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Research Article

MOLECULAR DOCKING STUDIES OF PHYTOCHEMICALS AGAINST *Leishmania donovani* TRYPANOTHIONE REDUCTASE

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ABSTRACT

Leishmaniasis is a parasitic disease which occurs as a co-infection with AIDS, and is spread by the bite of female sand flies possessing antibiotic resistance. To identify a potential lead compound by virtual screening, capable of inhibiting the target protein Trypanothione reductase (E.C 1.6.4.8) (TRP) thereby reducing the symptoms caused by leishmaniasis. The potential ligands were screened based on the MolDock score by docking and subjected to ADMET test to identify the suitable lead compound against leishmaniasis. In total 24 ligands were docked against TRP and 19 potential ligands were screened as hits based on the MolDock score.The ADME test identified hits based on Lipinski's rule of five; 6 ligands which had a good MolDock score but failed to obey Lipinski's rule was eliminated. Out of remaining 13 Hits only two ligands Taxifolin (1.29), and Emetine (1.0) which had good drug likeness score were considered. Toxicology studies revealed Emetine as a nontoxic compound. Docking studies showed that emetine shows greater affinity towards TRP due to hydrogen bond formation exactly at aforementioned positions: N (7) and ASP 326 has the minimal distance of 2.7 Å with -0.251 Kcal/mol, 2: O (8) and SER 178 has the minimal distance of 3.34 Å with – 1.049 Kcal/mol**.** This study reports the molecular interaction mechanism between emetine and TRP using molecular docking and identified the emetine as potential lead compound against leishmaniasis. Further study on emetine analogues, lead optimization and validation may lead to novel drug and drug targets for lead compounds against Leishmaniasis.

Keywords: ADME test, Lipinski's rule, Molecular Docking, Trypanothione Reductase, Toxicity test.

INTRODUCTION

Leishmaniasis is a zoonotic disease which is spread by the bite of female sand flies (phlebotomine) and it is caused by the parasites belonging to the genus Leishmania. Around 20 species of Leishmania are pathogenic for humans while 30 species of the sand fly act as a vector[1]. Three clinical forms of the disease are present: cutaneous, mucocutaneous and visceral leishmaniasis. Among these three forms cutaneous leishmaniasis is a major concern because it occurs as a co infection with AIDS[2]. Individuals suffering from leishmaniasis suffer from fever, low RBC count, skin ulcer and an enlarged liver[3].

More than 12 million people in 88 countries are known to be infected with leishmaniasis and expected to increase at a progressive rate, but the true burden remains largely hidden. Two million new cases -1.5 million of cutaneous leishmaniasis, 500,000 of the visceral leishmaniasis occurs annually. The declaration of this disease is only compulsory in 32 countries and a substantial number of cases are never recorded[4]. Approximately 20 to 50 thousand deaths occur every year. The disease is common in Asia, central America, southern Europe and Africa and around 200 million people live in this region[5].

The most severe form of leishmaniasis is visceral leishmaniasis(Kala Azar. It is caused by L. donovani complex which includes three species – L.donovani, L.infactum and L.chagasi[4]. Visceral leishmaniasis found in India is caused by Leishmania donovani. As of 2016, no successful vaccine was developed for leishmaniasis[6].

The chemotherapeutic treatments currently available have a number of limitations due to poor efficacy, unacceptable host toxicity and drug resistance, and new drug targets are required. Newer serological test for determining leishmaniasis infection (ELISA) do not function as well in immunocompromised patients who aren't making antibodies to infections. In these situations, two or more test must be used making the diagnostic procedure more expensive and less reliable[7]. Trypanothione reductase (E.C 1.6.4.8) is a member of the di-sulfide oxidoreductase family of enzymes that presents as an ideal target for structure based inhibitor drug design[8].

The aim of our work is to identify the lead compound against leishmaniasis by virtual screening of ligands. These ligands have the property of inhibiting the TRP enzyme thereby reducing the symptoms caused by leishmaniasis. Virtual screening is generally done in two ways viz., Ligand-based drug design and structure-based drug design.

In this study we have chosen structure-based drug design to identify the lead compound. Molecular docking is a structure-based drug design which has the ability to predict protein-ligand interaction[9]. It is a key tool in structural molecular biology and computer assisted drug design.

