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Original Article

IN SILICO PHARMACOKINETICS AND MOLECULAR DOCKING OF THREE LEADS ISOLATED FROM *TARCONANTHUS CAMPHORATUS* L

AMINA I. DIRAR[1,3], AYMAN Y. WADDAD[2], MAGDI A. MOHAMED[3]*, MALIK S. MOHAMED[4], WADAH J. OSMAN[5], MONA S. MOHAMMED[5], MOHAMED A. A. ELBADAWI[6], SAMI HAMDOUN[4]

[1]Department of Phytochemistry and Plant Sciences, Faculty of Pharmacy, National University, Sudan, [2]Department of Pharmaceutics, Faculty of Pharmacy, University of Gezira, Sudan, [3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Sudan, [4]Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum, Sudan, [5]Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum, Sudan, [6]Department of Pharmacology, Faculty of Pharmacy, University of Khartoum, Sudan
Email: mawadalla@uofk.edu

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ABSTRACT

Objective: To investigate the pharmacokinetic and toxicity profiles and spectrum of biological activities of three phytochemicals isolated from *Tarconanthus camphoratus* L.

Methods: Several integrated web based *in silico* pharmacokinetic tools were used to estimate the druggability of Hispidulin, Nepetin and Parthenolide. Afterward, the structural based virtual screening for the three compounds' potential targets was performed using PharmMapper online server. The molecular docking was conducted using Auto-Dock 4.0 software to study the binding interactions of these compounds with the targets predicted by PharmMapper server.

Results: The permeability properties for all compounds were found within the limit range stated for Lipinski's rule of five. Only Parthenolide proved to be able to penetrate through blood brain barrier. Isopentenyl-diphosphate delta-isomerase (IPPI), uridine-cytidine kinase-2 (UCK-2) and the mitogen-activated protein kinase kinase-1 (MEK-1) were proposed as potential targets for Hispidulin, Nepetin and Parthenolide, respectively. Nepetin and Parthenolide were predicted to have anticancer activities. The activity of Nepetin appeared to be mediated through UCK-2 inhibition. On the other hand, inhibition of MEK-1 and enhancement of TP53 expression were predicted as the anticancer mechanisms of Parthenolide. The three compounds showed interesting interactions and satisfactory binding energies when docked into their relevant targets.

Conclusion: The ADMET profiles and biological activity spectra of Hispidulin, Nepetin and Parthenolide have been addressed. These compounds are proposed to have activities against a variety of human aliments such as tumors, muscular dystrophy, and diabetic cataracts.

Keywords: *Tarconanthus camphoratus* L., Hispidulin, Nepetin, Parthenolide, *In silico* pharmacokinetic, Molecular docking, PharmMapper server, and Auto-Dock 4.0 software

INTRODUCTION

Drug discovery is concerned with finding new chemical entities, so-called leads, having a desired biological activity [1]. Certainly, bioactive molecules could be identified via different approaches [2], but the implementations of *in silico* ones have reduced the cost and time needed to bring a drug to the market [3]. The proper pharmacokinetics and toxicity profile along with efficacy are the major determinants for successful drug development [4]. Therefore, poor absorption, distribution, metabolism and excretion (ADME) along with the toxicity (T) are the primary causes of costly late-stage failures in drug development. Thus, it is mandatory that these areas should be considered at the early stages in the drug discovery process [5, 6]. Towards this goal, a variety of medium and high-throughput *in vitro* ADMET screens have been developed [4]. The high cost and lengthy time needed to conduct these *in vitro* screens have called for the development of *in silico* approaches. These approaches will render the ADMET profiles predictable resulting in a fast drug discovery process [3, 6].

The diversity of plant species has kept naturally-derived molecules at the core of the drug discovery process with many more molecules to be discovered [7]. Despite the massive researches on medicinal herbs, to scientifically validate their reputed curative properties, there are many concealed potential healing powers beyond their ethnomedicinal uses [8]. Herein, the medicinal plant *Tarconanthus camphoratus* L. (Family Asteraceae) is not an exception. The plant is known for its versatile ethnomedicinal uses including treatment of wounds, urinary tract infections, respiratory disorders and toothache relieve [9]. Moreover, it has a substantial role in the relief of a migraine and combating tumors [10].

