rot bonds (0 to 10), H-Bond Acceptors (0 to 10) and H-Bond Donors (0 to 5).

Prediction of Protein Active Site using Q-Site Finder from Modeled structure

Manually, modeled DHFR protein (Swiss-Prot ID: Q9UUP5) structure was used for virtual screening analysis. After the prediction of 3-dimensional model of dihydrofolate reductase, the possible Active sites of dihydrofolate reductase was determined using Q-Site Finder (Laurie ATR, Jackson RM 2005) online tool. Finally, amino acids and the atom molecules were notified.

Virtual Screening through Flex X

Flex X software was used for molecular docking along with ligand preparation. Flex X uses Monte Carlo (MC) simulated annealing and Lamarckian genetic algorithm. A receptor description file was built using a saved dot (.) pdb file. Ligands were docked as mol2 files and prepared as discussed below. All other parameters accepted default settings for docking runs. All molecules were used as input for docking into the protein active site and prioritized (ranked) using a series of scoring functions for protein-ligand interactions and it was highlighted through Pose View (Fig 2).

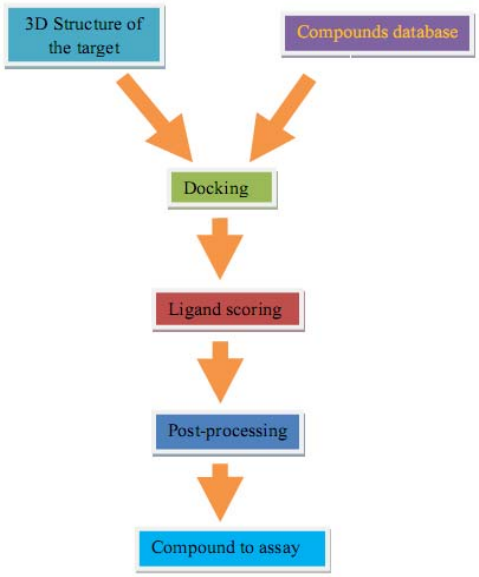


Figure. 2 Workflow of molecular docking-based virtual screening

A total of 3073 compounds from the ZINC database was interacted with the modeled structure of DHFR and evaluated the inhibitory action with score. Similarly the commercially available drugs such as TMP/SMX, Atovaquone, Dapsone and Pentamidine drugs were also checked with the DHFR protein by using the same procedure. Thus, from the 3073 molecules, although all showed interactions, the molecule (oxamide) was only considered for further analysis over Ligplot, since it was primarily a biomolecule that appeared in the first five list based on the scoring pattern.

Binding affinity of the selected lead compound

The LigPlot tool was used to generate molecular level interactions (Hydrogen bonds and hydrophobic interactions) in the docked complexes (Farmer R, et al, 2010). Labels, atoms and the bond option were satisfied and images were retrieved by LigPlot and Rasmol separately. The bond interactions were analyzed and explained below the result part.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

RESULTS AND DISCUSSION

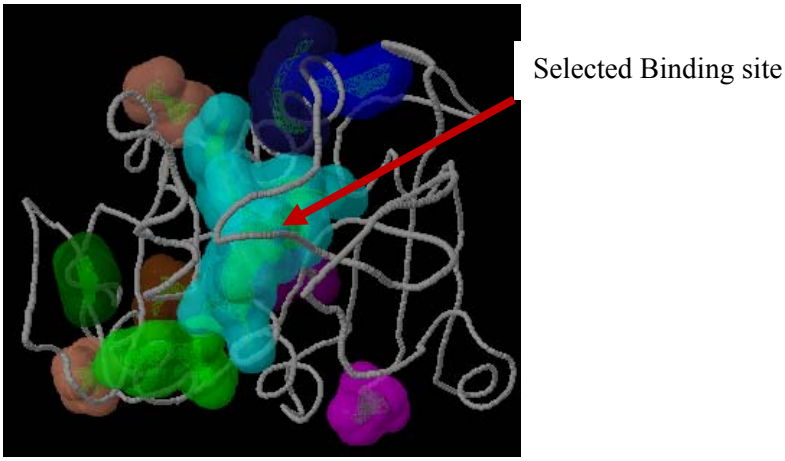
Filtering statistics of ADME/Tox properties with Lipinski's rule