The ligands selected are bioactive metabolites of plants which belong to these major classes: Alkaloids, Quinones, Terpenes and phenolic compounds. The evaluation of ADME and pharmacological properties of a drug is vital for successful drug development. Along with ADME, the toxicology test is done to ensure the drug cause no harm to patients[10]. Evaluation of ADME

is based on Lipinski's rule of five (Pfizer's rule of five) and drug likeness score.

MATERIALS AND METHODS

Target selection

Trypanothione Reductase (E.C 1.6.4.8) belongs to the di-sulfide oxidoreductase family of enzymes and is also identified as a NADPH dependent flavoprotein unique to protozoan parasites - Trypanosoma and Leishmania *sp.,* These protozoans do not possess Glutathione Reductase (E.C 1.6.4.2), So the enzymes such as Trypanothione, N – glutathionyl - spermidine and auxiliary enzyme trypanothione reductase (TRP) maintains the intracellular level of dihydro trypanothione and eventually results in the maintenance of reducing environment in the protozoan host.[11] The Trypanothione function was found to be essential for protozoan survival because of the dithiol trypanothione play vital role in the synthesis of DNA precursors, ascorbate homeostasis, hydroperoxides detoxification, and thiol conjugates export. The major peroxidases that eliminate the reactive oxygen species generated during the aerobic metabolism are trypanothione dependent. So, Enzymes such as Trypanothione, N – glutathionyl - spermidine and auxiliary enzyme trypanothione reductase (TRP) was identified as a potential target for developing drugs against Leishmaniasis, because these enzymes are essential for the survival of protozoan parasites and it is absent in mammals. In this study Trypanothione reductase (TRP) is used as a target for structure based drug design.

Retrieval and preparation of receptor model

The three-dimensional structure of the Homology-Modelled Structure of Trypanothione Reductase (TRP) of Leishmania donovani (PM0077559) was obtained from Protein Model Data Base (http://mi.caspur.it/PMDB/) and used as the receptor model[11]. The protein preparation was automatically done using the Molegro Virtual Docker tool (protein preparation module). During this preparation process it assigns missing bonds, flexible torsions, bond orders, and charges to the protein structure and make it available for the docking studies.

Retrieval and preparation of ligand

In this study, the screening of ligands is done by virtual screening. About 24 molecules were finally selected for docking studies. The screening was done based on structural similarity and the nature of the ligands. The ligands used in this study are Bernerine, Lapachol, 2-Benzoxazolinone, Plumbagin, Aloe emodin, Emetine, Ursolic acid, Shederagenin, Jacaranone, Obaberine, Xylopine, Minquartynoic acid, Piperine, Taxifolin, Curcumin, Hirsutine, Jatrophone, Harmane, Licochalcone a, Renieramycin, Nyasol, Picroside, Diospyrin, Sulfuretin. The three dimensional structure was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider database (http://www.chemspider.com/) The compounds were prepared using Molegro Virtual Docker (prepare molecule module), this module automatically assign necessary charges, assign bond, assign bond order and hybridization, detect flexible torsions, create explicit hydrogens and finally the energy minimized structures was obtained.

Molecular docking

The molecular docking was performed using Molegro Virtual Docker 4.0.0 (MVD), involving two major steps *viz*., the addition of molecular surface and predicting of binding sites. The molecular surface was added to the protein based on the default settings, which results in the formation of a double colored surface according to electrostatic properties. The potential binding sites for receptor protein was predicted using cavity prediction algorithm[12]. This algorithm predicts cavities in receptor protein model and visualizes it to the user in green color. The parameters were set to the molecular surface with expanded Van der Waals and number of cavities to five. The docking then carried out which works based on MolDock Simplex Evolution search algorithm with grid resolution 30 Å for grid generation and select predicted cavities as the origin for the binding site. We use MolDock SE as a search algorithm, many runs set to 10, maximum population 50 and maximum iteration 1500. The energy minimization was carried out after docking process. We docked all screened compounds using Molegro and binding efficiency is evaluated using the MolDock score, Re-rank score and H-bond score (Table 1). The ADME, Drug likeness test, Toxicity test was performed to check whether screened compounds (Hits) with the good MolDock score are suitable to use as a lead compound[13].

