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Insilico Characterization and Antifungal Activity of *Curcuma Longa* on DNA Topoisomerases of *Candida Auris*

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Abstract: *C.auris* is now an emerging and opportunistic pathogen with serious ailments both in terms of disease progression and increasing resistance to current antifungals.Topoisomerase I and II were actively used by the strain to multiply within the host. Though not very essential, but still could be used as drug targets. The emerging new fungal species is making the world to look for the development of new antifungal drugs which could lessen the people from symptoms. *Curcuma longa* (Turmeric) was effectively used against many fungal infections since ages, but still clinically not advised owing to its poorer absorption. In this study, a total of ten compounds from the turmeric were screened for their potent binding affinities to the above said receptors, using the *insilico*docking study, druglikeness properties, ADME/T test, PASS prediction along with P450 site of metabolism prediction.Form the experiment, micafungin and germacrene were found to be best docked ligands to the receptors, though micafungin was equally good in docking score to that of positive controls (Amphotericin B and caspofungin). Even the druglikeness properties, ADME/T test, PASS prediction along with P450 site of metabolism prediction all suggest germecrene to be more effective than micafungin. Moreover, germacrene was found to not toxic and nontumoric.

Key words:*C.auris*, Topoisomerase I, II, Caver web, Turmeric, germacrene.

1. Introduction:

Candida auris, a new *Candida* species reported for the first time in Japan seen to be mostly confined to five continents[Satoh K, 2009]. *C. auris* is mostly associated with nosocomial outbreaks in intensive care units even though infection prevention and control (IPC) measures were taken in serious concern which might be due to the increasing antifungal resistance among the strains. Due to the increasing exposure to the antifungals, antifungal resistance seems to be increasing which led to the development of variable antifungal susceptibility profiles [Deorukhkar SC, 2014]. Moreover, difficulty in screening for the phenotypic and molecular methods, uncertainity within the environmental niches and untraced spreading methods all contributed to the massive spread of this strain beyond the control.

Ever growing colonization and severity of infection among the non-*albicans* species is said to be the most driving reason for the increasing use of antifungal agents like fluconazole [Chowdhary A, 2013]. In the earlier decades, invasive candidiasis was caused by *Candida albicans*which led to the development of several susceptibility patterns. This eventually led to the development of multidrug-resistant species making fluconazole no more a good

antifungal agent.This made *C. auris*, a dominant opportunistic pathogen which could spread rapidly among the critically ill patients[Calvo B, 2016].This unclear evidences and increasing emergence of this pathogen, *C auris* made it resistant to most of the antifungal drugsunlike other *Candida* species. Even though a standard procedure and clinical experience was not available, CDC has put forward tentative antifungal breakpoints to treat this emerging *C. auris* infections [Bongomin F, 2017].

These breakpoints are defined basing on the *invitro* susceptibility studies and mutation sites distribution in antifungal resistance genes associated with other species of *Candida*.It was reported that about 90%, 30%, and 5% of clinical isolates of *C.auris* species from the United States are highly resistant to fluconazole, amphotericin B and echinocandins, respectively [Rudramurthy SM, 2017].

Most of the strains seem to be resistant to almost all the therapies available and about one-third of isolates are reported to be resistant 1 class of antifungal agents. Even though little work was done on the species and on its long-term impact, strategic developmental plans to be implemented to control and mitigate this pathogen. Physicians need to be extra invigilative on outbreak of *C auris* infections within their areas by monitoring the patients for their travel history and contacts and needs to plan good antimicrobial procedures to control the infection [Van Schalkwyk E, 2019].

DNA topoisomerases belong to a enzyme class which aids changing the topological structure of DNA, was said to be biological target to many therapeutic agents like antibacterial and anticancer agents. These drugs are reported to inhibit the enzyme by converting into a cellular poison. *Candida albicans* and *Aspergillus niger* are the two most opportunistic fungal infections [Shen LL, 1992]. Topoisomerases (TOP) I and II aids in the replication, transcription, repair, and chromosomal segregation [Shen LL, 1994] within cells. Liu *et al*, in his work confirmed that mammalian topoisomerase II is a critical enzyme and [Liu LF. 1989] and is known to be the drug target for anthracyclines and epipodophylotoxins.On the other hand fungal topoisomerase I though not very useful for the fungus, is reported to be acting as a target for the inhibitor camptothecin.

These inhibitors usually form complexes with the enzyme and ultimately halt the replication fork from functioning [Watt PM, 1994]. Even the alkaloid eupolauridine was found to inhibitthe *C. albicans*multiplication by stabilizing the cleavage complex formed by the organism topoisomerase [Fostel JM, 1992].

Ethanobotanical herbs or herbal remedy has gained more attention in the recent decades keeping in view of their effective management of symptoms.*Curcuma longa* (Turmeric), a spice which is used since ages for its invaluable medicinal properties was recently screened for its polyphenol curcumin which is present in large quantities. It not only helps in managing the oxidative and inflammatory conditions but also maintains the anxiety and hyperlipidemia. It also aids in managing exercise-induced inflammation and muscle soreness alleviating the recovery and performance among active people [Susan J, 2017]. Curcumin though effective as antibacterial, antifungal and anti-inflammatory still it is not used in clinical settings owing

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to its poor bioavailability. It is reported to be poorerin absorption with very high metabolism and faster elimination. On the other hand, US Food and Drug Administration (FDA) approved Curcuminoids as "generally recognized as safe" (GRAS) with good tolerability through clinical trials [Hewlings SJ, 2017].