Being involved in drug discovery from natural resources [11-13], we have recently isolated and identified three antimicrobial phytochemicals namely, the sesquiterpene lactone Hispidulin and the two flavones Nepetin and Parthenolide (fig. 1) [10, 14]. To further estimate the druggability of these phytochemicals, we herein report their ADMET profiles and potential biological activities using several *in silico* approaches.

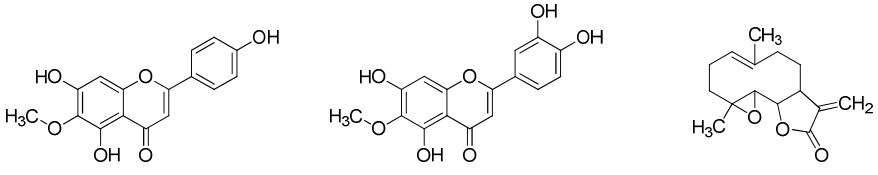

Fig. 1

MATERIALS AND METHODS

Generation of chemical structures' format

The chemical structure of each compound, unless otherwise stated, was submitted in the form of canonical simplified molecular input line entry system (SMILE), to estimate several *in silico* pharmacokinetic parameters.

Web based *in silico* pharmacokinetic tools

Molinspiration

Molinspiration online server (http://www.molinspiration.com/) [15] was used to estimate the molecular descriptors and drug likeliness properties for the lead candidate molecules. Estimated molecular properties were LogP, Topological polar surface area (TPSA), the number of hydrogen bond donors and acceptors. Drug likeliness property of the tested molecules was investigated as G-protein coupled receptor (GPCR) ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI) and enzyme inhibitors (EI).

Admet SAR

Admet SAR online server (http://www.admetexp.org) [16] was used to predict ADMET profiles for the candidate leads.

MetaPrint2D

MetaPrint2D online server (http://www-metaprint2d.ch.cam.ac.uk/) [17] was used to predict metabolic transformations obtained for the three leads.

PASS online tool

PASS Online tool (http://www.pharmaexpert.ru/passonline/) [18] was used to predict the potential pharmacological activities and toxicities of the leads submitted in .sdf format.

In silico molecular docking

Ligand preparation

The chemical structure of the compounds was drawn using ACD/ChemSketch v.12.0 software, and the obtained MDL format was converted into PDB format using Open Bable software [19].

Target identification

For each lead compound, the PharmMapper server (http://59.78.96.61/pharmmapper/) [20] was used to identify its potential target based on its fit score. Leads were submitted in MDL. sdf format, the target set was limited to human targets, and all other parameters were kept as default.

Protein structure retrieval and preparation

The 3-D structures of PharmMapper server identified targets were retrieved from protein databank (http://www. rcsb.org/ pdb/ home/home.do). Afterward, the protein files were prepared by removal of all water molecules and hetero groups except metals (if any). Target structure was further optimized and energy minimized using Swiss PDB viewer V.4.1.0 software [21].

In silico molecular docking

Molecular docking was performed using Autodock 4.0 software [22], based on Lamarckian Genetic Algorithm; which combines energy evaluation through grids of affinity potential to find the suitable binding position for a ligand on a given protein [23]. Polar hydrogen atoms were added to the protein targets and Kollman united atomic charges were computed. All hydrogen atoms were added to the ligands before the Gastiger partial charges were assigned. The co-crystal ligand was removed, and the bond orders were checked. The target's grid map was calculated and set to 60×60×60 points with grid spacing of 0.375 Å. The grid box was then allocated properly in the target to include the active residue in the center. The default docking algorithms were set in accordance with standard docking protocol. Finally, ten independent docking runs were carried out for each ligand, and results were retrieved as binding energies. Poses that showed lowest binding energies were visualized using UCSF chimera [24] and MOE [25].

RESULTS AND DISCUSSION

In silico pharmacokinetic estimation

Molinspiration

Physicochemical properties the discovery setting, 'Lipinski's rule of five' predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500 Da and the calculated LogP (CLogP) is greater than 5 (or MlogP>4.15) [26]. Moreover, good bioavailability is more likely for compounds with ≤10 rotatable bonds (nrotb) and total polar surface area (TPSA) of ≤140 Å [27]. In the current study, Hispidulin, Nepetin, and Parthenolide were predicted to have a high probability for good oral bioavailability where the calculated LogP values agreed with Lipinski's rule of five. Furthermore, TPSA, total hydrogen bond count and a number of rotatable bonds felt within the limit ranges (table 1). In addition, the number of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) for each lead were found to be within Lipinski's limit range from 7–3 and 3–0 i.e. less than 10 and 5, respectively. Drug molecules of molecular weight less than 500 Da are easily transported, diffuse and absorbed as compared to heavy molecules [28]. Fortunately enough, the three leads, have molecular weights (MW) in the range of 248–316.