RESULTS AND DISCUSSIONS

ADME and Drug likeness score prediction

The compounds with the good MolDock score (>=90) were identified as potential Hits. In this case 19 Hit compounds were Identified and subjected to ADME test to compute drug likeness and to identify whether the identified compounds follow Lipinski rule of 5, which is considered as an important parameter for selection of any compounds as a lead compound. The ADME test was performed using MOLSOFT (http://www.molsoft.com/mprop/) online tool. This tool predicts all molecular properties i.e. molecular weight (M.wt), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), polar surface area (PSA), Octane/water partition coefficient (LogP), and Drug likeness score (Table 2)[14].

The Hits such as Bernerine, Ursolic acid, Shederagenin, Obaberine, Renieramycin, Picroside showed good MolDock score but break the Lipinski rule of 5 which is considered as the important criteria for selecting a compound as lead compound (Table 2). Based on the drug likeness score, out of remaining 13 Hits only two ligands Taxifolin (1.29), Emetine (1.0) were considered and subjected to toxicity studies.

Toxicity test

The Identified ligands Taxifolin and Emetine were subjected to Toxicology tests to identify whether they possess any carcinogenic, skin sensitizing or hERG blocking properties. Toxicity test was conducted for each property separately using three specialized tools: Pred – Skin Web 1.0 (skin sensitizers predictor), Carcinopred – EL (Carcinogenicity predictor), hERG – Pred 4.0 (hERG blockage predictor).

Carcinopred – EL perform carcinogenicity prediction using 3 Ensemble machine learning models - XGBoost, Ensemble Support Vector Machine (SVM) and Ensemble Randon Forest (RF). It classifies compounds into carcinogen and non – carcinogen using only their 2D structures[15]. Pred-hERG is a predictive machine learning based QSAR models for prediction of hERG blockage. The models were built using the largest publicly available datasets retrieved from ChEMBL 21 database containing 16,932 associated bioactivity records for the hERG K_+ channel. It employs consensus models to achieving balanced accuracy, sensitivity, and specificity up to a range of 0.89-0.90 with the coverage of 0.63-1[16]. Pred – Skin Web 1.0 is a machine learning tool used to identify skin sensitizing potentials of

chemicals. The models were constructed using a largest database containing human and LLNA data. It was employed to check skin sensitizing property of Taxifolin and Emetine[17]. The ligands Taxifolin and Emetine have the valid MolDock score (-99.8747, -120.545), Re-rank score (-88.4713, -74.09) and Drug likeness score (1.29, 1.0) respectively and was selected and subjected to toxicity test. The Toxicity test was performed using three web based Machine learning tools: Pred – Skin Web 1.0, Carcinopred – EL, hERG – Pred 4.0. The Ligand Taxifolin was identified as skin sensitizer by Pred – Skin Web 1.0 and so not suitable for the lead compound. The ligand Emetine is bound within any one of the five cavities predicted (average cavity volume=720.384 $Å^3$). The interaction strength between the ligand and the protein largely depends on the number of H-bonds and the binding energy. When the analysing the hydrogen bond interactions between the ligand Emetine and amino acids present on the active site of the receptor protein i.e. SER 178, SER 162, ASP 326, TYR 198, GLY 197, GLY 286, LYS 60, GLU 202, SER 178 and VAL 55 revealed that there is a 2 hydrogen bonds interaction between Emetine and receptor protein – 1: N (7) and ASP 326 is having the minimal distance of 2.7 Å with -0.251 Kcal/mol, 2: O (8) and SER 178 is having the minimal distance of 3.34 Å with – 1.049 Kcal/mol. From analyzing the docking scores, hydrogen bond interaction data and toxicity test, it is clearly evident that Emetine has the best binding affinity towards receptor protein with least energy than other ligands and do not possess any toxicity and can be used as lead compound against Leishmaniasis.

