GC-MS analysis of phytoconstituents of *Acacia nilotica* and its molecular docking with key drug-targets of *Leishmania donovani* to combat leishmanaisis

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Abstract:

Acacia nilotica is an important medicinal plant, found in Africa, Middle East, and Indian subcontinent. Every part of the plant possesses a wide array of biologically-active and therapeutically important compounds and have been used in traditional-system of medicine. We reported the antileishmanial activity of *Acacia nilotica* (*A. nilotica*) bark methanolic extract

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through *in vitro* assays and dissected the mechanism of its action through *in silico* studies. Bark methanolic extract exhibited anti-promastigote and anti-amastigote potency with IC_{50} value of 19.6 ± 0.9037 µg/ml and 77.52 ± 5.167 µg/ml respectively in time and dose dependent manner. It showed very low cytotoxicity having CC_{50} value of 432.7 ± 7.71 µg/ml on human-macrophage cell line, THP-1. The major constituents identified by GC-MS analysis are 13-docosenoic acid (34.06%), lupeol(20.15 %), 9,12-octadecadienoic acid (9.92 %) and 6-octadecanoic acid (8.43 %) bind effectively with the potential drug-targets of *Leishmania donovani* (*L. donovani*) including sterol 24-c-methytransferase (SMT), trypanothione reductase (TR), pteridine reductase (PTR1) and adenine phosphorybosyl transferase (APRT); suggest the possible mechanism of its antileishmanial action. The highest affinity with all these targets was shown by lupeol. The pharmacokinetic studies, predicted bioactivity scores and acute toxicity studies of major extract constituents support safe antileishmanial drug candidate. This study proved the antileishmanial potential of bark-methanolic extract *A. nilotica* and its mechanism of action through the inhibition of potential drug targets of *L. donovani.*

Key words: *Leishmania*; *Acacia nilotica;* antileishmanial, drugs; host; infection; *in silico*

Running Title: Antileishmanial potential of *A. nilotica* bark extract

1. Introduction:

Visceral leishmaniasis (VL), also known as Kala-Azar in Indian subcontinent, is a fatal form of vector borne disease caused by protozoan parasites *L. donovani*. The disease remains endemic in more than 60 countries around the globe, while 95 % cases are concentrated in 7 to 8 countries. More than 50 % of the global burden of VL is found in the Indian subcontinent (India, Bangladesh, and Nepal) [1,2]. The disease is proved to be fatal if left untreated in more than 95 % of cases because of secondary infection and anemia [3]. VL is ranked second in mortality rate

among the neglected tropical diseases[4,5]. It is a significant problem for the economically weaker section of the society. Due to their unhygienic living environment, they are more vulnerable to the disease. Illiteracy is another factor which is directly proportional to the lack of awareness which leads to major morbidity and mortality. The available chemotherapy of visceral leishmaniasis is limited and undermined by drug resistance. Currently, in general the drug used in the Indian subcontinent sodium antimony gluconate (SAG) showed no response in more than 64 % of the patients due to development of resistance against the parasites [6]. Alternate drugs, miltefosine, amphotericin B and its lipid formulations have several limitations because of high toxicity, cost and unavailability, limit its use. The present scenario of disease and its limited treatment options demand an urgent need to develop a promising and cost-effective operational drug to overcome the disease. In case of parasitic disease, directly targeting parasites is found to be significant in disease recovery. Till date, large numbers of medicinal plants and their extracts had been studied for antileishmanial activity and proved to be potential therapeuticoptions[7,8]. Here, we planned to explore the antileishmanial activity of medicinal plant, *A. nilotica. A. nilotica* commonly known as babul, belongs to the family Fabaceae of genus Acacia, is an important medicinal plant, found in Africa, Middle East, and Indian subcontinent [9-11]. It is rich in secondary metabolites including condensed tannins, flavonoids, gums and phlobatannins [12,13].Every parts of the plant possess a wide array of biologically active, therapeutically potential compounds that have been used in traditional system of medicine, as remedy for various diseases. Its different parts are used in the treatment of different diseases like, floral parts for gastrointestinal disorders [14], leaves extracts for cancer and microbial infections [15,16], roots extract for tuberculosis and liver disorders [17] bark for bacterial infections including cold, bronchitis, dysentery, biliousness, cholera, bleeding piles [12,18-20]. The extract of bark contains the