Generally, ADME property needs to check the Lipinski's rule of 5 and some other parameters that comes under the FAF-Drugs tool for ADME/Tox prediction. So, here about 3073 compounds out of 13 million purchasable compounds from the ZINC library that passed the filters were used for eliminating the lead-like molecules. All the 3073 molecules were given as input to check the ADME/Toxicity property analysis. Among which, 540 molecules were identified as second copy. All (2533) molecules had molecular weight lower than 500; all molecules had less than 10 hydrogen bond acceptors; 4 molecules had more than 5 hydrogen bond donors, and 23 compounds had a partition coefficient (logP) value, greater than 5. The majority of the compounds came under the 95% confidence interval and most of the compounds followed Lipinski's Rule-of-Five. Visual inspection of randomly selected compounds revealed compounds to resemble drug compounds, as well as compounds being clearly nondrug-like (Poongavanam et al, 2009). Since, Lipinski's Rule-of-Five (Lipinski et al, 2001) is not a direct measure of drug-likeness; all molecules were subjected for docking interaction with the DHFR protein.

Drug-like molecules, according to Dr. Lipinski, refers to compounds that have sufficiently acceptable ADME properties and sufficiently acceptable toxicity properties to survive through the completion of human Phase I clinical trial. Yet, the rule of 5 only underlines properties that would make a compound a likely orally active drug in humans, but clearly these rules do not investigate functional groups that are reactive on the way in which it is metabolized. Over the years, many additional rules have thus been proposed (Oprea, 2002, Veber et al, 2002) and have been smartly combined with the rule of 5.

Active site determination from the modeled structure

The validated structure was examined for the presence of active sites through Q-site finder, which revealed about ten binding sites; first one was selected as a domain target to interact with lead molecules that were prepared from the library of ZINC database. Selected binding site was highlighted below (Fig 3) and the available amino acids and atom positions were listed below the Table 1.



* Selected binding pocket was highlighted with an arrow mark

Fig. 3 Analysis of binding sites in DHFR protein

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

This analysis showed the amino acids like Isoleucine, Leucine, Glycine, Serine and Phenylalanine to occupy at their atomic positions on the selected active site. Here, Pymol was used for graphics, labeling, and identifying H-bonds between ligand and residues at the binding site.

Table 1 Atomic composition of residues present in chosen active region

No.	Amino acid	Positions	Atomic positions of elements involved
1.	ILE	10	79 CA, 80 CB, 81 CG2, 82 CG1, 83 CD1, 84 C, 85 O
		19	143 CA, 144 CB, 145 CG2, 146 CG1, 147 CD1, 148 C, 149 O
		21	154 N, 155 CA, 160 C, 161 O
		123	962 CA, 963 CB, 964 CG2, 965 CG1, 966 CD1, 967 C, 968 O
		142	1111 CG2, 1114 C, 1115 O
2.	VAL	11	86 N, 87 CA, 88 CB, 90 CG2, 91 C, 92 O
		154	1199 N, 1200 CA, 1201 CB, 1202 CG1, 1203 CG2, 1204 C, 1205 O
3.	ALA	12	93 N, 94 CA, 95 CB, 96 C, 97 O
4.	GLY	20	150 N, 151 CA, 152 C, 153 O
		124	969 N, 970 CA, 971 C
		125	973 N, 974 CA, 975 C, 976 O
		126	977 N, 978 CA, 979 C
5.	LYS	22	163 CA, 169 C
		60	464 CA, 465 CB, 466 CG, 467 CD, 468 CE, 469 NZ, 470 C, 471 O
6.	ASN	23	171 N, 172 CA, 173 CB, 174 CG, 175 OD1, 176 ND2, 177 C, 178 O
7.	ASP	24	179 N, 180 CA, 185 C, 186 O
		32	249 CA, 250 CB, 251 CG, 252 OD1, 253 OD2, 254 C, 255 O
		153	1196 OD2
8.	LEU	25	187 N, 188 CA, 189 CB, 190 CG, 191 CD1, 192 CD2
		29	232 O
		65	508 N, 509 CA, 510 CB, 511 CG, 512 CD1, 513 CD2, 514 C
		72	564 CB, 565 CG, 566 CD1, 567 CD2
9.	TRP	27	207 CD1, 208 NE1, 211 CZ2
		62	479 N, 480 CA, 491 C, 492 O
10.	MET	33	256 N, 257 CA, 258 CB, 259 CG, 260 SD, 261 CE, 262 C, 263 O
		57	442 CB, 443 CG, 444 SD, 445 CE
11.	PHE	35	274 CB, 275 CG, 277 CD2, 279 CE2, 281 C
		199	1576 CE1
		36	283 N, 284 CA, 285 CB, 286 CG, 287 CD1, 288 CD2, 289 CE1, 290 CE2, 291 CZ
12.	SER	37	294 N, 295 CA, 296 CB, 297 OG
		64	502 N, 503 CA, 504 CB, 505 OG, 506 C, 507 O
		69	540 CB, 541 OG
13.	THR	61	472 N, 473 CA, 474 CB, 475 OG1, 476 CG2, 477 C, 478 O
		144	1121 N, 1123 CB, 1124 OG1
14.	PRO	66	516 N, 518 CD
15.	ARG	75	593 CZ, 594 NH1, 595 NH2
16.	GLU	127	981 N, 982 CA, 983 CB, 986 OE1
17.	TYR	129	1003 CD2, 1005 CE2, 1006 CZ, 1007 OH

