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Silico Docking Studies of Few Antitrypanosomal Inhibitors Obtained from *Eucalyptus Tereticornis* by using Bioinformatics Softwares

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Abstract : Human's trypanosomiasis or sleeping sickness is a protozoan disease which is caused primarily by *Trypanosoma brucei* in India. Trypanosomiasis is re-emerging in India due to the proximity of domesticated animals with the people. Though the human infection through animal vector is unlikely, still there are number of report of a distinctive human infection caused by animal trypanosomes in recent times. As the available medication is either too expensive or possess side effects, there is an urgent need for the discovery of novel natural drugs. In this research, a study was performed to identify an efficient drug molecule possessing antitrypanosomal activity. From the literature study, plant Compounds of *Eucalyptus tereticornis* which is well known for its antiparasitic property have been identified and its structure was retrived and tested for its drug likeness activities. The proteins responsible for causing Trypanosomiasis were identified, their structures were retrieved and their binding sites were predicted. *In Silico* molecular docking was performed and the result demonstrated a particular target revealing exceptional binding affinity with the inhibitors in contrast to other targets. Further, clinical studies will confirm the inhibitors efficiency as a superior contender for development of improved drug against Trypanosomiasis.
Keywords: Human's trypanosomiasis, *Eucalyptus tereticornis, Trypanosoma brucei,* Trypanosomal Inhibitors, Molecular Docking.

Introduction:

In India Trypanosomiasis is caused by *Trypanosoma brucei* and remains as the world's most neglected parasitic diseases, endangering millions of people and livestock in India and sub-Saharan Africa[1]. Even though encouraging lead compounds to fight the disease have been revealed in the recent times, the current treatments are still proving to be unproductive and have serious adversarial effects. Thus, hunting for antitrypanosomal agents is need of the hour and requires constant effort[2]. In third world countries, protozoal infections particularly trypanosomiasis is a foremost health problem and the available drugs for the treatment are Suramin, Pentamidine, Melarsoprol and Eflornithine, but these drugs have side effects and are prone to resistance, so therefore treating trypanosomiasis has been a big challenge for long time[3,4,5.] A number of drug targets have been identified in *T. brucei* like kinase ,pteridine reductase, dihydrofolate reductase, trypanothione reductase, cathepsin B , heat shock protein 90, as well as sterol 14α-demethylase, nucleoside hydrolase, triose phosphate isomerase, nucleoside 2-deoxyribosyltransferase, UDP-galactose 4′epimerase and ornithine decarboxylase [6,7,8,9,10]

The small molecules were taken from plant *Eucalyptus tereticornis*. The *Eucalyptus* is a traditional Aboriginal remedy for a variety of ailments[11]. Internally *Eucalyptus* appears to help relieve symptoms of colds,

flu, chest congestion, sore throat, bronchitis, pneumonia, and respiratory infections. *Eucalyptus* can be made into a tea or tincture. Externally, the antiseptic, slightly anaesthetic, anti-bacterial, and warming properties of *Eucalyptus* make it a valuable resource treatment of burns, sores, ulcers, scrapes, boils, and wounds.[12]

Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest[13]. Lately the scientific focus has turned on the application of virtual docking methods which are less intensive on labour, time and cost[14, 15]. This work presents the molecular docking of antitrypanosomal natural products into *T. brucei* drug targets with a view of obtaining small molecules that indeed interact with these targets. This will not only help in designing natural drugs but also in compound isolation schemes for target-based bioassays, which could lead to the isolation of potent antitrypanosomal agents that can become leads for drug development.

Materials and Methods:

Protein Preparation

The three dimensional crystal structures for various trypanosomal proteins were retrived from the PDB database were used as the target for docking studies. All solvent molecules and the co-crystallized ligands were removed from the structures in order to be used as a receptor for docking, protein structures should be processed. Some of the typical operations include (i) addition of hydrogen atoms, (ii) elimination of water molecules that are not involved in ligand binding (iii) making binding groups were done using arguslab. For all the above target proteins, its active site was predicted using Metapocket[27].