different form of tannins ((-) – epigallocatechin-7-gallat, (-) – epigallocatechin-5,7-digallat, dicatechin) [21,22] , flavonoids ((+)-catechin-5-gallate , (+)-catechin-5,7-digallate, (+)-catechin-3′,5-digallate, (+)-catechin-4′,5-digallate, (+)-mollisacacidin, apigenin-6,8-bis-C-β-D-glucopyranoside (vicenin), leucocyanadin, kaempferol-7-glucoside, acacetin, umbelliferon, caffeic acid, protocatechuic acid and m-catechol [12,23,24]. Keeping the rich antimicrobial bioactive collection of bark of *A. nilotica*, in mind, we planned to study its antileishmanial potential here. We also tried to dissect the mechanism of its antileishmanial action through different *in silico* approaches. SMT, TR, PTR1 and APRT are prerequisite enzymes for survival, pathogenicity and transmission of *L. donovani*. Therefore, we selected these potential drug-targets for molecular docking study of major constituents of bark-extract identified by GC-MS, with these mentioned essential enzymes of *Leishmania.*

2. Materials and method

2.1 Chemicals: M199 media, Roswell park memorial institute (RPMI) 1640 media, penicillin streptomycin antibiotic cocktail, fetal bovine serum (FBS) were purchased from Gibco. HEPES, sodium bicarbonate and paraformaldehyde were purchased from Sigma Aldrich. Miltefosine, MTT assay reagents, DMSO and different solvents were procured from Merck. Propidium iodide and Annexin V apoptosis kit were procured from Thermo scientific. All the other chemicals and reagents were purchased from Sigma Aldrich or Merck unless stated otherwise.

2.2 Parasites and cell culture:

Infective strain of *L. donovani* (MHOM/IN/83/AG83)was obtained from Dr. Rentala Madhubala (School of life science JNU, New Delhi; India). THP-1, Human monocytic cell line was procured from Cell Repository of National Centre for Cell Science, Pune, India. It was further maintained in M199 media. Human monocytic cell line, THP-1 was maintained in RPMI 1640

media supplemented with 10 % FBS and 1 % penicillin streptomycin antibiotic medium in a humidified environment at 5 % CO_2 and 37^0C temperature. THP-1 monocytic cell was differentiated to macrophages by using phorbol myristate acetate (PMA) at a concentration of 20 ng/ml.

2.3 Extract preparation and antileishmanial activity:

A. nilotica was collected from natural habitats. Bark identification was done at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. The selected plant material was washed and air dried in shade at room temperature. The powdered plant materials were soaked in methanol and placed on the rotary shaker at room temperature for 24 h. The extract was filtered and concentrated using rotatory evaporator under vaccum at 35^0C. The dried plant extract was stored at -20^0C until used for bioassay. To evaluate anti-promastigote potential of *A nilotica*, stationary phase (2 x 10^6 cells/ml) promastigotes were incubated with plant extract for 48 h followed by fixing using 1 % paraformaldehyde and counting through hemocytometer at 22^0C. Miltefosine a known antileishmanial drug used as the positive control. Percent viability was determined using the formula:

$$\%\ Viability = \frac{Average\ parasite\ count\ per\ ml\ (treated)}{Average\ parasite\ count\ per\ ml\ (control)} x100$$

50 % inhibitory concentration (IC_{50}) at which parasite growth was reduced by 50 % was assessed by GraphPad Prism 7.00, nonlinear regression curve fit.

2.5 Cytotoxicity assessment and anti-amastigote evaluation of extract:

The cytotoxicity of *A. nilotica* on THP-1 differentiated macrophages were assessed by MTT [3-(4,5 dimethyl- thiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. Briefly, 2 x 10^6 THP-1 monocytes were seeded in 96 well tissue culture plate (200 µl/well) in RPMI1640 complete