The present study was designed to elucidate the binding affinity of the turmeric phytochemcial constituents to the *C.auris* topoiosmerase I and II pockets and to compare them with the commercialized and currently used antifungals like Amphotericin B and caspofungin. Along with molecular Docking, the compounds were screened for their pharmacological properties like absorption, distribution, Metabolism and excretion along with their toxicity levels.

2. Methodology:

2.1 Ligand preparation and retrieval: Protein was prepared and cleaned up using the protein parameter like removing waters and free ions. Amphotericin B, Micafungin and Caspofungin analogues (positive controls) were prepared along with test ligands in the study. Alpha turmerone, Beta turmerone, Cymene, Phellandrene, Terpinolne, undecanol, Tumerone, Germacrene, Selinene and Zingiberene are sued as test ligands.Ligands are tiny molecules which bind to the Protein's binding sites. All the above mentioned structures were obtained from Pubchem database in mol file format. They are later converted into pdb format using Arguslab software. The compounds were converted to three-dimensional structure with the help of PyMol version 1.3 [Seyed Mahdi Sadati, 2019], along with physic-chemical properties from the Pubchem database.

2.2. Retrieval of target enzyme structures: The three-dimensional (3D) structures of the enzymes for the species were not available in the RCSB database, hence their structures were modelled using homology modelling. The primary sequences were obtained from the UniprotKB database were obtained in FASTA format. The homology protein templates of the Topoisomerase I (1ois.1.A) and II (3l4j.1.A) were screened using SWISS model and models were for the templates using MODELLER software (version: 9.0). The models were now used for the selective docking with the positive and test ligands. The generated 3D structure of the macromolecule or model protein were validated by Ramachandran Plot using PROCHECK online server tool.

2.3 Protein-ligand docking:*In silico* docking studies were performed using PatchDock server **[**Schneidman-Duhovny D**, 2005]**, which uses the local geometric characteristics of proteins by limiting the search to certain areas of the molecular surface.

2.4 Prominent binding site prediction: Prior to docking, prominent binding sites were predicted for both the receptors in the study using MetaPocket 2.0 server [Bingding Huang, 2009]. Out of these only the top 3 major binding pockets were used in analysing the active binding residues and in comparing to the docking results.

2.5 Active binding site: Recognition of protein binding sites was identified by Caver web server. CAVER 3.0, (https://loschmidt.chemi.muni.cz/caverweb/?action=example&) was

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used for analysing the tunnels in a molecular dynamics simulation of the receptors (Topoisomerase I and II). We used it to identify and estimate the role of published tunnels to predict the bottleneck residues which could be very critical for the catalytic function of this enzymes. The caver default settings (minimum probe radius 0.9, Shell depth 4, Shell radius 3, clustering threshold 3.5, maximal distance 3, Desired radius 5) were used in the study.

2.6 Drug likeness and ADMET prediction: Among the 10 ligands in the study, two ligands Micafungin and Germacrene with lowest binding affinity and high patchdock score was filtered out and evaluated for pharmacokinetic and physicochemical properties using SwissADME server [http://swissadme.ch/index.php]. Drug-likeness of the ligands in the study were calculated to trace the pharmacokinetic properties like drug likeness, scorability of the drug, mutagenicity, irritancy, and reproductive effect. Moreover, it is very essential to trace for the possible health effects in terms of blood/brain barrier penetration, HIA (Human IntestinalAbsorption), Caco-2 cell permeability and aqueous solubility. Lipinski filter was used in ligand prediction. SWISS-ADME was used in drug investigation and other ligand properties like adsorption, distribution, metabolism, and excretion (ADME). BOILED-EGG (Brain or Intestinal Estimated permeation method) is also used in screening for the accurate model [Antoine Daina, 2016].
The molecular docking solutions obtained were screened on the PyMol platform for active sites and ligand binding confirmation. All the dock solutions confirms that the ligand actively binds within the active binding sites obtained from metapcket and Caver Web platforms.

2.7 PASS (Prediction of Activity Spectra for Substances): The PASS (Prediction of Activity Spectra for Substances) prediction of the two best selected ligands were determined using the PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/) using canonical SMILES from PubChem server (https://pubchem.ncbi.nlm.nih.gov/) [Filimonov, D, 2014]. PASS prediction, P_a (probability "to be active") was kept higher than 70%, since $P_a > 70\%$ threshold is said to give reliable prediction [Geronikaki, 1999]. Using this possible biological activities along with possible adverse effects were predicted for the selected ligands.

3. Results and Discussion:

3.1 Ligand preparation and enzyme structures: Proteins were prepared and cleaned up using the protein parameter like removing waters and free ions. Physico-chemical properties of the ligands were retrived from the Pubchem database and shown in table 1. The best model receptors for the Topo I and Topo II were analysed using Prockeck for Ramachandran plot [Figure 1].

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Table 1: Physico-chemical properties of the ligand.