Ligand Preparation:

The five small molecules (p-cymene, Euglobal, Betulonic acid, Beta-pinene, Alpha-pinene) were identified from the Pubmed literatures which have good inhibitory effects towards protein trypanosome[28, 29, 30.31.32.] They were downloaded from the Pubchem database in (.SDF format)[33] using PYMOL[34], all the 3D structure of the molecules were viewed and converted to PDB format and these molecules are further tested for its ADMET (Druglikness test) properties using AdmetSAR[35] which was done base on Lipinski's rule of five. The rule states that an active oral drug should not violate more than one following criteria i,e., Molecular Weight < 500 Daltons, Log P >5, H-bond Donor >5, H-bond Acceptor >10, No of Rotatable Bonds >5.

Protein-Ligand Interaction Using Autodock vina PyRx:

The compounds obtained from the plants were docked against the proteins using AutoDock Vina, to find the reasonable binding geometries and explore the protein-ligand interactions.[36] Docking of the protein-ligand complex was mainly targeted to the predicted active site only. The selected residues of the receptor were defined to be a part of the binding site. After completion of docking, the docked protein (protein-ligand complex) was analysed using Pymol to investigate the type of interactions. The docking poses saved for each compound were ranked according to their dock score function and best docking result were further analysed using Arugus lab.

Results and Discussion:

Preparation of targets & ligands for docking studies.

The three Dimensional structures of the trypanosomal protein used as targets for docking studies were shown in the table 1. For further study, the binding site of the protein was predicted using Metapocket. The binding sites of target protein comprises of amino acids which has been listed below in table 2a & b. The Three dimensional ligand structures obtained through literature search are shown in table.3 and all the above compounds pass its drug likeness test which was performed based on Lipinski's rule of five using AdmetSAR[35]

Table 1: PDB ID for target trypanosomal proteins with its Function

Sl.no	Protein name	PDB ID	Function
1.	TbAMP - *T.brucei* Adenosine Monophosphate	2XTB[16]	Catalysis the phosphorylation of ingested adenosine to form adenosine monophosphate as the preferred phosphoryl donor
2.	TbAK - *T.brucei* Adenosine Kinase	3OTX[17]	Key player in purine salvage
3.	TbPTR1 - *T. brucei* Pteridine Reductase 1	3JQ7[18]	Catalyse a two-step reduction of oxidisedpterins to active tetrahydropterin
4	TbTR - *T.brucei* Trypanothione Reductase	3QFX[19]	Immunity and hypersensitivity
5.	TbDHFR - *T.brucei* Dihydrofolate Reductase	3RG9[20]	Its role in thymidine biosynthesis is the reduction of dihydrofolate to tetrahydrofolate using the cofactor NADPH
6.	TbDHFD- *T. brucei* Dihydrofolate Dehydrogenase	3MHU[21.]	Pyrimidine biosynthesis
7.	TbHSP90 - *T. brucei* Heat Shock Protein 90	3OPD[22]	It is involved in the folding of key molecules of the cellular signal transduction system such as kinases and steroid receptors F HSP90
8.	*T.brucei* Tyrosine Kinase Nonreceptor tyrosine	3FZ0[23]	It is implicated in multiple signallingpathways.It is a negative regulator of osteogenesis
9.	TbTIM - *T.brucei* Triosephosphate Isomerase	1IIH[24]	It catalyses the interconversion of dihydroxyacetonephosphate and glyceraldehyde-3-phosphate
10.	TbODC - *T.brucei* Ornithine Decarboxylase	1NJJ[25]	They are important for stabilizing DNA structure, the DNA double strand-break repair pathway

Note: A prefix present in the PDBID represents the references of the particular protein.

Table 2a: The active site of proteins predicted using Metapocket

SL No	Amino Acid	Active Sites of Target Proteins with Its Positions									
		3JQ7	**2XTB**	**1NJJ**	**3RG9**	**3QFX**	**3MHU**	**3OPD**	**3OTX**	**1IIH**	**3ZFO**
1	Cystine	-	25, 33,140	-	20, 37, 78, 102, 111, 136, 153, 198	59, 84, 95, 100, 107, 183	25, 33,140	-	12, 123,239	25, 33,140	20, 56, 88
2	Argenine	89, 98, 106, 108, 192, 216	57, 60, 68, 71, 98, 109, 283	33, 35, 106, 108, 192, 216, 289	223, 245, 265, 316, 332	-	265, 316	133	7, 34, 58 70, 94, 132, 156, 265, 316, 332	57, 60, 68, 71, 98, 109, 283,	-
3	Asparagine	-	125, 278, 291, 329	-	13, 56, 67, 195, 222,	-	225, 279, 268, 241, 228,	90, 305	13, 56, 67, 195, 222,	125, 278, 291, 329	-