Table 1: Physicochemical parameters predicted by Molinspiration for each lead

Lead	Mi logP	TPSA	MW	HBA	HBD	nrotb
Hispidulin	2.48	100.13	300.27	6	3	2
Nepetin	1.99	120.36	316.26	7	4	2
Parthenolide	2.09	38.83	248.32	3	0	0

Druggability likeliness property of Hispidulin, Nepetin and Parthenolide as GPCR ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI) and enzyme inhibitors (EI) were studied, and results were retrieved as bioactivity scores. In this context, scores>0.00 indicate high activity, between 0.00 to −0.5 indicate moderate activity and<−0.5 indicate inactivity [29]. The results of the present study demonstrated that the three leads showed high bioactivity scores as NRLs and EIs (score>0.00) with Parthenolide being the most active (1.16 and 1.10, respectively) (table 2). Nuclear receptors (NRs) are important pharmaceutical targets because they are key regulators of many metabolic and inflammatory diseases, including diabetes, dyslipidemia, cirrhosis and fibrosis [30]. Based on our results, Parthenolide, Hispidulin, and Nepetin could be envisioned as potential ligands for NRs representing interesting and promising therapeutic alternatives to cure relevant disorders. Considerable bioactivity against GPCR was revealed by Parthenolide (0.43); Hispidulin and Nepetin, on the other hand, produced moderate activities (−0.07 and −0.08, respectively). Hispidulin, Nepetin and Parthenolide exhibited moderate activities as ion channel modulators (−0.22, −0.23 and −0.07, respectively). Moderate protease inhibition was predicted for Hispidulin and Nepetin (−0.33 and −0.31, respectively); Parthenolide, on the other hand, was proposed to have satisfactory activity (0.04). While Hispidulin and Nepetin possessed considerable activity, Parthenolide was found inactive as kinase inhibitor (0.21, 0.22 and −0.55, respectively).

Table 2: Drug likeliness property estimations by Molinspiration for each lead

Compound	GPCR	ICM	KI	NRL	PI	EI
Hispidulin	− 0.07	− 0.22	0.21	0.20	− 0.33	0.17
Nepetin	− 0.08	− 0.23	0.22	0.17	− 0.31	0.16
Parthenolide	04.3	− 0.07	− 0.55	1.16	0.04	1.10

AdmetSAR predictions

The ADMET properties of the studied leads were calculated using admetSAR. Blood-Brain Barrier (BBB) penetration, HIA (Human Intestinal Absorption), Caco-2 cell permeability and AMES test were calculated. The results obtained for BBB penetrability greatly agreed with structures of the studied compounds. Only Parthenolide, a less polar sesquiterpene lactone, was predicted to cross BBB. It was also found that all tested compounds could be absorbed by the human intestine, and they could penetrate to Caco-2 (table 3). Nevertheless, the tested compounds proved to be potential substrates for P-glycoprotein (P-gp) which effluxes drugs and various compounds to undergo further metabolism and clearance [31] resulting in therapeutic failure because the drug concentration would be lower than expected [32]. Many of the human microsomal P450s aromatase catalyze the metabolism of a wide variety of compounds including xenobiotic and drugs [33].

Thus, inhibition of cytochrome P450 isoforms might cause drug-drug interactions in which co-administered drugs fail to be metabolized and accumulate to toxic levels [34]. Notwithstanding, some of the cytochrome P450 isoforms could be inhibited by one or more of the tested compounds. Fortunately, all compounds did not show any acute toxicity and mutagenic effect with respect to the AMES test data.