Ligand	Molecular formula	Molecular weight in g/mol	Monoisotropic mass in g/mol	Heavy atom count	Pubchem ID	LogP
Amphotericin B	$C_{47}H_{73}NO_{17}$	924.1	923.487854	65	5280965	0.8
Micafungin	$C_{56}H_{71}N_9O_{23}S$	1270.3	1269.438351	89	477468	-1.5
Caspofungin	$C_{52}H_{88}N_{10}O_{15}$	1093.3	1092.643062	77	1.6E+07	-3.88
Alpha turmerone	$C_{15}H_{22}O$	218.33	218.167065	16	1.5E+07	4.05
Beta turmerone	$C_{15}H_{22}O$	218.33	218.167065	16	196216	4.06
Cymene	$C_{10}H_{14}$	134.22	134.10955	10	7463	4.1
Phellandrene	$C_{10}H_{16}$	136.23	136.125201	10	7460	4.4
Terpinolne	$C_{10}H_{16}$	136.23	136.125201	10	11463	4.47
undecanol	$CH_3(CH_2)_9CH_2OH$	172.31	172.182715	12	8184	4.72
Germacrene	$C_{15}H_{24}$	204.35	204.187801	15	9548705	6.566
Selinene	$C_{15}H_{24}$	204.35	204.187801	15	442393	6.01
Zingiberene	$C_{15}H_{24}$	204.35	204.187801	15	92776	5.77

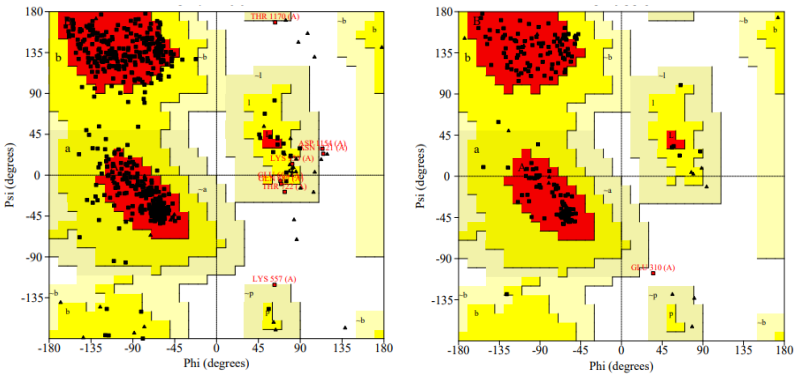

Figure 1: Images of Ramachandran plot generated using Procheck. Left: Topoisomerase 1 (3l4j.1.A); Right: Topoisomerase 1 (1ois.1.A).

3.2 Molecular docking: Docking was initially screened using Patchdock server.Amphotericin B and Caspofingin were used as positive control throughout the study.

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From the patchdock scores, it was found that Micafungin was found to as potent as positive controls (Scores). The scores for Micafungin were found to 8608 and 9960 for Topoisomerase 1 and 2 respectively. Patchdock scores Amphotericin and Caspofungin with topoisomerase 1 were found to be 7538 and 7848 respectively. And scores of Amphotericin and Caspofungin with topoisomerase 2 were found to be 7860 and 9044 respectively. Among all the ten ligands in the study, Micafungin was found to be as potent as the current positive controls. Second ranking was shared by germacrene with good score compared to Micafungin. The scores for Germacrene were found to 6500 and 6124 for Topoisomerase 1 and 2 respectively [Figure2].

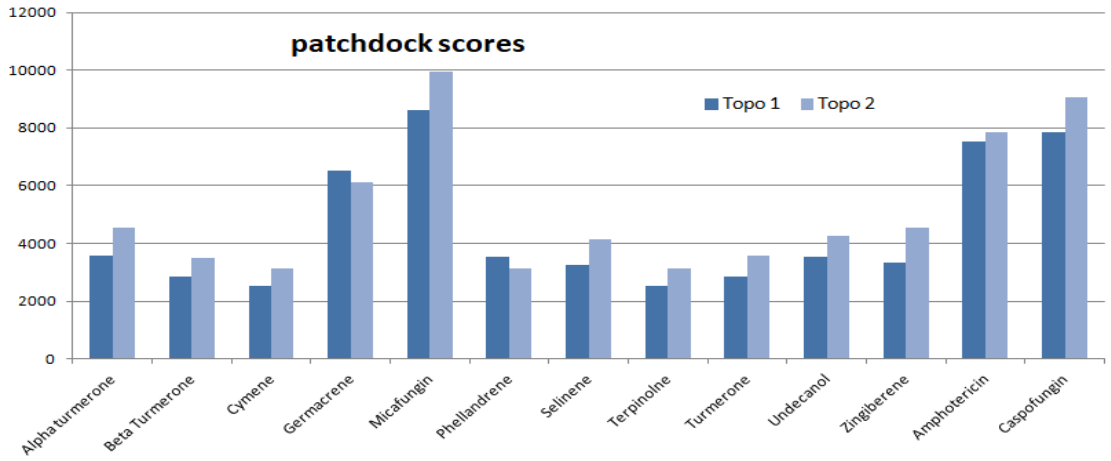

Figure 2: Graph showing the Patchdock scores of the ligands in the study with both the receptors (Topoisomerase 1 and 2).