					231, 295				231, 295		
4	Leucin	90, 97, 105, 137, 168	-	105, 137, 168, 190,	7, 34, 58 70, 94, 132, 156	90, 97, 105	-	85, 93, 168	15, 16, 39, 134, 138, 286	-	-
5	Aspergine	46, 86, 164, 184	-	46, 86, 164, 184	17, 92, 238, 266, 287, 289, 293,2 99	43, 45, 54, 88, 120	-	-	17, 92, 238, 266, 287, 289, 293, 299	-	-
6	Serine	43, 45, 54, 88, 120	-	43, 45, 54, 88, 120	15, 16, 39, 134, 138	89, 98, 106, 108, 192, 216	-	-	19, 64, 197, 269	-	-
7	Alanine	34, 226	19,6 4, 197, 269,	34, 226	28619 , 64,19 7, 269,1 23,23 9	34, 226	108, , 3301 25, 278, 291, 329	-	20, 37, 78, 102, 111,1 36, 153,1 98, 157,2 21,	19, 64, 197, 269	34, 56, 123, 226
8	Histidine	52, 60, 101	21, 105, 114, 224, 323	52, 60, 101	21, 105, 114, 224, 323	182	71, 98, 109, 283, 240,	-	21, 105, 114, 224, 323	21, 105, 114, 224, 323	136, 161, 162, 163
9	Glucine	-	225, 279, 268, 241, 228, 328, 339	-	33, 101, 104,1 06, 131,1 60, 225,2 79, 268,2 41, 228,3 28, 339	-	200, 74	28,51 , 118, 160, 165	33, 101, 104,1 06, 131,1 60, 225,2 79, 268,2 41, 228,3 28, 339	160, 225, 279, 268, 241,	-

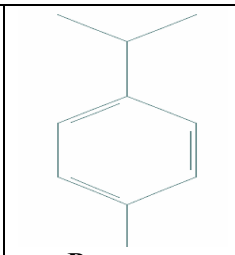
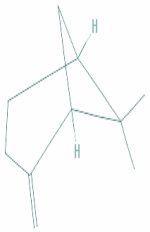
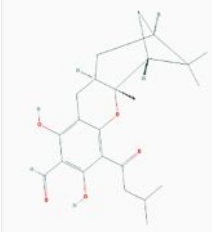
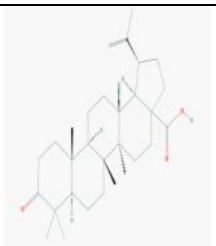
10	Glycine	42, 44, 45, 83, 136, 161, 162, 163	35, 107, 129, 298, 296	42, 44, 136, 161, 162, 163	35, 62, 63, 81, 107, 129, 298, 296	42, 44, 45, 83, 136, 161, 162, 163	80, 285, 288, 327340	-	35, 62, 63, 81, 107, 129, 298, 296	35, 62, 63, 81, 107, 129, 298, 296	42, 44, 45, 83
11	Threonine	46, 86, 164, 184	36, 85, 172, 264, 270, 280,	46, 86, 164, 184	36, 85, 172, 264, 270, 280, 325	46, 86, 164, 184	169, 110, 294, 302	-	36, 85, 172, 264, 270, 280, 325	36, 85, 172, 264, 270, 280, 325	46, 86, 164, 184
12	Isolucine	41, 47, 51, 118, 160, 165	38, 90, 108, , 330, 125,	50, 41, 47, 51,	38, 90, 108, 127, 267, 292	41, 47, 51, 118, 160, 165	59, 79, 95, 165	-	38, 90, 108, 127, 267, 292, 330	33, 101, 104, 106, 131	28,51, 118, 160, 165

Table 2b: The active site of proteins comprises of amino acid were predicted using Metapocket