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Selection of Active compounds by Docking

After 100 iterations, 137070 docked poses and 98 non docked molecules were retrieved through the Lead IT (Flex X). The compounds selection was made by ranking of the dock score and ADME/Toxicity analysis (Choowongkomon K, et al 2010). Lead IT- Docking finds a perfect solution on rank 1 (http://www.biosolveit.de/LeadIT/). Hydrogen bindings were proved to play an important role for the structure and function of biological molecules, especially for inhibition in a complex. Docking results indicated that out of 3073 compounds, highly scored molecules were analyzed sequentially. Thus our study confirmed that ligand molecules with the following to be ordered as 1. ZINC ID: 05830289 (3-amino-2-azanidyl-propyl) azanide score: -34.4024, 2. ZINC ID: 05848879 (2-azanidylcyclobutyl) azanide score: -31.9839, 3. ZINC ID: 06424824 (2-azanidylethyl-ethyl-azanide) score: -21.0290, 4. ZINC ID: 34140506 (1-N-methylpropane-1, 2-diamine) score: -20.7645 and 5. ZINC ID: 05177750 (oxamide) score: -20.3173 (Table 2).

Table 2 ADME/Tox properties of the best 5 ligands from Library of compounds

ZINC ID	05830289	05848879	06424824	34140506	05177750
Name	(3-amino-2-azanidyl-propyl) azanide	(2-azanidylcyclobutyl) azanide	(2-azanidylethyl-ethyl-azanide)	(1-N-methyl propane-1, 2-diamine)	(oxamide)
MW (KD)	87.12	84.12	87.14	88.15	88.07
Log P	-2.53	-1.03	-0.78	-0.71	-1.55
Log Sw	1.35	0.29	0.31	0.19	0.59
tPSA	59.22	33.20	28.63	38.05	**86.18**
Rotatable Bonds	2	0	3	2	0
Rigid Bonds	0	4	0	0	4
H Bond Donors	4	2	2	3	4
H Bond Acceptors	3	2	2	2	4
H Bonds	7	4	4	5	8
n_System Ring	0	1	0	0	0
Max Size System Ring	0	4	0	0	0
Num Charges	3	2	2	2	0
Total Charge	3	2	2	2	0
n_Heavy Atoms	6	6	6	6	6
n_carbon	3	4	4	4	2
n_hetero	3	2	2	2	4
ratioH_C	1.00	0.50	0.50	0.50	2.00
n_Lipinski Violations	0	0	0	0	0
Solubility	334642.17	112123.26	118708.00	106891.84	158947.51
Veber Rule	Good	Good	Good	Good	Good
Egan Rule	Good	Good	Good	Good	Good

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

The polar surface area (PSA) of a molecule is defined as the surface sum over all polar atoms, primarily oxygen and nitrogen, including also their attached hydrogen. PSA calculated the number of polar fragments based on 3D molecular structure (Ertl.P, et al 2000). Since oxamide has more number of Polar Regions (86.18) then the other four molecules were considered to be best among four as evidenced (Bannister D, et al 2010) was further concentrated (Fig 4). Drug distribution coefficient (LogP) strongly determines how easily the drug can reach its intended target in the body and what kind of effect could be accomplished once it reaches its target, and how long it will remain in the body in an active form (Leo et al 1971). Atom-additive method was used to predict LogP values of chemical compounds (Wang et al, 2000) and no violations against Lipinski's rule. Here, LogP values showed to be less than 5 in oxamide and therefore could be effective in *Pneumocystis* DHFR (Leeson PD, Springthorpe 2007, Edwards MP, Price DA, 2000).

Library of compounds computed descriptors:

Click on the compound ID to to get detailed compound results.