3.3 Prominent binding site prediction: The potential binding sites(PBS) of proteins are those residues or atoms which bind to ligands directly on protein surface, they are near to the ligand binding sites. The first metapocket site (MPK1) consists of 6 and 6 for Topoisomerase 1 (Z score: 20.28) and topoisomerase 2 (Z score: 15.67) respectively. The second metapocket pocket (MPK2) consists of 3and 3 pockets for Topoisomerase 1 and topoisomerase 2 respectively. The third metapocket consists of 4 and 3 pockets for Topoisomerase 1 and topoisomerase 2 respectively [Table 2].

Table 2: table showing the metapocket results of the two receptors in the study.

				Ranking				**Size of Cluster**	**Total Z - score**
Topo 1	ATOM 1	C3	MPT	1	-12.86	55.805	40.884	6	20.28
	ATOM 2	C3	MPT	2	-23.323	52.857	40.527	3	3.59
	ATOM 3	C3	MPT	3	-16.743	42.701	43.853	4	2.79
Topo 2	ATOM 1	C3	MPT	1	-11.16	53.456	41.342	6	15.67
	ATOM	C3	MPT	2	-	51.234	36.789	3	3.12

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	2				27.311				
	ATOM 3	C3	MPT	3	-19.743	41.235	42.123	3	2.12

3.4 **Active binding sites:**From the web tool, we could estimate the druggability score of the two receptors. This score represents how likely the drug-like molecules can bind to the active pockets.

Table 3: Table showing the druggability and bottle neck radius of the two receptors as viewed from Caverweb.

	Volume	Druggability	Bottle neck radius	Curvature	No of bottle necks	Length	No.of residues
Topo 1	2743	0.53	1.6	1	1	1.8	11
Topo 2	2700	0.53	3	1.3	1	5.9	20

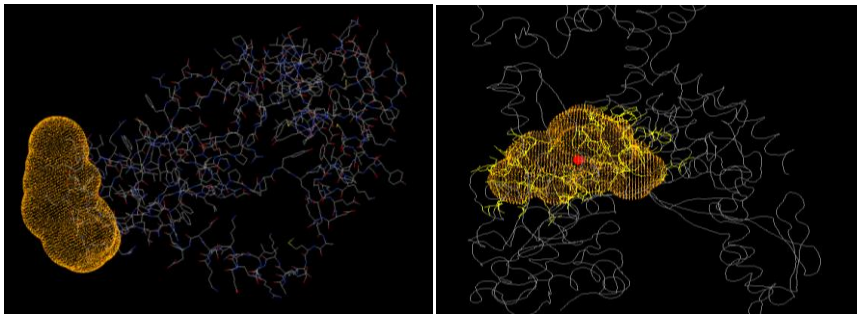


Figure 3: Active pockets as visualized on Caver web 1.0. left: Topoisomerase 1 (3l4j.1.A); Right: Topoisomerase 1 (1ois.1.A).

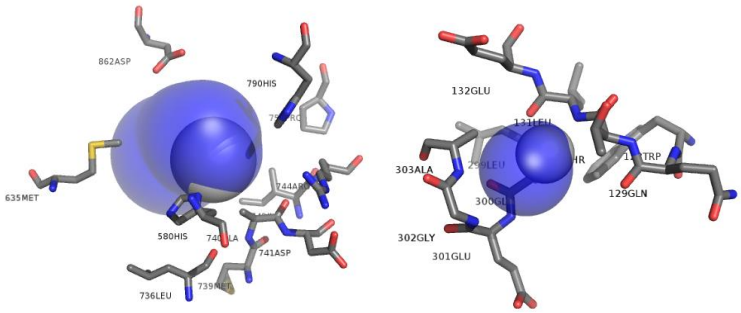
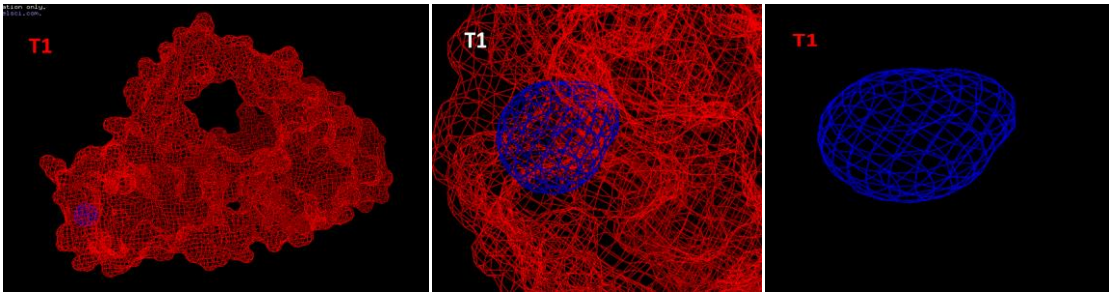


Figure 4: Images of the tunnel and the bottleneck residues lining the bottleneck. Images retrieved from Caver web server.