Sl No	**Amino Acid**	**Active Sites of Target Proteins with its Positions**									
		3JQ7	**2XTB**	**1NJJ**	**3RG9**	**3QFX**	**3MHU**	**3OPD**	**3OTX**	**1IIH**	**3ZFO**
13	Proline	-	55, 61, 199, 282, 284, 338, 127, 267,	-	55, 61, 199, 282, 284,	48, 52, 91, 92,119	55, 61, 199, 282,	42, 44, 45, 83	55, 61, 199, 282, 284, 338	33, 81, 27, 267, 292	-
14	Valine	-	57, 60, 68, 71, 98, 109, 283, 240,	-	57, 60, 68, 71, 98, 109, 283	32, 33, 195	284, 338, 127, 267, 292	-	57, 60, 68, 71, 283,240, 125,278, 291, 329	57, 60, 68, 71, 109, 283, 240	-
15	Tyrosine	-	59, 79, 95, 165	-	125, 278, 291	-	-	-	59, 79, 95, 165	59, 79, 95, 165	-
		50, 234	73, 77, 203, ,		73, 77,	50, 234	33, 101,	-	73, 77,	73, 77,	52, 60,

16	Glutamine		82, 97, 100, 130, 227	234	203, 285, 288, 327		104, 106, 131		203, 285, 288, 327	203, , 82, 97, 100, 130	101 50, 234
17	Tyrpsin	30, 168	301	30, 168	294, 302	57, 166	-	-	74	11, 33, 62	30, 123, 235
18	Lysine	85, 93, 123, 235	80, 285, 288, 327340	55, 98, 133	80, 82, 97, 100, 130, 227, 340	85, 93, 123, 235	-	136, 161, 162, 163	80, 82, 97, 100, 130, 227, 340	80, 285, 288, 32, 73, 40	85, 93, 168
19	Methionine	55, 90, 100, 105	200, 74	55, 90, 100,	111, 265	55, 82	-	-	110, 294, 302	-	155, 2 90, 305
20	Phenylalanine	55, 98, 133	169, 110, 294, 302	85, 93, 123, 235	169, 200, 301	58, 94, 233	-	34, 56, 123, 226	169, 200, 301	-	133

Table 3: Shows the 3 D structure of compound with its name and pubchem ID

 **Alpha Pinene CID: 6654**	 **P-cymene CID: 7463**	 **Beta Pinene CID: 14896**	 **Euglobal CID: 10068739**	 **Betulonic Acid CID: 9933683**

Docking Analysis using Autodock vina PyRx;

The interactions between the ligands and the trypanosomal proteins were explored to check their binding affinity using docking software Autodock vina PyRx. The docking results exhibited the interaction between proteins (10) and plant compounds (5) *Eucalyptus terticornis*. The Interaction between all the 50 docking complexes were compared with each other based on their binding energy which in turn depends on the interaction of hydrogen bond between protein and ligands. The binding energy obtained by interaction between the ten trypanosomal proteins and the five plant compounds were compiled in the Table4.

By using Arguslab the docking results were compared to see which interaction yielded the best binding energy, out of fifty dockings executed only two docking results exhibited the binding energy with its hydrogen bond interaction as expected with the binding energy of < 12 shown in Table 5. The best docking interaction were further analysed for finding their interaction. The best docking interaction between betulonic acid with trypanothione reductase were shown in Figure 1, exhibiting a binding energy of -15.66 kcal/mol with hydrogen bond interaction of 2.9. The next best docking interaction between Euglobal and Adenosine kinase were shown in Figure 2 giving a binding energy of -12.24 kcal/mol with hydrogen bond interaction of <2.4.

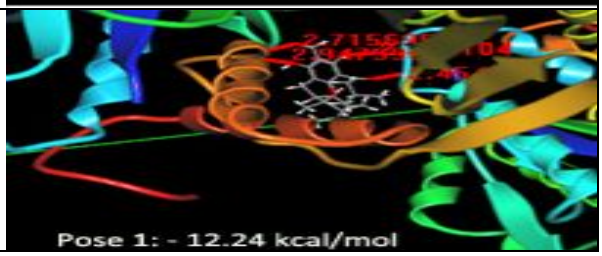
On analyzing the docking result it was predicted that among 10 trypanosomal protein, two protein (trypanothione reductase Adenosine kinase) play a mojor role in causing Humans trypanosomiasis. The protein

trypanothione reductase play a mojor role in the process of Immunity and hypersensitivity of the human body[14] and the other protein, Adenosine kinase is the key player in purine salvage[16], *Trypanosoma brucei* when it enters in the human body, first affect immune system where trypanothione reductase plays a mojor role[38] and later terminate the Nucleotide salvage by inhibiting the purine salvage where Adenosine kinase act as the main enzyme[39]. The two small molecules predicted by this study were Betulonic acid and Euglobal. Betulonic acid is the the most potent derivative which act as antitrypanosomial agents[40] and another derivative euglobal also exhibit good antitrypanosomial activity[41] when compared with other three derivative.