media for 24 h in CO_2 incubator at 37^0C and 5% CO_2. After treatment to THP-1 differentiated macrophages, freshly prepared 5 mg/ml MTT was added (20 µl/well) with 50 µl of blank media and further incubated for 2 to 3 h in CO_2 incubator. Precipitated formazan was dissolved in dimethyl sulfoxide (DMSO) absorbance was recorded at 570 nm in an ELISA plate reader and percent viability was calculated. To determine the effect of *A. nilotica* on the parasite burden of the host macrophages**,** 0.5 x 10^6 THP-1 cells were seeded on the cover slip, placed in the six well plates in CO_2 incubator at 37^0C. THP-1 macrophages were plated, infected with *L. donovani* at the ratio of 1:10 (macrophages to *Leishmania*) for 48 h then cells were fixed with chilled methanol and parasite counting was performed under microscope after Giemsa staining. From the different focus, 100 macrophages were counted to determine the parasite burden of the macrophages. Parasite burden in the infection control was considered 100 %, with respect to parasite load in treated samples.

2.6 GC-MS analysis of extract:

GC-MS analysis was performed to identify the secondary metabolites that may be responsible for the antileishmanial efficacy of *A nilotica*,. Bark was crushed, powdered, and extracted in methanol and then analyzed on Shimadzu QP2010 armed with a DB-5MS column. The mass spectrums of the sample were produced in an electron impact ionization mode of 70 eV and the phytochemicals were identified after correlation of the recorded mass spectrum with the reference library WILEY8.LIB and NIST14.LIB supplied with the software of the GC-MS system.

2.7 Molecular docking studies:

To begin with structure-based virtual screening and docking, we used various bioinformatic tools, such as PyRx [25], AutoDock Vina [26], PyMOL [27] and BIOVIA Discovery Studio [28]. The

online resources used in the retrieval, analysis and evaluation of the data are PubChem database and RCSB Protein Data Bank (PDB) [29]. The target proteins of *L. donovani* and the phytochemical compounds were uploaded into the virtual screening program PyRx. The target protein was changed into macromolecule, which converted the atomic coordinates into pdbqt format. Molecular docking was performed by selecting the grid box around the crystal structures and rest of the parameters were left as default. AutoDock Vina was used to predict the binding mode and the best binding affinity of the phytochemicals. The algorithm used by AutoDock Vina is a hybrid scoring function that is inspired by X-score, which accounts for hydrogen bonding, hydrophobic effect, van der Waals forces and deformation penalty. In addition, for computing the binding energy AutoDock Vina combines both the conformational preferences of receptor–ligand complex and experimental affinity measurements. The results of molecular docking were screened for binding affinity and then all possible docked conformations were generated for different constituents. After analyzing with PyMOL and Discovery Studio, only those conformations were selected which specifically interact to the active-site residues of *L. donovani* targeted proteins. Discovery Studio was used to analyze detailed interactions and their types including hydrogen bonds, alkyl, pi-alkyl, halogen, and the van der Waals interactions formed with between different constituents and the target proteins. The most favorable binding poses of the rutin were analyzed by choosing the lowest free energy of binding (ΔG) and the lowest inhibition constant (*K*i) which is calculated using the following formula:

$$Ki_{pred} = \text{exponential}^{(\Delta G/RT)}$$

where ΔG is binding affinity (kcal/mol), R (gas constant) is 1.98 $calK^{-1}mol^{-1}$, and T (room temperature) is 298.15 Kelvin.

2.8 Sequence analysis, template identification, homology modeling, and receptor and ligand preparation

The protein sequences of trypanothione reductase (XP_003858222.1) and sterol 24-c-methyltransferase (XP_003865366.1) from *L. donovani* were retrieved from NCBI. The blastp [30] was performed against Protein Data Bank for the identification of similar templates. The alignment of the query sequences and template sequences was performed using CLUSTAL Ω [31].The crystal structure of trypanothione reductase from *L. infantum* 2.95 Å resolution (PDB id: 2JK6_A) and X-ray diffracted crystal structure 1.34 Å resolution (PDB id: 5WP4_A) were used as template structures to model the 3D structures of trypanothione reductase and sterol 24-c-methyltransferase, respectively. PDB was used to retrieve the template structure. Homology modelling was carried out using Modeller 9.24 [32] and PyMol was used for the visualization of the 3D structures. The energy minimization was performed using Discovery studio. The PROCHECK program, Ramachandran plots was also used for the assessment of the model [33].