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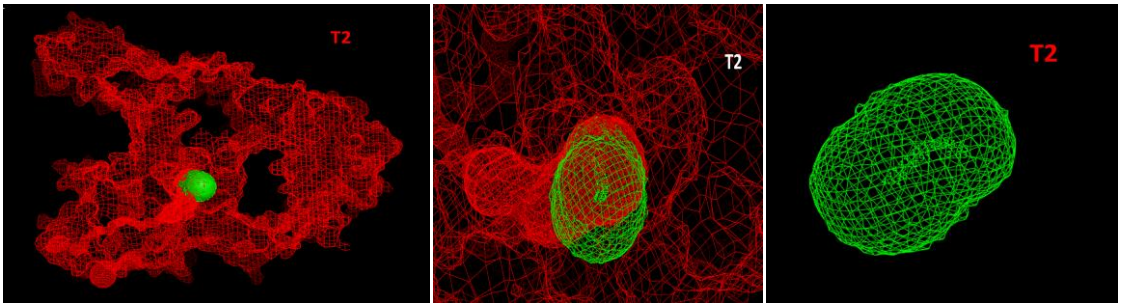

Figure 5: Images of the tunnels as visualized under Pymol. T1: Topoisomerase I; T2: topoisomerase II. Shape of the tunnels are showin in blue and green colour respectively for TI and TII.

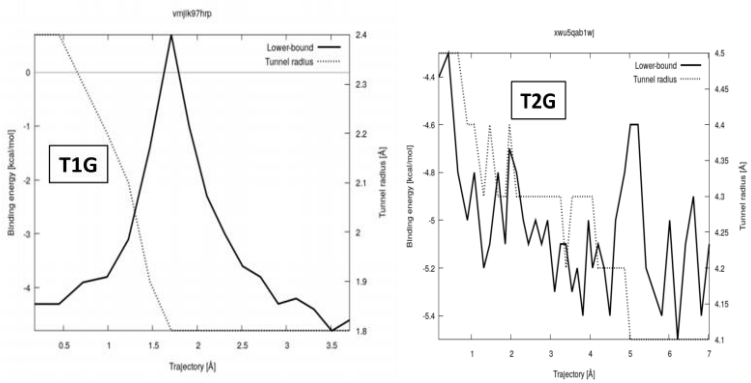

Figure 6: Images showing the energy profiles of the two receptors with the best ligand (Germacrene). T1G: Topoisomerase I and germacrene; T2G: Topoisomerase II and germacrene.

Table 4: Table showing the energy bound and surface energy of the docked solutions between the receptors and Germcrene as viewed under CaverWeb.

	T1 and G	**T2 and G**
EBound [kcal/mol]	-4.8	−5.1
EMax [kcal/mol]	0.7	−4.3
ESurface [kcal/mol]	-4.3	−4.4
Ea [kcal/mol]	5	0.1
ΔE_{BS} [kcal/mol]	-0.5	−0.7

E_{Bound}represents the binding energy of the ligand within the binding site. Lesser is the binding energy, more is the binding affinity. From the energy profiles obtained, E_{Bound} was found to be less (-5.1 kcal/mol) for T2 and G interaction than the T1 and G interaction (-4.8 kcal/mol).$E_a$represents activation energy of association [Table 4] . Ea of T1 and G was more (5 kcal/mol) when compared to T2 and G (0.1 kcal/mol). This suggests that there are less barriers in the T2 and G tunnel than the T1 and G tunnel. E_{Max} - $E_{Surface}$for reactants describes the difficulty of getting through the tunnel. The value is very high for T1 and G (5) when compared to T2 and G (0.1). This states that T2 and G tunnel interaction is better.

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3.5 Drug likeliness:

From the results it was observed that all the 2 ligands (Micafungin and Germacrene) of good score were poor in GI absorption and none of them are BBB permeant. The same was observed with positive control Amphotericin and caspofungin. Micafungin was good in terms of docking scores when compared to positive control. In terms of the docking scores, the second one in ranking is the germacrene which is also same like the above mentioned ligands but skin permeation (Log K_p) is better (-4.18cm/s). This permeability coefficient (K_p) prediction is based on the transport of compounds via the epidermal layer in mammals [Potts RO, 1992]. The more negative the log K_p value, the less skin permeant is the ligand. Hence, germacrene can be used as a good topical antifungal agent with a predicted log K_p = -4.18cm/s when compared to Micafungin (-15.19 cm/s). On the contrary though Micafungin is having a good docking score, it has little chance to cross the skin with a predicted log K_p = -15.19 (cm/s).

Moreover, in case of lipinskis fliter, there is only one violation for germacrene and three violations for Amphotericin, caspofungin and micafungin. Even the veber filter for TPSA and rotable bonds suggest the same as like Lipinskis filter. Germacrene has no violations but Amphotericin, caspofungin and micafungin have one, one and two violations respectively [Table 5].

Table 5: Table showing the drug likeliness properties of the best ligands and positive controls.

	Amphotericin B	**Caspofungin**	**Micafungin**	**Germacrene D**
TPSA	319.61 Å²	412.03 Å²	518.52 Å²	0.00 Å²
Log $P_{o/w}$	-0.65	-1.91	-2.94	4.3
Water Solubility	Yes	Moderate	Moderate	Yes
GI absorpiton	Low	Low	Low	Low
BBB permeant	No	No	No	No
Log K_p (skin permeation)	-11.94 cm/s	-12.73 cm/s	-15.19 cm/s	-4.18 cm/s
Lipinski filter	3 violations	3 violations	3 violations	1 violation
MW	>500	>500	>500	
N or O	>10	>10	>10	
NH or OH	>5	>5	>5	
MLOGP				>4.15
Veber filter	1 violation:	1 violation:	2 violations	Nil
TPSA	>140	>131.6	>140	
Rotatable bonds			>10	

Lipinski filter analysis states that a ligand is best (MW ≤500, MLOGP ≤ 4.15, N or O ≤ 10 and NH or OH≤5) and not ideal if it deviates from the above parameters [Lipinski CA.et al. 2001].