Table 1: MolDock and Re-rank scores of 24 screened ligands against TR receptor model

Compound Name	CID	MolDock Score	Re-rank Score	H-bond
Bernerine	2353	-106.209	-87.0388	-1.72618
Lapachol	3884	-90.247	-5.70199	-6.39813
2-benzoxazolinone	6043	-71.9674	-60.974	-4.06347
Plumbagin	10205	-72.2478	-63.4981	-6.79323
Aloe emodin	10207	-80.0719	-79.8821	-11.0436
Emetine	10219	-120.545	-74.09	-0.25148
Ursolic acid	64945	-94.6925	-41.4494	-4.54601
Shederagenin	73299	-104.022	-13.5159	-5.42212
Jacaranone	73307	-86.6918	-72.6083	-6.94471
Obaberine	100231	-139.29	-92.1034	-1.43176
Xylopine	160503	-104.315	-88.4619	-2.35478
Minquartynoic acid	183614	-134.496	-110.735	-5
Taxifolin	439533	-99.8747	-88.4713	-9.18194
Piperine	638024	-127.67	-107.665	-1.07187
Curcumin	969516	-141.19	-110.876	-5.23538
Hirsutine	3037884	-126.398	-103.136	-0.04164
Jatrophone	5281373	-113.535	-74.4576	-0.88724
Harmane	5281404	-80.6387	-63.2534	-1.08239
Licochalcone a	5318998	-124.296	-98.6003	-5.09838
Renieramycin	6326666	-109.019	-92.8851	-3.56863
Nyasol	6438674	-110.591	-88.7248	-4.49263
Picroside	6440892	-140.715	-102.807	-12.5325
Diospyrin	308140	-100.122	-95.3456	-7.18244
Sulfuretin	5281295	-91.3212	-71.1665	-0.42917

Table 2: ADME and Drug likeness score prediction results

Compound Name	CID	M.wt (g/mol)	Log p (-4.0 - 5.6)	HBA (<= 10)	HBD (<= 5)	PSA (0-150) $Å^2$	Drug likeness score
Bernerine	2353	336.12	5.05 (> 5)	4	0	33.45	0.91
Lapachol	3884	242.09	3.24	3	1	41.52	0.44
Emetine	10219	480.3	4.32	6	1	45.8	1.00
Ursolic acid	64945	456.36	7.84 (> 5)	3	2	44.14	0.65
Shederagenin	73299	472.36	6.67 (> 5)	4	3	61.25	0.45
Obaberine	100231	622.30 (> 500)	7.27 (> 5)	8	0	50.42	1.25
Xylopine	160503	295.12	3.09	4	1	36.32	-0.56
Minquartynoic acid	183614	284.14	4.57	3	2	45.37	0.13
Taxifolin	439533	304.06	1.02	7	5	103.49	1.29
Piperine	638024	285.14	3.96	3	0	33.47	-0.02
Curcumin	969516	368.13	3.41	6	2	73.83	-0.66
Hirsutine	3037884	368.21	3.21	4	1	44.31	0.54
Jatrophone	5281373	312.17	2.84	3	0	34.72	-1.68
Licochalcone a	5318998	338.15	4.87	4	2	55.01	-0.16
Renieramycin	6326666	566.23 (> 500)	-1.18	11 (> 10)	1	110.51	0.40
Nyasol	6438674	252.12	4.51	2	2	35.2	-0.38
Picroside	6440892	492.16	-0.99	11 (> 10)	5	131.82	-0.76
Diospyrin	308140	374.08	3.41	6	2	84.73	-0.66
Sulfuretin	5281295	270.05	2.74	5	3	72.08	0.14