Crystal structures of the Adenine phosphoribosyl transferase and Pteridine reductase proteins were downloaded from PDB [IDs: 1QB7 (APRT) and 2XOX (PTR1)]. The PDB files used for docking-based virtual screening study were processed by removing water molecules and adding hydrogen atoms. The proteins were finally prepared by Discovery Studio keeping all the parameters at default. The identification of the critical residues of the binding pockets were taken from the native binding pockets of available crystal structure of proteins, various submitted literatures, from their homologous template proteins andinvestigation in the mechanism of inhibition. The 3D structure of 9,12-Octadecadienoic acid, 6-Octadecenoic acid, 13-Docosenoic

acid and Lupeol was retrieved from PubChem database in SDF format. The atomic coordinates of all the ligands were changed to pdbqt set-up using Open Babel GUI, an open source chemical toolbox for the interconversion of chemical structures [34]. Universal Force Field (uff) was used for the energy minimization [35].

2.9 Pharmacokinetics studies

The selected ligands were evaluated for their pharmacological profiles by analyzing for Lipinski's rule of violation-5, which was analyzed by Molsoft L.L.C.: Drug-Likeness and molecular property prediction for drug-likeness. (http://www.molsoft.com/mprop/) The bioactivity of the selected inhibitors was checked by Molinspiration. (https://molinspiration.com/cgi-bin/properties). The successfully screened ligands were further evaluated for ADMET (absorption, distribution, metabolism, excretion and toxicity) properties by GUSAR [36] and SwissADME database [37].

3. Results:

3. 1 Antileishmanial activity of *A. nilotica* on *L. donovani* promastigotes:

The growth inhibitory effects of *A. nilotica* bark methanolic extract fraction was assessed against exponentially growing *L. donovani* promastigotes. *A. nilotica* treatment reduced the promastigotes proliferation in a time and dose dependent manner. Growth kinetics was assessed for seven days; there was a gradual decrease in the promastigote proliferation at all the doses (Figure. 1A). The promastigote culture was completely shattered at the dose concentration of 250 and 500 µg/ml of *A. nilotica,* after the three days of treatment. Miltefosine, an established antileishmanial drug rapidly shattered the promastigote parasites *in vitro*. The *Leishmania* promastigotes without any treatment or with 0.5 % DMSO (solvent control), exponentially grow till 4th days of parasites seeding, conforming no antileishmanial potential of solvent. After 4th day

of experimental setup, the culture even in the control and the solvent control were gradually decreased, because of media exhaustion. IC_{50} value of *A. nilotica*on *Leishmania*-promastigotes was calculated as 19.6 $\pm$ 0.9037 µg/ml and the miltefosine treated positive control have the IC_{50} of 3.118 $\pm$ 0.2395 (Figure. 1B). *A. nilotica* treatment exhibits the morphological changes in the promastigote stage parasites, though at lower doses the parasites retained normal morphology. At the higher concentrations there is reduction in size and shortening of flagella. Miltefosine treatment also exhibited the similar morphological changes as extract showed at higher doses (Figure. 1C).

3.2 Growth reversibility assay after extract treatment:

A. nilotica, treated and untreated parasites were washed with PBS after 7 days and old media was removed and supplemented with fresh media. The samples were further incubated at 22^0C for next 72 h to study the growth reversibility of parasites. Parasites treated with higher doses do not revert back though parasites in flasks of lower dose plant-extract treatment show slower growth reversion (Figure. 1D). Suppression of growth reversion was observed significant ($P < 0.001$) at 250 µg/ml of *A. nilotica* in comparison to untreated sample (Figure. 1D).

3.3 Cytotoxicity and antileishmanial activity of *A. nilotica* on intra-macrophagic amastigotes

Upon internalization promastigotes are transformed into the amastigote form inside the parasitophorous vacuoles of macrophages. Theses amastigote forms of the parasites are non-motile and define the parasite pathogenicity. Thus, being the biologically and clinically relevant form it was important to check the anti-amastigote efficacy of *A. nilotica* methanolic extract. THP-1 differentiated macrophages were parasitized by *L. donovani* promastigotes and treated with different doses of extract. Plant extract treatment reduced the intra-macrophagic parasites in