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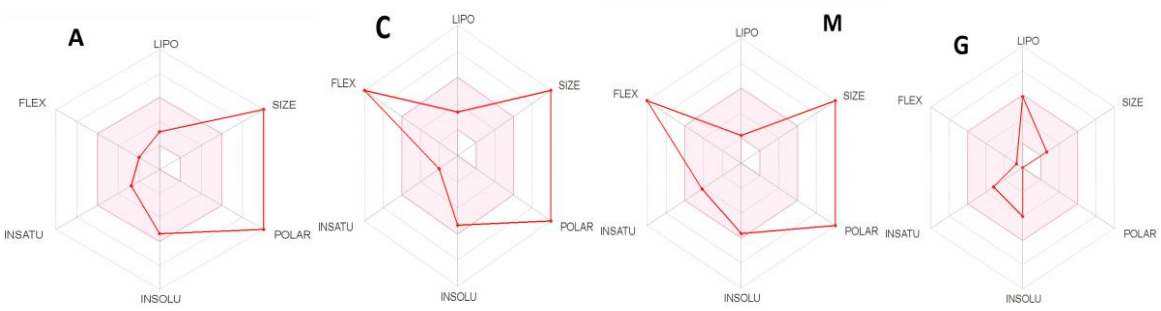

Figure 7: Images showing the bioavailability radar at the drug likeliness of a molecule for the 4 ligands in the study. A: Amphotericin;C: Caspofungin;M: Micafungin; G: Germacrene.

The pink area in the radar represents the optimal values for each of the properties specified. (lipophilicity: XLOGP3 between −0.7 and +5.0, Size: MW between 150 and 500g/mol, Polarity: TPSA between 20 and 130 $Å^2$, Solubility: log *S* not higher than 6, saturation: fraction of carbons in the sp^3 hybridization not less than 0.25, and Flexibility: no more than 9 rotatable bonds. From the radar analysis done with SWISS ADME, it was found that Germacrene was orally acceptable than the other three. Amphotercin was too large in size and is more polar, Caspofungin too is large and more polar and more flexible. Micafungin is also not orally bioavailable owing to its more polar and more flexible in addition to large size [Figure 7].

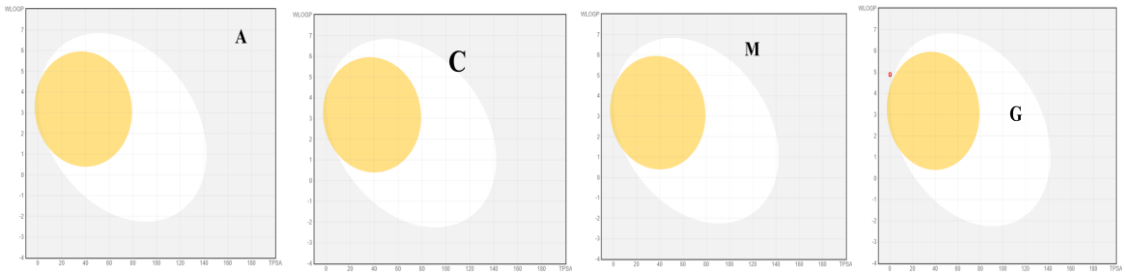

Figure 8: Images of the Boiled egg plot, water partition coefficient (WlogP) vs. Topological polar surface area (TPSA) of the hits Legends (A: Amphotericin; C: Caspofungin; M: Micafungin; G: Germacrene).

Boiled egg model was proposed as most accurate model to compute the lipophilicity and polarity of the ligands in the study [Rahma Muhammad Adamu, 2018]. The Boiled egg analysis of the four molecules (Figure 8) states that all of them are low or not absorbable by the gastrointestinal tract. Among them Germacrene shows within the range, whereas other molecules are out of range. White area suggests the area of highest probability of being absorbed by the gastrointestinal tract and area under yellow shade suggests highest probability to permeate to the brain. In case of germacrene (G), a red colour dot almost near to the white shaded area suggests it is more likely to be absorbable by the GI tract than the other 3 ligands, but still best as topical agent.

3.6 Molecular Modelling of the docking solutions: The docking solutions with good patch dock score [**Duhovny D, 2002]** were alone used for visualization in the PyMol web tool [Figure 9]. The binding residues were screened and selected which are in align with the active

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sites. The ligands docked to the receptor were also cross checked whether they lie in the active site pockets as designed using Caver web tool.