Show 10 entries Search:

#	ID	MW	logP	logSw	tPSA	Rotatable Bonds	Rigid Bonds	HB Donnors	HB Acceptors	HBonds	Rings	Max Size Ring	Charge	To Ch
1	ZINC15633215	31.06	-0.71	0.41	26.02	0	0	2	1	3	0	0	1	
2	ZINC15633213	45.08	-0.20	0.01	12.03	0	0	1	1	2	0	0	1	
3	ZINC25783052	46.07	0.08	-0.18	9.23	0	0	0	1	1	0	0	0	
4	ZINC12358605	46.07	-0.09	-0.07	20.23	0	0	1	1	2	0	0	0	
5	ZINC00901212	53.06	0.57	-0.53	23.79	0	2	0	1	1	0	0	0	
6	ZINC00895973	54.05	0.20	-0.30	17.07	0	2	0	1	1	0	0	0	
7	ZINC06072456	55.08	0.45	-0.46	23.79	0	1	0	1	1	0	0	0	
8	ZINC00895974	56.06	-0.34	0.03	20.23	0	1	1	1	2	0	0	0	
9	ZINC00897143	56.06	0.32	-0.32	17.07	1	2	0	1	1	0	0	0	
10	ZINC42989589	56.07	-0.93	0.40	49.81	0	1	2	2	4	0	0	1	

Showing 1 to 10 of 2,533 entries

First Previous 1 2 3 4 5 Next Last

Fig. 4 ADME/Toxicity result page showed the entries of filtered compounds

Among the 5 compounds, first four were of synthetic in nature, while the fifth one alone (oxamide) was identified as a natural compound that is obtained from the herb *Peganum harmala*. Since, existing drugs are synthetic chemicals that give more side effects in *Pneumocystis* affected patients (Kottom TJ, Limper AH, 2000) and fact that Oxamide passed the ADME/Tox property analysis was consequently selected a potential inhibitor for dihydrofolate reductase in *Pneumocystis jiroveci.*

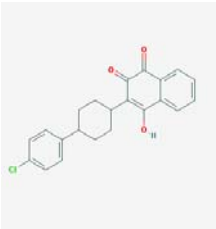
Comparison of the Physico-chemical properties of the filtered ligands

The 2D and 3D structures (Fig 5) of the known drugs - Atovaquone, Dapsone and Pentamidine were downloaded from Pubchem compound database and compared. Polar surface area value of oxamide (86.18) was predicted to be in the ranges of existing drug values of atovaquone (54.4), dapsone (94.6) and pentamidine (118) (Table 3).

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

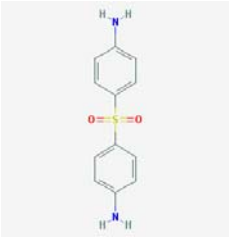
Atovaquone

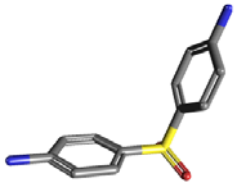
Two dimensional structure Three dimensional structure



Dapsone

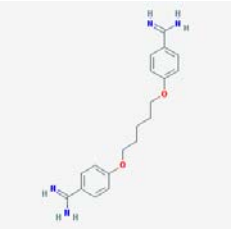
Two dimensional structure Three dimensional structure





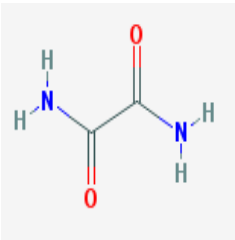
Pentamidine

Two dimensional structure Three dimensional structure



Oxamide

Two dimensional structure Three dimensional structure



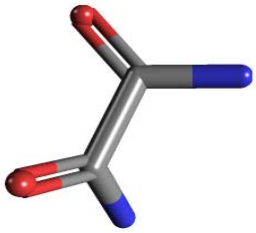


Fig. 5 2D and 3D structures of the Known drugs and screened compound

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Table 3 Pubchem compound information of Known drugs

Properties	Synthetic drugs			Natural compound
	Atovaquone	**Dapsone**	**Pentamidine**	**Oxamide**
Compound ID	CID 74989	CID 2955	CID 4735	CID 10113
Mol. Wt [g/mol]	366.83746	248.30088	340.41946	88.06536
Mol. Formula	$C_{22}H_{19}ClO_3$	$C_{12}H_{12}N_2O_2S$	$C_{19}H_{24}N_4O_2$	$C_2H_4N_2O_2$
XLogP3-AA	5.2	1	2.6	-1.6
H-Bond Donor	1	2	4	2
H-Bond Acceptor	3	4	4	2
Rotatable Bond	2	2	10	0
Tautomer Count	1000	---	---	3
Exact Mass	366.102272	248.061948	340.189926	88.027277
TPSA	**54.4**	**94.6**	**118**	**86.2**
Heavy Atom	26	17	25	6
Complexity	595	306	376	75.5
3D Ring	4	2	2	0.4
IUPAC Name	3-[4-(4-chlorophenyl) cyclohexyl]-4-hydroxynaphthalene-1,2-dione	4-(4-aminophenyl) sulfonylaniline	4-[5-(4-carbamimidoylphenoxy) pentoxy] benzene	oxamide
Canonical SMILES	C1CC(CCC1C2=CC=C(C=C2)Cl)C3=C(C4=CC=CC=C4C(=O)C3=O)O	C1=CC(=CC=C1N)S(=O)(=O)C2=CC=C(C=C2)N	C1=CC(=CC=C1C(=N)N)OCCCCCOC2=CC=C(C=C2)C(=N)N	C(=O)(C(=O)N)N