Table 4: Docking result of ligand and receptor using Autodock vina PyRx

Protein	Ligand (kcal/mol)				
	Betulonic Acid	**Euglobal**	**Beta pinene**	**Alpha pinene**	**P- Cymene**
3JQ7	-7.698	-8.24	-8.313	-10.18	-8.6045
2XTB	-9.544	-9.042	-10.31	-8.844	-8.386
1NJJ	-8.386	-7.24	-9.313	-10.18	-10.31
3RG9	-8.313	NALP	-8.6045	-10.41	-10.15
3QFX	-15.66	-9.24	-9.10	-9.121	NALP
3MHU	NALP	-10.24	NALP	-8.18	NALP
3OPD	-6.325	-6.589	NALP	-9.122	-8.045
3OTX	-7.66	-12.24	-10.313	-10.3418	-10.604
1IIH	-7.38	-7.20	-9.114	-9.364	-8.857
3ZFO	-10.12	-9.982	NALP	-10.3418	NALP

Table 5: Best docking result of ligand and receptor using Arguslab

Sl No	Protein	Ligand	Binding Energy with H bond interaction
1	Trypanothione Reductase	Betulonic acid	
2	Adenosine kinase	Euglobal	

Conclusion:

The *In silico* docking results finally predicted the compounds that best fits with target proteins by interacting to each other with higher affinity in the active site of the target protein. The two small active molecules predicted by this study Betulonic acid and Euglobal exhibits strong inhibitory activity against trypanosomal Proteins (Trypanothione Reductase & Adenosine kinase). Those two molecules can be further tested in wet lab experiment for the designing and development of novel compounds having better inhibitory activity against several type of trypanosomiasis.[42] These potential drug candidates can be further be validated in wet lab studies for its proper function.

Reference:

1. Barrett, M.P., Boykin, D.W., Brun, R., Tidwell, R.R. 2007. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *British Journal of Pharmacology. 152*, 1155-1171.

