Eucalyptol, sabinene and cinnamaldehyde: potent inhibitors of salmonella target protein L-asparaginase

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Abstract *Salmonella typhimurium* is a severe threat to human life. The treatment became more difficult with the emergence of multidrug resistance. In the present in silico study, a novel drug target L-asparaginase was tested against three ligands eucalyptol, sabinene, and cinnamaldehyde, major components of cardamom, nutmeg, and cinnamon, respectively. The lowest docking score was obtained for sabinene followed by eucalyptol and cinnamaldehyde i.e. −5.648, −3.939 and −3.469. The docking score of sabinene is also better than the standard drug, Ciprofloxacin (−4.661) and natural substrate L-asparagine (−5.497). The amino acid residues involved in interactions inside the binding pocket are threonine 115 and threonine 35. The ADMET profile studied, also suggests the potency of the test ligands as a drug candidate. The results suggest they could be safe alternatives of chemical compounds to treat infections and combat multidrug-resistant bacteria.

Keywords *S. typhimurium* · L-Asparaginase · Pathogenicity · Spices · Antimicrobial

Introduction

L-Asparaginase is an enzyme of extensive investigations for last four decades for its remarkable chemotherapeutic application. The enzyme also has a significant role in the food industry as a food processing aid (Batool et al. 2016). Recent studies show that pathogen like *Salmonella typhimurium*, *Shigella flexneri*, *Yersinia* spp., and *Helicobacter pylori* use this enzyme to overcome pathways of the adaptive immune system and cause severe infections (Kullas et al. 2012). *Salmonella* causes all-inclusive mortality through various infections ranging from gastroenteritis to typhoid fever. It is reported that L-asparaginase-II produced by *S. typhimurium* inhibits T-cell responses and mediates virulence in the host. The enzyme hydrolyses L-asparagine, a vital nutrient for clonal expansion of T cells. The nutrient deprivation results in down-modulation of T-cell receptor (TCR)- β expression, T-cell blastogenesis suppression, cytokine production, and ultimately inhibition of T-cell proliferation (Kullas et al. 2012). A further detailed study showed that the pathogen uses the hydrolyzing property of L-asparaginase to suppress activation-induced mammalian target of rapamycin signaling, autophagy, Myc expression, and L-lactate secretion. The enzyme also participates in the inhibition of cellular processes and pathways that regulate protein synthesis, metabolism, and immune response (Torres et al. 2016).

Development of multi-drug resistance suggest immediate need to identify novel drug target against this parasite. L-asparaginase-II could be a potent drug target as it is involved in the virulence mechanism of the pathogen.

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There is an equal necessity to search for inhibitors which effectively blocks this target to check the pathogenicity. Along with food additive, Indian spices are also acknowledged for their valuable medicinal application. Essentials oil extracted from the spices have many secondary metabolites that possess antimicrobial activity against a wide range of pathogens (Nazzaro et al. 2013). In the present study, we consider three ligands eucalyptol, sabinene, and cinnamaldehyde, major components of cardamom, nutmeg and cinnamon, respectively. There are two prime reasons for selecting these ligands. Firstly, their herbal nature makes them comparatively safe and economical drug candidate. Secondly, the literature supports the antimicrobial activity of many spices against *S. typhimurium* (Naveed et al. 2013; Piaru et al. 2012; Rafiq et al. 2016).

Drug discovery is a complex, time consuming and expensive process and many uncertainties are associated with it that are lack of effectiveness, side effects, poor pharmacokinetics, etc. According to a report, it takes approximate US \$802 million and 12 years starting from research to marketing of a drug (Wadood et al. 2013). However, presently the application of in silico approach in drug discovery eases the process, and reduces the cost and time. The technique like homology modeling helps in the prediction of the 3-D structure of a protein with the help of sequence whose structure is unknown due to experimental limitations. Similarly, molecular docking predicts the binding free energy of a receptor–ligand complex and ADMET profiling predicts the pharmacokinetic behavior of the lead molecules (Debnath and Ganguly 2014). The present in silico study suggests the potency of these ligands as L-asparaginase-II inhibitor and provides insights to combat multi-drug resistant *S. typhimurium*.

Materials and methods

Software

The present study was carried out using different modules/tools of Maestro interface (Maestro, version 10.5, Schrödinger, LLC, New York, NY, 2016).

Homology modeling

The 3-D structure of L-asparaginase-II from *S. typhimurium* was modeled by Glide (version 7.0). The sequence (Accession No. ALE64056) for homology modeling was acquired from National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov/) (Silva et al. 2016). The L-asparaginase from *Erwinia carotovora* (PDBID: 2JK0) was used as template (Papageorgiou et al. 2008).

The modeled structure was validated through Ramachandran plot.

Ligand selection

The ligand selected as L-asparaginase inhibitor were eucalyptol (1,8-cineole or 1,8-Epoxy-*p*-menthane or 1,3,3-Trimethyl-2-oxabicyclo[2,2,2]octane), sabinene (4-methylene-1-(1-methylethyl)bicyclo[3.1.0]hexane) and cinnamaldehyde ((*2E*)-3-phenylprop-2-enal). Ciprofloxacin an established antibiotic against *S. typhimurium* was used as reference and vinblastine (an anti-cancer drug) was used as a negative control. L-asparagine and L-aspartic acid were also tested that are natural substrate and inhibitor of L-asparaginase enzyme, respectively.

Molecular docking

It is multi-step process starting with protein and ligand preparation using protein preparation wizard (version 4.3) (Sastry et al. 2013) and LigPrep (version 3.7), respectively. Then, drug binding sites were created using SiteMap application (version 3.8) (Halgren 2007, 2009) followed by grid generation. The final step of docking was performed through Glide (version 7.0) (Friesner et al. 2004, 2006; Halgren et al. 2004).