Synthetic drugs such as Atovaquone, Dapsone and Pentamidine were docked with the protein target DHFR by using Lead IT and compared with the virtually selected molecule oxamide. Even though docking does not in any way replace the need for *in vitro* and *in vivo* testing, it could contribute to a molecular understanding of bioactivity. Oxamide docking score showed highest than the synthetic drugs score (Table 4). Although, Pentamidine was proved to be a more efficient ligand based on the dock score of -26.3398 still could not be considered as the natural compound oxamide also was highly comparable with the value of -20.3173.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

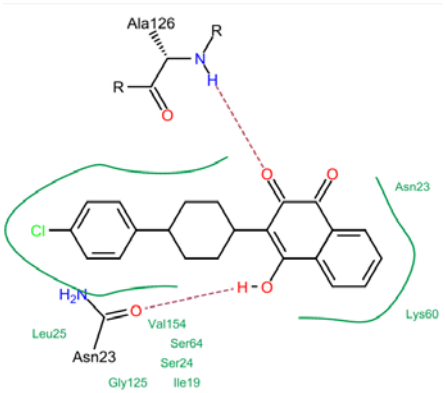
Table 4 Comparison of *in silico* interactions

Name	Atovaquone	Dapsone	Pentamidine	Oxamide
Total Poses	319	400	535	147
Rank	1			
Score	-17.2127	-14.0201	**-26.3398****	**-20.3173***
Match	-12.6783	-13.9809	-29.9910	-20.9235
Lipo	-10.4069	-7.0945	-13.9785	-2.3276
Ambig	-4.9578	-4.0520	-6.8983	-3.8226
Clash	1.2303	2.9073	7.9281	1.3564
Rot	4.2000	2.8000	11.2000	0.000
# Match	7	9	13	5

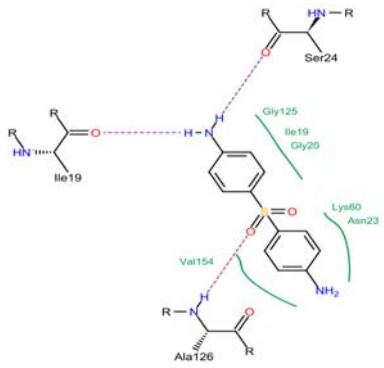
**** - highly significant value * - significant value**

Protein-Ligand efficacy analyzed in Lead IT and LigPlot

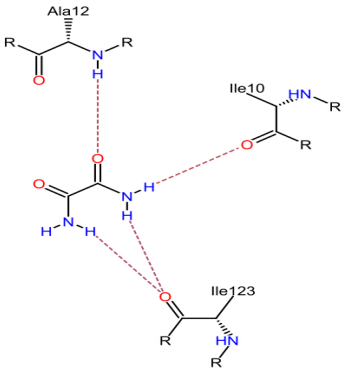
In the previous reports informed that, all the existing drugs cause many side effects. However, at this time pentamidine is suggesting for patients to cure the disease of *P. jiroveci* with HIV. So, the selected lead molecule (oxamide) was interacted with the protein target (DHFR) and compared with the existing drugs interactions (Fig 6). All the hydrogen bonds (Desiraju GR, 1996, Panigrahi SK, Desiraju GR, 2007, Panigrahi SK, 2008) and hydrophobic interactions were analyzed through the Lead IT and LigPlot (Wallace AC, 1995) tools.