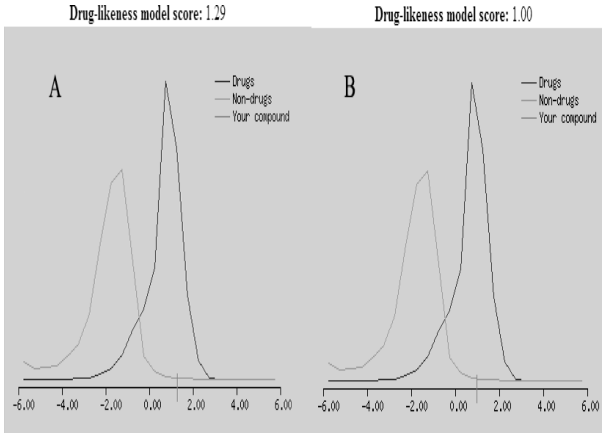

Figure 1: Predicted Drug likeness score of (A) Taxifolin and (B) Emetine

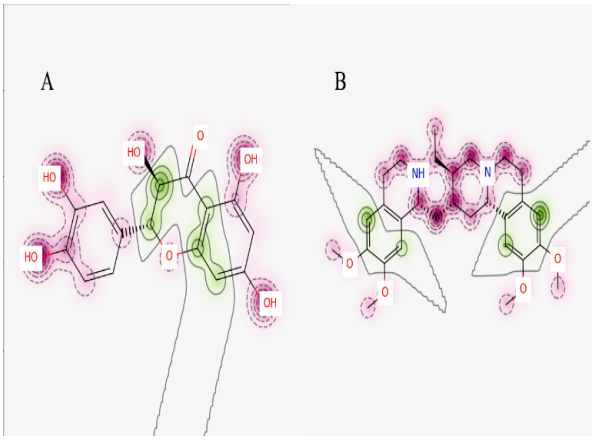

Figure 2: The computational simulated model of hERG – ligand interaction for (A) Taxifolin and (B) Emetine.

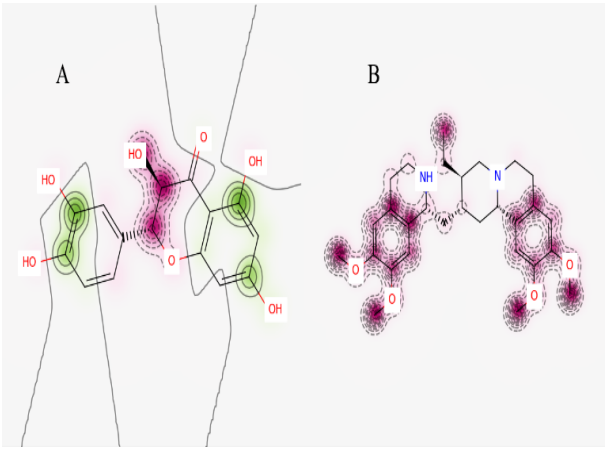

Figure 3: The computational simulated model of murine local lymph node assay for (A) Taxifolin and (B) Emetine

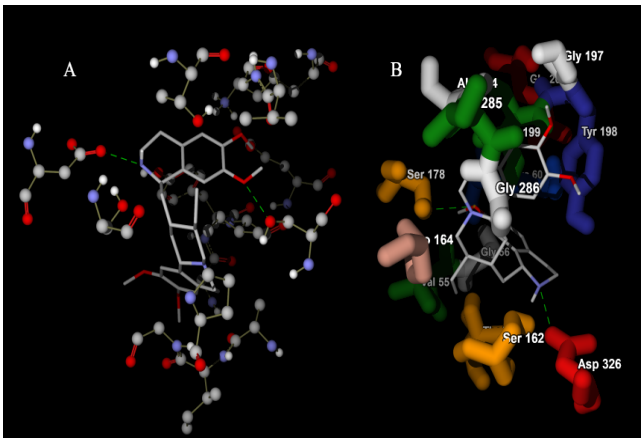

Figure 4: (A) Docking view of Emetine and Trypanothione reductase, (B) H-Bond interaction between Emetine and Asp 326,Ser 178 of target protein present in the active site.

CONCLUSION

The enzyme trypanothione reductase plays a major role in the thiol metabolism and therefore is one of the important proteins in the life cycle of *L. donovani*. The inhibition of this enzyme with chemotherapeutic drugs found be the best way to treat Leishmaniasis. The major drawback in the drug discovery is the longevity of the process in lead identification. Virtual screening may act as a vital alternative in lead identification, thereby speeding up the process involved in drug discovery. Future studies involve further screening using clinical trials, pharmacology, bioavailability and efficacy of drugs. The future scope may involve bringing out a cost-effective drug into the commercial market.

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ABBREVIATION USED

AIDS: Acquired Immunodeficiency Syndrome, TRP: Trypanothione Reductase
ADMET: Adsorption, Distribution, Metabolism, Excretion and Toxicity
RBC: Red Blood Cells
ELISA: Enzyme-Linked Immunosorbent Assay
DNA: Deoxyribonucleic Acid
MVD: Molegro Virtual Docker
M.wt: Molecular weight
HBD: Hydrogen Bond Donors
HBA: Hydrogen Bond Acceptors
PSA: Polar Surface Area
SVM: Support Vector Machine
QSAR: Quantitative Structure Activity Relationship
LLNA: Local Lymph Node Assay