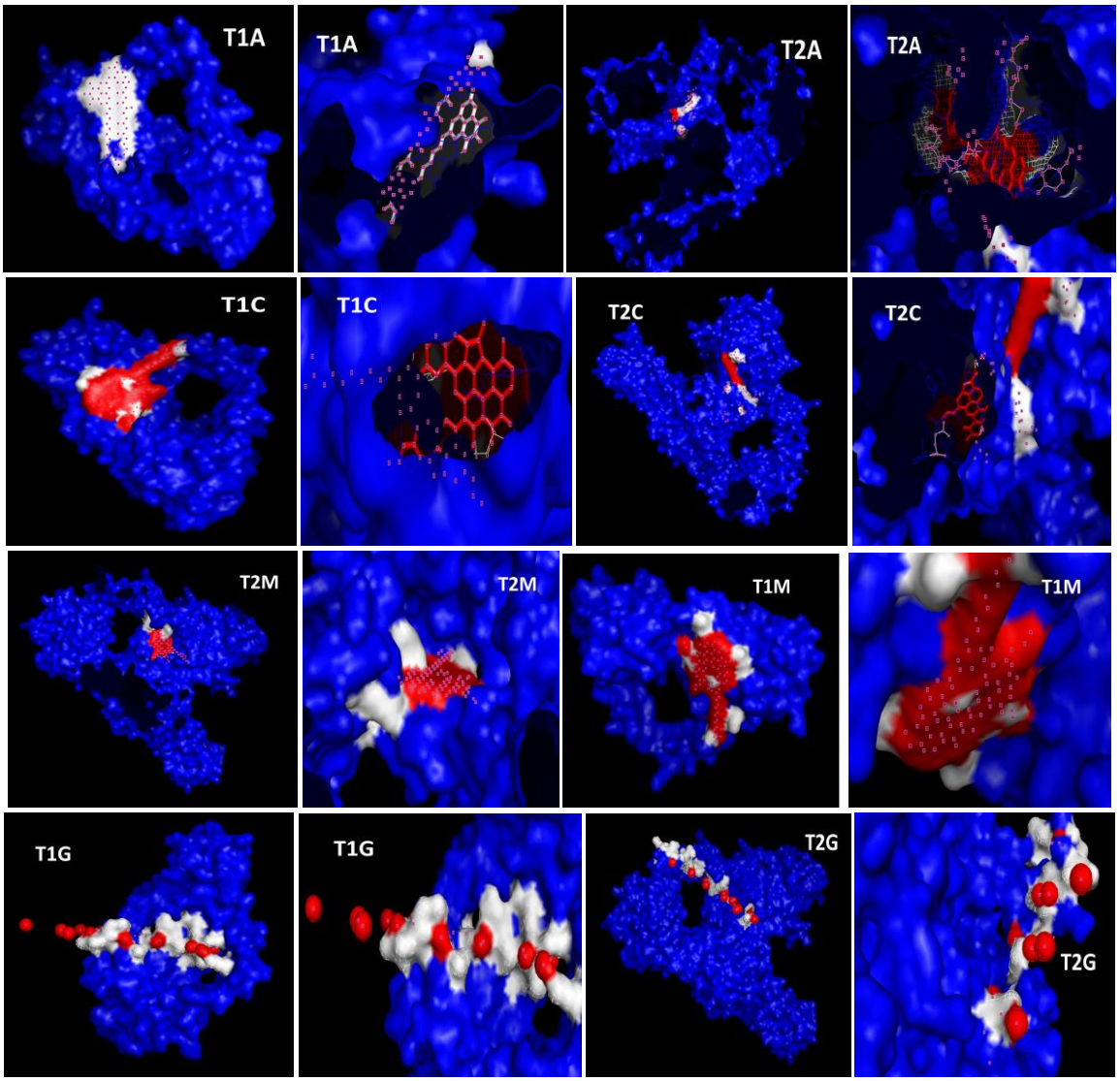

Figure 9: Images of the docking solutions of the receptors and ligands as visualized by PyMol. T1: Topoisomerase I; T2: Topoisoemrase 2; A: Amphotericin B; C: Caspofungin; M: Micafungin; G: Germacrene. White: Surface of the active binding sites; Red: Ligand.

3.7 Drug likeliness: The ADME/T for each of the best ligand molecules (Micafungin and germacrene) were carried out using online based servers, admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) and ADMETlab (http://admet.scbdd.com/) for predicting various pharmacokinetic and pharmacodynamic properties. Absorption, distribution, metabolism, excretion and toxicity of the above ligands of choice were determined by admetSAR server (78, 79). Toxicity properties were screened using the ADMETlab server [Table 6].

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Table 6: Table showing the drug toxicity levels of the best ligands (germacrene and Micafungin).

	Germacrene	**Micafungin**
Ames mutagenesis	-	-
Acute Oral Toxicity (c)	III	III
Androgen receptor binding	-	+
Aromatase binding	-	+
Biodegradation	+	-
Estrogen receptor binding	-	+
Glucocorticoid receptor binding	-	+
Hepatotoxicity	-	+
Acute Oral Toxicity	2.393196344	3.418991327
P-glycoprotein inhibitior	-	+
P-glycoprotein substrate	-	+
Plasma protein binding	0.704254806	0.929190338
Thyroid receptor binding	-	+
UGT catelyzed	-	+

From the report it was found that Germacrene could be the most potent molecule in terms of safety and degradation.Germacrene was found to be negative for hepatotoxicity with an oral toxicity value (2.393196344) which is much lesser than the Micafungin (3.418991327).