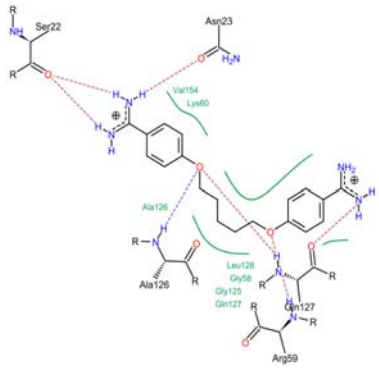
Atovaquone



Dapsone



Oxamide



Pentamidine

Fig. 6 Protein-Ligand interaction diagrams retrieved from the Flex X (Pose View)

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

The results presented here, demonstrate the hydrogen bonding and optimized hydrophobic interactions both stabilize the ligand at the protein site and help to alter the binding affinity and drug efficacy. Hydrogen bonds were manually analyzed for existing and selected molecule; the observed interactions (H…O) with the H...O distance of < 1.85 Å (Patil et al, 2010); therefore all the molecular bonds are strong interactions and cannot be broken by another bond depending upon the chemical environment.

Comparatively, Pentamidine docking score was identified as higher than the other drugs; although oxamide docking score was higher than the Atovaquone and Dapsone, has comparable variations to pentamidine score. However, both pentamidine and oxamide interactions was matched and was highlighted in the Fig 7a and b. The docked complex showed the binding affinity and the hydrophobic interactions. The energy minimized structure predicts the reliability of the docked results and the accuracy of the prediction. The binding affinity and drug efficacy related with hydrophobic interactions and it can be optimized by Qian (Qian SB et al, 2009).

Oxamide

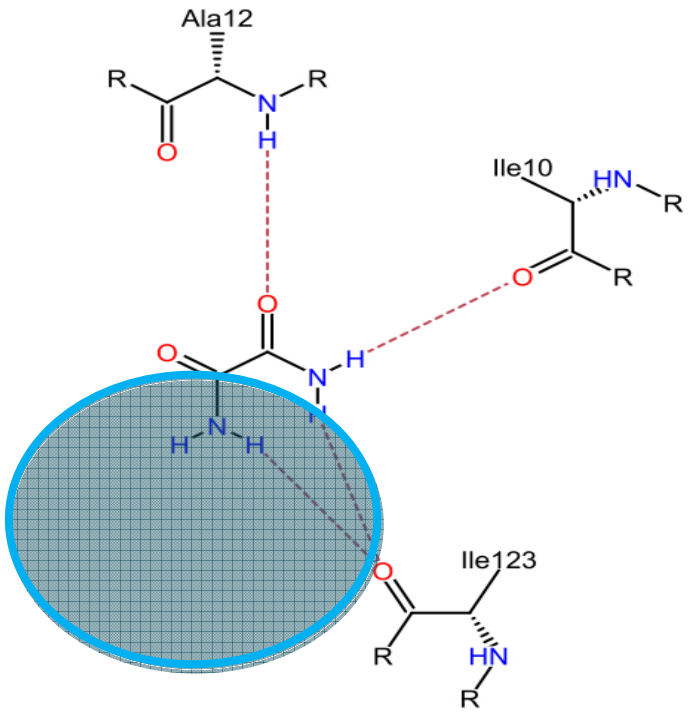


Fig. 7a Selected lead compound (oxamide) with DHFR protein interaction diagram

Pentamidine was bonded with 7 poses similar to that SER 22 oxygen (O) bound with two positions of hydrogen (H) into the pentamidine, ASN 23 Oxygen (O) bound with a single position of hydrogen (H) into the pentamidine, ALA 126 and GLN 127 hydrogen's (H) bonded with a same position of oxygen (O) into the pentamidine, ARG 59 hydrogen (H) bound with a single position of oxygen (O) into the pentamidine and finally GLN 127 oxygen (O) bound with single position of nitrogen (N) in pentamidine. Four interactions were identified in the docked complex of DHFR - Oxamide; to facilitate ALA 12 hydrogen (H) bound with single position of oxygen (O) into the oxamide, ILE 10 oxygen (O) bound with single position of hydrogen (H) into the oxamide, finally the ILE 123 oxygen (O) bonded with two hydrogen (H) positions of oxamide compound.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Pentamidine