Table 3: ADMET predictions using AdmetSAR

ADMET	Lead		
Absorption	**Hispidulin**	**Nepetin**	**Parthenolide**
BBB	BBB−	BBB−	BBB+
HIA	HIA+	HIA+	HIA+
Caco-2	Caco-2+	Caco-2+	Caco-2+
P-gp substrate	Substrate	Substrate	Substrate
P-gp inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
ROC transporter	Non-inhibitor	Non-inhibitor	Non-inhibitor
Distribution and metabolism			
CYP450 substrate			
CYP450 2C9	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4	Non-Substrate	Non-Substrate	substrate
CYP450 inhibitor			
CYP450 1A2	Inhibitor	Inhibitor	Inhibitor
CYP450 2C9	inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19	inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4	inhibitor	inhibitor	Non-inhibitor
CYP IP (Inhibitory promiscuity)	High	High	Low
Excretion and Toxicity			
HERG	Weak inhibitor	Weak inhibitor	Weak inhibitor
Inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor
AMES toxicity	Non toxic	Non toxic	Non toxic
Carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen
Fish toxicity	High	High	High
T. P toxicity	High	High	High
H. B toxicity	High	High	High
Biodegradation	Not ready	Not ready	Not ready
Acute Oral Toxicity	Category III	Category III	Category III
ADMET Predicted profile---regression			
Aqueous solubility (logS)	−3.2219	−3.0097	−3.4998
Caco2 permeability (logPapp, cm/s)	0.91621	0.3244	0.2060
Toxicity			
RAT(LD_{50} mol/kg)	2.71922	2.6388	0.2241
FT (pLC_{50} mg/l)	0.6628	0.5863	0.4893
TPT ($PIGL_{50}$µg/l)	1.3073	1.1489	0.3812

Key: BBB: Blood Brain Barrier, HIA: Human Intestinal Absorption, P-gp: P-Glycoprotein, ROC: Renal Organic Cation, HERG: Human Ether-a-go-go-Related Gene, TP: Tetrahymena Pyriformis, HB: Honey Bee, RAT: Rat acute toxicity, FT: Fish toxicity.

Metaprint2D predictions

MetaPrint2D predictions revealed that the red colored allylic methine's carbon atom of Parthenolide and most hydroxyl groups for the two flavones represented good sites for metabolism (fig. 2). Metabolic transformations (dehydroxylation, hydroxylation, phosphorylation, glucuronidation, sulfation, alkylation, methylation and esterification) were predicted for the hydroxyl groups. Oxidation, on the other hand, was proposed for the methine moieties.

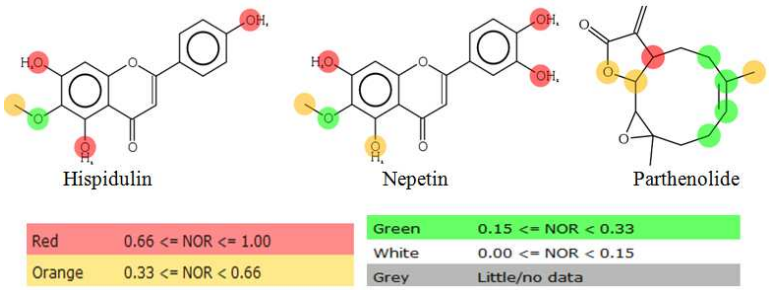

Fig. 2: Metabolic predictions using Metaprint2D for each lead

The color highlighting an atom indicates its Normalized Occurrence Ratio (NOR). A high NOR indicates a more frequently reported site of metabolism in the metabolite database. Atoms are colored according to the likelihood of a metabolic site; High: red, Medium: orange, Low: green, very low is not colored, and No data: grey.

PASS online server

The chemical structure is described in PASS by original descriptors called Multilevel Neighbourhoods of Atoms (MNA). For each investigated activity, PASS calculates two probabilities, Pa and Pi, based on statistics of MNA descriptors. Pa and Pi values vary from 0.000 to 1.000 indicating the probabilities of the compound to be active or inactive, respectively. Our findings revealed that both Hispidulin and Nepetin are potential chlordecone reductase inhibitors (Pa = 0.965 and 0.964, respectively) (table 4). The clinically important chlordecone reductase belongs to a family of enzymes known as aldo-ketoreductases. These enzymes are involved in the metabolism of xenobiotics and have been implicated in the pathogenesis of diabetic cataracts and muscular dystrophy [35]. Nonetheless, chlordecone reductase inhibition could be a drawback in the sense that risk of developing prostate cancer is positively associated with the chlordecone concentration in blood [36]. Parthenolide showed a higher probability to function as TP53 expression enhancer (Pa = 0.980). TP53 helps protect genome integrity and maintain general cell homeostasis. In the presence of chromosomal mutations or damage, TP53 acts through multiple mechanisms such as apoptosis, cell cycle arrest, or cellular senescence halting the propagation of any mutated DNA [37]. It is therefore thought that the known anticancer activity of Parthenolide could be mediated through TP53 upregulation.