P-glycoprotein acts as a critical mediator of drug-drug interactions which can be inhibited or induced by compounds which can cause alteration in the pharmacokinetics of a drug. Some such inhibitors are clarithromycin, erythromycin, ritonavir and verapamil [Andrew Finch, 2014]. Germacrene was found to be negative for P-glycoprotein inhibition, whereas Micafungin was positive for the same. This reveals the potency of the molecule. Plasma protein binding depicts the degree to which xenobiotics get attached to blood proteins. The lesser bound a drug is, the more efficient it is in traversing across the cell membranes [Tonika Bohnert, 2013].As such it clearly states that Germacrene was not P-glycoprotein inhibitor. But Micafungin is positive for the P-glycoprotein inhibition.This confirms that germacrene was more efficientthan Micafungin.

UGT proteins (UGT1, UGT2, UGT3 and UGT8)are the isoforms are seen in liver, intestine, kidneys, lung and skin(Rowland *et al.*, 2013). Both the endogenous (e.g. bilirubin) and xenobiotics (e.g. drug) are metabolized by this enzymes to yield water-soluble products which are easy to be excreted (Miners and Mackenzie, 1991). Glucuronidation modifies the pharmacokinetic profile during lead optimization. Germacrene was found to be negative for UGT catalysed and micafungin was found to be positive. This states that Germacrene was more efficient than Micafungin.

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The P450 Site of Metabolism (SOM) of the best selected ligand molecule was determined by online tool, RS-WebPredictor 1.0 (http://reccr.chem.rpi.edu/Software/RS-WebPredictor/) [Zaretzki, J, 2012]. Prediction of Activity Spectra for Substances or PASS prediction is a process that is used to estimate the possible profile of biological activities associated with drug-like molecules. Two parameters are used for the PASS prediction: Pa and Pi. The Pa is the probability of a compound "to be active" and Pi is the probability of a compound "to be inactive" and their values can range from zero to one (80). If the value of Pa is greater than 0.7, the probability of exhibiting the activity of a substance in an experiment is higher. However, if the Pa is greater than 0.5 but less than 0.7, the probability of exhibiting a particular activity in an experiment is good, although less than the activity determined when Pa > 0.7 threshold is used. And if Pa is less than 0.5, then the probability of exhibiting the activity is the least (130).

3.8 PASS prediction: The PASS prediction was performed for 10 intended biological activities at Pa > 0.7 threshold. From the report obtained, it was found that the ligand Germacrene was able to show more than 560 biological activities. The activity is said to pass the test, if its value of Pa is more and lesser Pi value [Filimonov, D., A, 2014]. Increasing Pa and decreasing Pi values depicts the activity to be passed.

Germacrene is said to be a good antifungal agent, anticarcinogenic, and best molecule to treat adenomatous polyposis treatment. On the other hand it was very high as anticancer agent Antimutagenic and Antineoplastic 0.817). It was found to be a good carminative [Table 7].

Table 7: Table showing the Prediction of Activity Spectra for Substances (PASS) for the ligand of choice germacrene.

Activity	**Pa>0,7**	**Pi**
Adenomatous polyposis treatment	0,644	0,014
Anticarcinogenic	0,242	0,087
Antifungal	0,570	0,022
Antifungal enhancer	0,116	0,028
Antimetastatic	0,571	0,008
Antineoplastic	0,817	0,010
Chemopreventive	0,332	0,032
Hepatoprotectant	0,306	0,058
TP53 expression enhancer	0,616	0,045
Carminative	0,885	0,002

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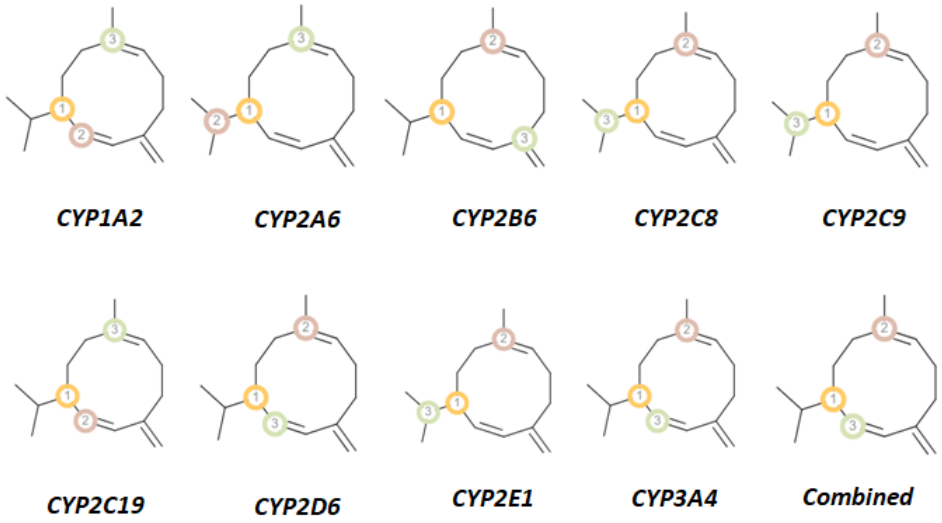

Figure 10: The P450 site of metabolism prediction of the best ligand molecule (Germacrene). Prediction was done by server RS-WebPredictor 1.0 (http://reccr.chem.rpi.edu/Software/RS-WebPredictor/). Circles denotes the possible sites of metabolism.

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