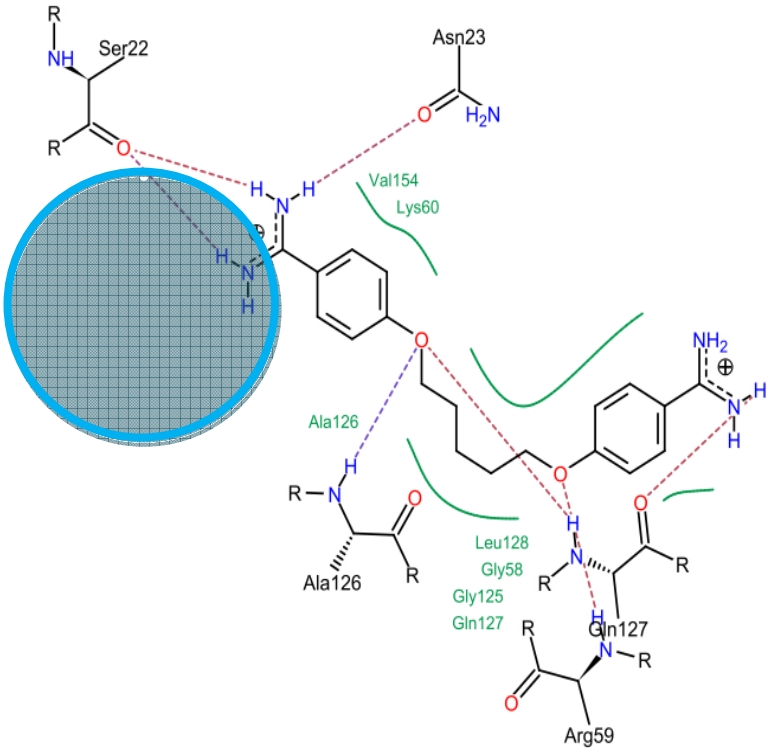
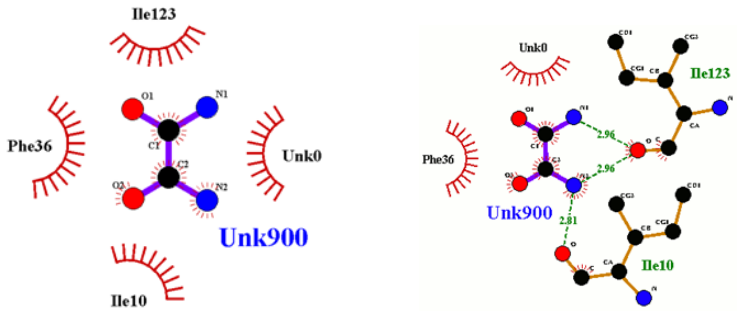


Fig. 7b Known drug (Pentamidine) with DHFR protein interaction diagram

The LIGPLOT diagram displayed the schematic diagram of all the interactions (hydrogen bonds and non bonded contacts) made between the ligand and the residues of DHFR protein molecules in the structure. Hydrogen bindings were showed as green color dashed lines labeled with the length of the bond in Å. They are calculated by the HBPLUS program which computes all possible hydrogen atoms. The complex diagram showed the bonds between oxamide and DHFR protein. Totally, it has interacted with three positions; two with Isoleucine 123 and remaining one with Isoleucine 10. The distances were found in Isoleucine 123 oxygen (O) interacted with two positions of oxamide N1 (2.96Å) and N2 (2.96Å) and single interaction of Isoleucine 10 at N2 (2.81) of oxamide. These all are hydrogen bond acceptors; which were highlighted (Fig 8).

A) **B)**



DHFR with Oxamide complex

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

C)

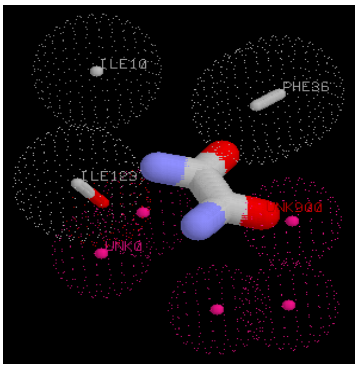


Fig. 8 Protein-Ligand interactions diagram retrieved from the LigPlot

Phenylalanine has non-bonded interaction with the oxamide. Here non-bonded contacts are defined as any contacts between ligand and protein involving either a carbon or a sulphur atom, where the interaction distance is ≤3.9Å (Chakrabarti P, Bhattacharyya R 2007). These interactions are also computed by HBPLUS. Protein residues are shown as dark red "eyelashes" whose spokes point in the general direction of the calculated atom.

CONCLUSIONS

In conclusion, these docked atoms of selected ligand (oxamide) molecule help to increase the binding affinity of the protein target-ligand molecule and optimize the hydrophobic interactions by enchanting the hydrogen bonding at the hydrophobic group of the complex. From these interaction analyses conceited oxamide to be suitable molecule to interact the DHFR functions in *P. jiroveci;* since it could be activate to the level of pentamidine functions. Overall analysis declared the newly identified screened compound value was nearest to all the known drugs. So, oxamide could be suggested for further research. Oxamide is available in *Peganum harmala* plant which has selected for preliminary analysis. Yet to confirm it to be promising, Pharmacological studies need to be performed.