Table 4: Biological activity spectrum predicted by pass for each lead

Compound name	Pa	Pi	Activity
Hispidulin	0.965	0.002	Chlordecone reductase inhibitor
Nepetin	0.964	0.002	Chlordecone reductase inhibitor
Parthenolide	0.980	0.002	TP53 expression enhancer

Pa: probablyactive; Pi: probablyinactive

***In silico* molecular docking**

PharmMapper server has predicted that isopentenyl-diphosphate delta isomerase (IPPI) (PDB ID: 1PPV), uridine-cytidine kinase-2 (UCK-2) (PDB ID: 1UDW) and the mitogen-activated protein kinase kinase-1 (MEK-1) (PDB ID: 1S9J) are the best targets, in terms of fit scores (3.958, 4.702 and 4.338), for Hispidulin, Nepetin and Parthenolide, respectively.

IPPI is expressed in both prokaryotic and eukaryotic organisms [38]. The enzyme catalyzes isomerization of isopentenyl diphosphate (IPP) to dimethylallyl pyrophosphate (DMAPP) [39], which are important precursors for several compounds involved in signaling pathway and components of the cell membrane [40]. The IPPI key active-site residues are Glu-116, Tyr-104, and Cys-67, with Glu-116 being thought to protonate the IPP double bond while the Cys-67 thiolate removes a proton from C-2 of IPP. The active residue Tyr-104, which is hydrogen bonded to Glu-116 [41] is also thought to be involved with Glu-116 in protonating the double bond in IPP [42]. Hispidulin was successfully docked into IPPI with a binding energy of −8.36 kcal/mol. Analysis of the binding interactions revealed that Hispidulin formed a hydrogen bond with the active residue Tyr-104 (fig. 3). Contrary to other known IPPI inhibitors [42], Hispidulin did not interact directly with the active residues Glu-116 or Cys-67. Nevertheless, the above-mentioned interaction of Hispidulin with Tyr-104 altered the Glu-116/Tyr-104 hydrogen bonding resulting in a recognizable conformational change in the active pocket. Thus, the active pocket will no longer be complementary with the natural substrate. Apart from the active residues, Hispidulin formed hydrogen bonds with Cys-118, Phe-35, and Gly-68 that helped to allocate Hispidulin in the vicinity, between Glu-116 and Cys-67, where IPP isomerizes. In addition, Hispidulin-IPPI complex was further stabilized through interactions with both divalent metals and hydrophobic residues (fig. 3).

Being an IPPI inhibitor, Hispidulin can be considered as a potential lead for development of novel drugs for infectious [43, 44] and non-infectious [45] diseases with which IPP pathway is associated. Nevertheless, host toxicity should be taken into consideration regarding the development of Hispidulin as an anti-infectious drug.

Docking of Nepetin into UCK-2 enzyme (binding energy = −10.05 kcal/mol) showed hydrogen bonding with Arg-176 and His-117, which are known binding residues for the inhibitor cytidine triphosphate (fig. 4). In addition, Nepetin interacted via hydrogen bonding with Arg-166 and Arg-169.

These residues are important binding sites for other UCK-2 inhibitors [46]. The UCK-2 enzyme is normally expressed in human placenta and testis and overexpressed in many neoplasias of blood and solid tissues [47]. It is worth noting that Nepetin has been shown to have anticancer activity [48]. It appears that this activity could be mediated through UCK-2 inhibition.

Parthenolide docked nicely within the active pocket of MEK-1 enzyme (binding energy = −6.42 kcal/mol). Literature revealed that MEK-1 inhibitors form an essential hydrogen bond with catalytic Lys-97 in addition to hydrophobic interactions with the deep hydrophobic pocket formed by Met-143, Ile-141, Leu-118 and Phe-209 [49]. Interestingly, Parthenolide showed similar interactions with MEK-1 active pocket (fig. 5).

These interactions would render MEK-1 catalytically inactive by stabilizing the inactive conformation of the activation loop. It is worth noting that MEK-1 plays a curial role in normal cell survival, however, altered expression level was detected in various types of cancer [50]. Thus, the known anticancer activity of Parthenolide [51] can be explained by MEK-1 inhibition.