REFERENCES

1. Desjeux P. Leishmaniasis: Current situation and new perspectives. Comp Immunol Microbiol Infect Dis. 2004;27(5):305-318. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15225981
2. Cruz I, Chicharro C, Nieto J, et al. Comparison of New Diagnostic Tools for Management of Pediatric Mediterranean Visceral Leishmaniasis Comparison of New Diagnostic Tools for Management of Pediatric Mediterranean Visceral Leishmaniasis. 2006. Available from: http://jcm.asm.org/content/44/7/2343.full
3. Barrett MP, Croft SL. Management of trypanosomiasis and leishmaniasis. Br Med Bull. 2012;104(1):175-196. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23137768
4. Malla N, Mahajan RC. Pathophysiology of visceral leishmaniasis - Some recent concepts. *Indian J Med Res*.

2006;123(3):267-274. Available from: https://www.ncbi .nlm.nih.gov/pubmed/16778309
5. Ejazi SA, Ali N. Developments in diagnosis and treatment of visceral leishmaniasis during the last decade and future prospects. Expert Rev Anti Infect Ther. 2013;11(1):79-98. Available from: https://www.ncbi.nlm.nih.gov/pubmed /23428104
6. Srivastava S, Shankar P, Mishra J, Singh S. Possibilities and challenges for developing a successful vaccine for leishmaniasis. Parasit Vectors. 2016;9(1):277. Available from:https://parasitesandvectors.biomedcentral.com/articles /10.1186/s13071-016-1553-y
7. Davies CR. Leishmaniasis: new approaches to disease control. Bmj. 2003;326(7385):377-382. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12586674
8. Singh BK, Sarkar N, Jagannadham M V, Dubey VK. Modeled structure of trypanothione reductase of Leishmania infantum. *BMB Rep*. 2008; 41:444-447. Available from:https://www.ncbi.nlm.nih .gov/pubmed/18593527
9. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov*. 2004; 3(11):935-949. Available from: https://www.ncbi.nlm .nih.gov/pubmed/15520816
10. Hodgson J. ADMET—turning chemicals into drugs. Rapidly resolving the pharmacokinetic and toxicological properties of drug candidates remains a key challenge for drug developers. Nat Biotechnol. 2001; 19: 722-726. Available from: https://www.nature.com/articles/ nbt0801_722
11. Ram K, Damodaran AP. Homology Modeling Of Trypanothione Reductase of Leishmania donovani. J Sci Technol. 2011;1(7):3-6. Available from: https://www.researchgate.net/publication/272567514_HO MOLOGY_MODELING_OF_TRYPANOTHIONE_RED UCTASE_OF_Leishmania_donovani
12. Molegro APS. MVD 5.0 Molegro Virtual Docker. DK-8000 Aarhus C, Denmark. 2011. Available from: https://insilicogen.com/
13. Sahu PK, Raval MK. Virtual screening for inhibitors of shikimate kinase of Mycobacterium tuberculosis Virtual screening for inhibitors of shikimate kinase of Mycobacterium tuberculosis. 2016; (June). Available from: http://www.onlinepbe.com/index.php/PBE/article/view/125
14. Molsoft LLC. Drug-Likeness and Molecular Property Prediction. Available from: http://molsoft.com/mprop/
15. Zhang L, Ai H, Chen W, et al. CarcinoPred-EL: Novel models for predicting the carcinogenicity of chemicals using molecular fingerprints and ensemble learning methods. *Sci Rep*. 2017;7(1):2118. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28522849
16. Alves VM, Braga RC, Silva MB, et al. Pred-hERG: A novel web-accessible computational tool for predicting cardiac toxicity of drug candidates. In: abstracts of papers of the american chemical society. Vol 248. Amer chemical soc 1155 16th street, NW, Washington DC 20036 USA; 2014. Available from: https://www.ncbi.nlm.nih .gov/pmc/articles/PMC5720373/
17. Braga RC, Alves VM, Muratov EN, et al. Pred-Skin: A Fast and Reliable Web Application to Assess Skin Sensitization Effect of Chemicals. J Chem Inf Model. 2017; 57(5): 1013-1017. Available from: http://pubs.acs.org/doi/abs/10.1021/acs.jcim.7b00194?journ alCode=jcisd8

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