ADME/T (absorption, distribution, metabolism, excretion/toxicity) profiling

The ADME studies were executed using QikProp program (version 4.7). It is an ADME prediction program that predicts the physically significant descriptors and pharmaceutically relevant properties of organic molecules by comparing it with 95% of known drugs. The toxicity was studied through online server PreADMET (<https://preadmet.bmdrc.kr/>).

Result and discussion

The 3-D structure of L-asparaginase-II from *S. typhimurium* was obtained through homology modeling. The structure was validated by Ramachandran plot that predicts 90.1% residues are in most favored regions while 9.0, 0.5 and 0.4% are in additionally allowed, generously allowed and disallowed regions, respectively. There were five binding sites generated; only one having score above 1, i.e., 1.002 and is suitable for drug binding. The three ligands, ciprofloxacin (positive control), vinblastine (negative control), L-asparagine (natural substrate) and L-aspartic acid (natural inhibitors) were docked one by one against the binding site generated. The highest docking score amongst the test

ligands is obtained for sabinene, i.e., -5.648 followed by eucalyptol and cinnamaldehyde having score -3.939 and -3.469 , respectively. It has even better score than the

Fig. 1 Ligand docked inside the binding pocket of target protein **a** eucalyptol **b** sabinene **c** cinnamaldehyde

standard drug ciprofloxacin (-4.661). Vinblastine (an anti-cancer drug) was taken as a negative control and showed no interaction with the target protein. The docking score for the natural substrate L-asparagine was -5.497 and for the natural inhibitor, L-aspartic acid was -5.721 that supports the results obtained for test ligands. The ligands docked in the binding site are shown in Fig. 1. It is found that threonine residue present in binding pocket is interacting in all the test cases in the form of hydrogen bonds. Threonine 35 is interacting in the case of eucalyptol and sabinene while threonine 115 is involved in interaction in cinnamaldehyde and ciprofloxacin. The potency of a lead molecule depends on ADMET profile along with the high docking score. ADMET profiling suggests the pharmacokinetic behavior of the lead molecule. It is done to avoid wastage of time, energy and money due to late stage failure of drug (Hou et al. 2004). The assessment of in silico ADME profile and toxicity of test ligands was done and results are found to be in the defined range. The #star is a parameter that suggests the drug likeliness of a lead molecule. Lower the value more suitable is the lead molecule as a drug candidate. The maximum permissible limit of it is five. For the entire lead molecule, it is within permissible limit. Sabinene having highest docking score among all has #stars value 2, suggesting its potency as a drug candidate. All other ADME parameters tested also falls within the recommended range of employed software for the ADME testing (http://gohom.win/ManualHom/Schrodinger/Schrodinger_2015-2_docs/qikprop/qikprop_user_manual.pdf). After ADME profiling, the toxicity of the lead molecules are predicted by PreADMET server. The predicted values are summarized in Table 1. The Ames test predicts the mutagenicity of test ligand on the basis of the toxicity studies on different strains of *S. typhimurium*. The generally used strains are TA98, TA100 and TA1535. The PreADMET server also predicts the

Table 1 In silico prediction of toxicities of test ligands

Tests	Eucalyptol	Sabinene	Cinnamaldehyde	Ciprofloxacin
Algae_at	0.0415166	0.0319527	0.166933	0.0757514
Ames_test	Mutagen	Mutagen	Mutagen	Mutagen
Carcino_Mouse	Positive	Negative	Negative	Negative
Carcino_Rat	Positive	Positive	Negative	Negative
Daphnia_at	0.666532	0.132964	0.556563	0.298979
hERG_inhibition	Medium risk	Medium risk	Medium risk	Low risk
Medaka_at	0.435724	0.0206289	0.342976	0.140013
Minnow_at	0.352191	0.0167968	0.136716	0.134985
TA100_10RLI	Negative	Positive	Positive	Negative
TA100_NA	Negative	Negative	Negative	Negative
TA1535_10RLI	Positive	Negative	Negative	Positive
TA1535_NA	Negative	Positive	Positive	Negative

mutagenicity (in silico) for the same strains. The result obtained showed ligands mostly have non-mutagenic effect on different strains. Another toxicity that the server predicts is the carcinogenic effects of the test compounds on rodents. The positive prediction means that the ligand is having no carcinogenic effect. Sabinene showed positive result in the case of the rat while eucalyptol showed positive effect on both rat and mouse model. All the other values tested for toxicity prediction are comparable to reference (ciprofloxacin) or even better for some parameters signify their safety for human use.

Conclusion

Salmonella typhimurium is the causative agent of severe human infections and mortality throughout the world. Pacing advent of new resistance mechanisms in this microorganism is witnessed, rendering treatment of infectious disease difficult. Therefore, the need of scenario is to find out novel drug target and its potential inhibitor to fight against this pathogen. It is reported that L-asparaginase is exploited by the pathogen for its survival benefit. Therefore, it is targeted to fight against lethality caused by *Salmonella* infections. The plant-derived molecules hold remarkable potential and therefore, their antimicrobial properties are explored in the present study (Naveed et al. 2013; Piaru et al. 2012; Rafiq et al. 2016). The ligands tested are found to be effective inhibitor(s) against novel target L-asparaginase especially sabinene (major component of nutmeg). However, there is need of in vivo validation of in silico result. The study could be useful in designing effective and safe drug against *S. typhimurium* in future. This can be possible by exploring the medicinal property of phytoconstituents of other spices as well as natural and synthetic derivative of the test ligands. The present work also inspires for designing drug against another pathogen like *Shigella flexneri*, *Yersinia* spp., and *Helicobacter pylori* that uses L-asparaginase in their virulence mechanism.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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