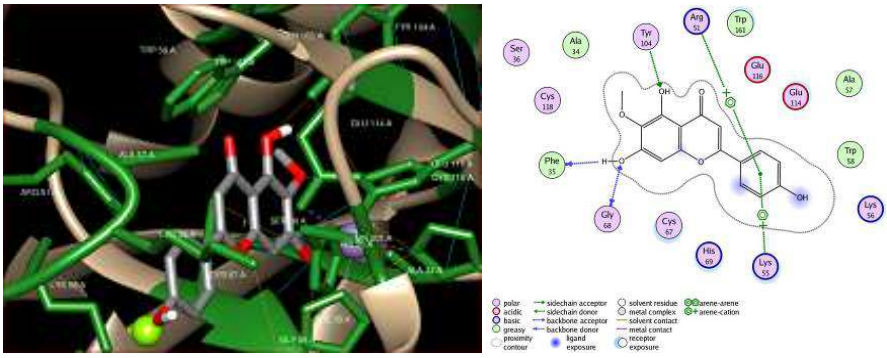

Fig. 3: Hispidulin-IPPI interactions visualized by Chimera (left) and MOE (right)

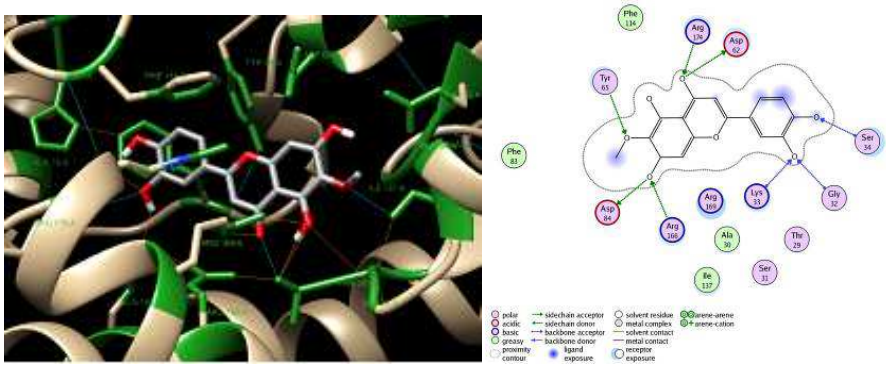

Fig. 4: Nepetin-UCK-2 interactions visualized by Chimera (left) and MOE (right)

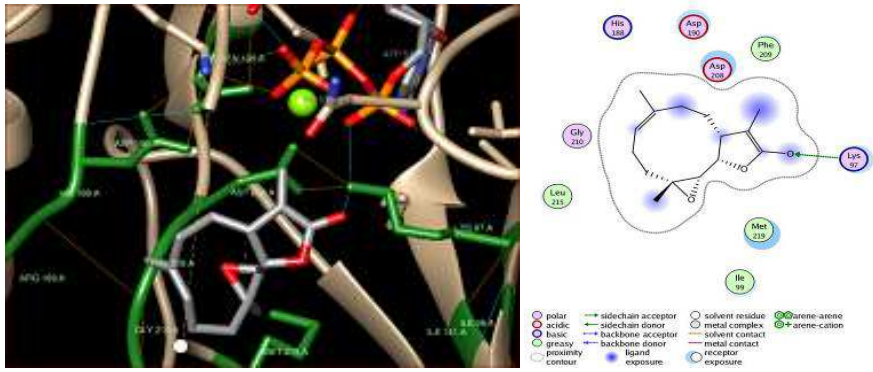

Fig. 5: Parthenolide-MEK-1 interactions visualized by Chimera (left) and MOE (right)

CONCLUSION

The ADMET profiles for the antimicrobials; Hispidulin, Nepetin and Parthenolide, isolated from *Tarconanthus camphoratus* L., have been estimated using different web based *in silico* tools. In addition, their biological activity spectra were also investigated. The sesquiterpene lactone Parthenolide was proposed to have potential antitumor activity through inhibition of MEK-1 or enhancement of TP53 expression. The flavone Nepetin was also predicted to have anticancer activity mediated by UCK-2 inhibition. Nepetin and the other flavone Hispidulin were proposed to play a role in the treatment of diabetic cataracts and muscular dystrophy through inhibition of chlordecone reductase enzyme.

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CONFLICT OF INTERESTS

We declare no conflict of interest

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