2. Koehn, F.E and Carter, G.T., 2005. The evolving role of natural products in drug discovery. *Nature Reviews Drug Discovery. 4*, 206-220.
3. Peter Babokhov., Adekunle O Sanyaolu., Wellington A Oyibo., Adetayo F Fagbenro-Beyioku, and Nnaemeka C Iriemenam., 2013. A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis *Pathog Glob Health.* 107, 242–252.
4. Wafo, P., Nyasse, B., Fontaine, C., Sondengam, BL., 1999. Aporphine alkaloids from *Enantia chlorantha*. *Fitoterapia* 70. 157–160.
5. Wink, M., 2007. Molecular modes of action of cytotoxic alkaloids: From D37A intercalation, spindle poisoning, topoisomerase inhibition to apoptosis and multiple drug resistance. Alkaloids *ChemBiol.* 64, 1–47.
6. William, N., Setzer, Ifedayo, V., Ogungbe, 2012. *In-silico* Investigation of Antitrypanosomal Phytochemicals from Nigerian Medicinal Plants. *PLoS Negl Trop Dis.* 6, e1727.
7. González, AG., Aguiar, ZE., Grillo, TA., Luis, JG., Rivera, A., et al. 1991. Methoxyflavones from *Ageratum conyzoides*. *Phytochemistry,* 30, 1269–1271.
8. Iwu ,M., Igboko, O., (1982) Flavonoids of *Garcinia kola*. *J Nat Prod*. 45, 650–651.
9. Zhang, B., Yang, SP., Yin, S., Zhang, CR., Wu, Y., et al. 2009. Limonoids from *Khayai vorensis. Phytochemistry* 70, 1305–1308.
10. Cimanga, K., De Bruyne T., Lasure, A., Li, Q., Pieters, L., et al. 1995. Flavonoid O-glycosides from the leaves of *Morinda morindoides*. *Phytochemistry.* 38, 1301–1303.
11. Dawson, A, Gibellini, F, Sienkiewicz, N, Tulloch, LB, Fyfe, PK, et al., 2006. Structure and reactivity of *Trypanosoma brucei* pteridine reductase: Inhibition by the archetypal antifolate methotrexate. *MolMicrobol.* 61, 1457–1468
12. Alexandra Lüscher, Pinar Önal, Anne-Marie Schweingruber, and Pascal Mäser. 2007. Adenosine Kinase of Trypanosoma brucei and Its Role in Susceptibility to Adenosine Antimetabolites. Antimicrob Agents Chemother. 51, 3895–3901
13. Gamarro, F, Yu PL, Zhao J, Edman, U, Greene, PJ, et al., 1995. *Trypansoma brucei* dihydrofolate reductase-thymidylate synthase: Gene isolation and expression and characterization of the enzyme. *MolBiochemParasitol.* 72, 11–22.
14. Krieger S, Schwarz W, Ariyanayagam MR, Fairlamb AH and Krauth-Siegel RL, et al. 2000. Trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidativestress. *MolMicrobiol* 35, 542–552.
15. Mackey, ZB, O Brien, TC, Greenbaum, DC, Blank, RB and McKerrow, JH., 2004. A cathepsin B-like protease is required for host protein degradation in *Trypanosoma brucei*. *J BiolChem,* 279, 48426–48433.
16. Tulloch, LB., Martini, VP., Iulek, J., Huggan, JK., Lee, JH., et al. 2010. Structure-based design of pteridine reductase inhibitors targeting African sleeping sickness and the leishmaniases. *J Med Chem* 53, 221–229.
17. S. Kuettel, J. Greenwald, D. Kostrewa, S. Ahmed, L. Scapozza, and R. Perozzo, "Crystal structures of T. b. rhodesiense adenosine kinase complexed with inhibitor and activator: implications for catalysis and hyperactivation," PLoS Neglected Tropical Diseases, vol. 5, no. 5, Article ID e1164, 2011.
18. Vandemeulebroucke, A., Minici, C., Bruno, I., Muzzolini, L., Tornaghi, P., et al. (2010) Structure and mechanism of the 6-oxopurine nucleosidase from *Trypanosoma brucei*. *Biochemistry* 49, 8999–9010.
19. Patterson, S., Alphey, MS., Jones, DC., Shanks, EJ., Street IP., et al. 2011. Dihydroquinazolines as a novel class of *Trypanosoma brucei* trypanothione reductase inhibitors: Discovery, synthesis, and characterization of their binding mode by protein crystallography. *J Med Chem.* 54, 6514–6530.
20. Vanichtanankul J, Taweechai S, Yuvaniyama J, Vilaivan T, Chitnumsub P, et al. (2011) Trypanosomal dihydrofolate reductase reveals natural antifolate resistance. ACS Chem Biol 6: 905–911.
21. Kerr, ID., Wu, P., Marion-Tsukamaki R., Mackey, ZB., Brinen, LS., (2010) Crystal structures of TbCatB and rhodesain, potential chemotherapeutic targets and major cysteine proteases of *Trypanosoma brucei*. *PLoSNegl Trop Dis.* 4, e701.
22. JC PizarroAK WernimontA. HutchinsonH. SullivanK. Chamberlain 2011 Crystal structure of the N-terminal domain of an HSP90 from Trypanosoma brucei, Tb10.26.1080 in the presence of a benzamide derivative. To be published, DOI:10.2210/pdb3opd/pdb
23. Seungil Han , Anil Mistry, Jeanne S. Chang, David Cunningham, Matt Griffor, et al., 2009. Structural Characterization of Proline-rich Tyrosine Kinase 2 (PYK2) Reveals a Unique (DFG-out) Conformation and Enables Inhibitor Design. *Journal Of Biological Chemistry.* 284, 13193 - 13201