ACKNOWLEDGEMENTS

We thank University Grants Commission (UGC-RGNF) at India for providing fellowship for this study.

REFERENCES

Leach RE (2009) Acute and Critical Care Medicine at a Glance (2nd edition). *Wiley-Blackwell*

Pommerville JC (2010) Alcamo's Fundamentals of Microbiology (9 edition). Sudbury, Mass: Jones & Bartlett Publishers 323

Ryan KJ, Ray CG (2004) Sherris Medical Microbiology (4th edition). McGraw Hill 247-9

Agarwal R (2005) The Acronym PCP is Pneumonia, Not Pneumonia-Pneumocystis carinii. American Journal of Roentgenology 185(6):1653-1654

Laurie ATR, Jackson RM (2005) Q-Site Finder: an energy-based method for the prediction of protein-ligand binding sites. Bioinformatics 21(9):1908-1916

Farmer R, Gautam B, Singh S, Yadav PK, Jain PA (2010) Virtual screening of AmpC/β-lactamase as target for antimicrobial resistance in Pseudomonas aeruginosa. Bioinformation 4(7):290-294

Poongavanam V, Taboureau O, Oostenbrink C, Vermeulen NPE, Olsen L, Jorgensen FS (2009) Classification of Cytochrome P450 1A2 Inhibitors and Noninhibitors by Machine Learning Techniques. Drug Metabolism and Disposition 37(3):658-664.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 46(1-3):3-26

Oprea TI (2002) Virtual screening in lead discovery: a view point. Molecules 7:51-62

Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry 45:2615-2623

Choowongkomon K, Sawatdichaikul O, Songtawee N, Limtrakul J (2010) Receptor-Based Virtual Screening of EGFR Kinase Inhibitors from the NCI Diversity Database. Molecules 15:4041-4054

Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum fragment-based contributions and its application to the prediction of drug transport properties. Journal of Medicinal Chemistry 43:3714-3717

Bannister D, Popovic B, Sridharan S, Giannotta F, Filee P, Yilmaz N, Minter R (2010) Epitope mapping and key amino acid identification of anti-CD22 immunotoxin CAT-8015 using hybrid b-lactamase display. Protein Engineering, Design and Selection 24(4):351-60

Leo A, Hansch C, Elkins D (1971) Partition coefficients and their uses. Chemical Reviews 71(6):525-616

Wang R, Gao Y, Lai L (2000) Calculating partition coefficient by atom-additive method. Perspectives in Drug Discovery and Design 19:47-66

Leeson PD, Springthorpe B (2007) The influence of drug-like concepts on decision-making in medicinal chemistry. Nature Reviews Drug Discovery 6:881-890

Edwards MP, Price DA (2010) Role of Physicochemical Properties and Ligand Lipophilicity Efficiency in Addressing Drug Safety Risks. Annual Reports in Medicinal Chemistry 45:381-391

Kottom TJ, Limper AH (2000) Cell Wall Assembly by Pneumocystis carinii: Evidence for a Unique Gsc-1 Subunit Mediating Beta-1, 3-Glucan Deposition. The Journal of Biological Chemistry 275(51):40628-40634

Desiraju GR (1996) The C-H…O hydrogen bond: Structural implications and supramolecular design. Accounts of Chemical Research 29:441-449

Panigrahi SK, Desiraju GR (2007) Strong and weak hydrogen bonds in the protein-ligand interface. Proteins 67(1):128-141

Panigrahi SK (2008) Strong and weak hydrogen bonds in protein-ligand complexes of kinases: a comparative study. Amino Acids 34(4):617-633

Wallace AC, Laskowski RA, Thornton JM (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Engineering 8(2):127-134

Patil R, Das S, Stanley A, Yadav L, Sudhakar A, Varma AK (2010) Optimized Hydrophobic Interactions and Hydrogen Bonding at the Target-Ligand Interface Leads the Pathways of Drug-Designing. PLoS ONE 5(8):e12029

Qian SB, Waldron L, Choudhary N, Klevit RE, Chazin WJ, Patterson C (2009) Engineering a ubiquitin ligase reveals conformational flexibility required for ubiquitin transfer. Journal of Biological Chemistry 284(39):26797-26802

Chakrabarti P, Bhattacharyya R (2007) Review: Geometry of non-bonded interactions involving planar groups in proteins. Progress in Biophysics and Molecular Biology 95(1-3):83-137.

Webography

http://zinc.docking.org/
http://www.biosolveit.de/LeadIT/