24. Vandemeulebroucke, A., Minici, C., Bruno, I., Muzzolini, L., Tornaghi, P., et al. (2010) Structure and mechanism of the 6-oxopurine nucleosidase from *Trypanosoma brucei*. *Biochemistry* 49, 8999–9010.
25. Noble, MEM., Verlinde, CL., Groendijk, H., Kalk, KH., Wierenga, RK., et al. 1991. Crystallographic and molecular modeling studies on trypanosomal triosephosphate isomerase: A critical assessment of the predicted and observed structures of the complex with 2-phosphoglycerate. *J Med Chem* 34, 2709–2718.
26. Abdelouahab C and Abderrahmane. 2008. Docking Efficiency Comparison of Surflex, a Commercial Packageand Arguslab, a Licensable Freewar. *J Comput Sci Syst Biol* 1: 081-086.
27. Bingding Huang. MetaPocket: A Meta Approach to Improve Protein Ligand Binding Site Prediction. *OMICS: A Journal of Integrative Biology*. 2009, 13(4): 325-330.
28. Salem, MM, Davidorf, FH, Abdel-Rahman, MH., 2011. In vitro anti-uveal melanoma activity of phenolic compounds from the Egyptian medicinal plant *Acacia nilotica*. *Fitoterapia* 82, 1279–1284.
29. Bourdat-Deschamps, M., Herrenknecht, C., Akendengue, B., Laurens, A., Hocquemiller, R., 2004. Separation of protoberberine quaternary alkaloids from a crude extract of *Enantia chlorantha* by centrifugal partition chromatography. *J Chromatogr A,* 1041, 143–152.
30. Vyas, AV., Mulchandani, NB., 1986. Polyoxygenated flavones from *Ageratum conyzoides*. *Phytochemistry*. 25, 2625–2627.
31. Ogbadoyi, EO., Garba, MH., Kabiru, AY., Mann, A., Okogun, JI., 2011. Therapeutic evaluations of *Acacia nilotica (Linn)* stem bark extract in experimental African trypanosomiasis. *Int J Appl Res Nat Prod* 4, 11–18.
32. Nour, AMM., Khalid, SA., Kaiser, M., Brun, R., Abdalla, WE., et al. 2010. The antiprotozoal activity of methylated flavonoids from *Ageratum conyzoides* L. *J Ethnopharmacol* 129, 127–130.
33. Yanli Wang, Jewen Xiao, Tugba O. Suzek, Jian Zhang, Jiyao Wang and Stephen H. Bryant 2009, PubChem: a public information system for analyzing bioactivities of small molecules. *Nucleic Acids Research,* 37, W623–W633.
34. Daniel Seeliger and Bert L. de Groot. 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des*. 24, 417-422
35. Christopher A. Lipinski,2000, Drug-like properties and the causes of poor solubility and poor permeability; Journal of Pharmacological and Toxicological Methods; 44,235-249.
36. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. *Methods Mol Biol*. 2015; 1263:243-50. doi: 10.1007/978-1-4939-2269-7_19.
37. Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24: 1596–1599.
38. Stephen Patterson., Magnus S. Alphey., Deuan C. Jones., Emma J. Shanks., Ian P. Street., et al 2011Dihydroquinazolines as a Novel Class of *Trypanosoma brucei T*rypanothione Reductase Inhibitors: Discovery, Synthesis, and Characterization of their Binding Mode by Protein Crystallography. *J. Med. Chem*, 54. 6514–6530.
39. David W. Parkin 1996. Purine-specific Nucleoside N-Ribohydrolase from *Trypanosoma brucei brucei* purification, specificity, and kinetic mechanism. *The Journal Of Biological Chemistry*. 271, 21713–21719.
40. Sami Alakurtti., Pia Bergstro., Nina Sacerdoti-Sierra., Charles L Jaffe and Jari Yli-Kauhaluoma. 2010. Anti-leishmanial activity of betulin derivatives *The Journal of Antibiotics*. 63, 123–126.
41. Sandip B. Bharate., Kamlesh K. Bhutani., Shabana I. Khan., Babu L. Tekwani.,Melissa R. Jacob., 2006. Biomimetic synthesis, antimicrobial, antileishmanial and antimalarial activities of euglobals and their analogues. *Bioorganic & Medicinal Chemistry*. 14, 1750–1760.
42. Prakash N, Patel S, Faldu NJ, Ranjan R, Sudheer DVN. 2010. Molecular Docking Studies of Antimalarial Drugs for *Malaria. J Comput Sci Syst Biol*. 3, 070- 073.

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