dose dependent manner with IC_{50} value of 77.52 ± 5.167 µg/ml (Figure. 2A). Miltefosine was taken as positive control (Figure. 2A). Cell cytotoxicity (CC_{50}) of *A. nilotica* methanolic extract was evaluated along with miltefosine as positive control on THP-1 differentiated macrophages to study its safe dose. THP-1 differentiated macrophages were incubated with different concentrations of extract/miltefosine (0 to 1000 µg/ml) and the cell viability was assessed using MTT assay. It was observed that *A. nilotica* has least cytotoxic effect on the viability and morphology of the macrophages with CC_{50} value of 432.7 ± 7.71 µg/ml while miltefosine showed higher toxicity with CC_{50} value of 8.219 ±0.6337 µg/ml (Figure 2B). A significant reduction in intra-macrophagic parasite count was observed in micrographs of Giemsa stained infected and extract treated macrophages (Figure. 2C).

3.4 TLC-bioautography identification and GC-MS analysis of *A. nilotica* bark methanolic extract:

Plant secondary metabolites present in *A. nilotica* bark methanolic extract fractions that may have been responsible for the observed antileishmanial effects were identified throughTLC-bioautography and GC-MS analysis. The total constituents found in were 25 (Table. 1) out of which the major constituents were 13-docosenoic acid (34.06 %), lupeol(20.15 %), 9,12-octadecadienoic acid (9.92 %) and 6-octadecanoic acid (8.43 %).

3.5 Molecular docking of *A. nilotica* methanolic extract of major constituents with potential drug-targets of *L. donovani*

The TR and SMT enzymes were modelled using Modeller 9.24 and the energy minimization was carried out by BIOVIA Discovery studio. The three-dimensional cartoon representation of TR and SMT enzymes are shown in Supplementary Fig 1A & 2A. The models were selected by analysing its stereochemical quality using PROCHECK program. The generated models of TR

and SMT show a good quality structure having 99.8 % and 99% residues in the allowed regions of Ramachandran plot respectively (Supplementary Fig. 1B & 2B). PDBsum tool was used to analyze and found that the 3D structure of the enzyme is composed of mixed α-helices and β-strands (α+β) secondary structures [33]. The structural topology of TR and SMT showed 5 sheets, 23 strands, 18 helices and 34 beta turns and 2 sheets, 10 strands, 14 helices and 41 beta turns, respectively (Supplementary Fig. 1C, 1D, 2C & 2D). Multiple sequence alignments were performed and Discovery studio was used in order to find the key residues and region around the binding cavity of TR and SMT. The active site residues of the SMT, TR, PTR11 and APT enzymes making different numbers of hydrogen bonds as well as hydrophobic bonds with the ligands were also identified. On the basis of binding affinity Lupeol; 9,12-Octadecadienoic acid; 6-Octadecenoic acid; and 13-Docosenoic acid have binding energies of −8.5 kcal/mol, −5.7 kcal/mol, −5.7 kcal/mol, and −5.6 kcal/mol ; −8.4 kcal/mol, −4.9 kcal/mol, −4.9 kcal/mol, and −4.7 kcal/mol ; −7.9 kcal/mol, −5.3 kcal/mol, −4.4 kcal/mol, and −5.4 kcal/mol ; −6.2 kcal/mol, −6.1 kcal/mol, −5.9 kcal/mol, and−5.9 kcal/mol with SMT, TR, PTR11 and APT enzymes, respectively (Table 2). The binding pattern of lupeol with SMT, TR, PTR11 and APT may hinder the substrate accessibility and its subsequent inhibition as shown in (Fig. 3A, 4A, 5A & 6A) where the binding energies and inhibition constants are −8.5 kcal/mol, −8.4 kcal/mol, −7.9 kcal/mol, −6.2 kcal/mol, and 6.25 μM, 6.12 μM, 5.81 μM, 4.56 μM respectively (Table 2). It shows favorable interactions with SMT through two pi-alkyl bonds with Arg347 and Lys351, TR via a pi-alkyl bond with Tyr198, PTR11 by two pi-alkyl bonds with Val83 and Arg88 and APRT through a hydrogen bond with Thr151. (Fig. 3B, 4B, 5B & 6B). The binding interaction shown by 9,12-Octadecadienoic acid with SMT, TR, PTR11 and APRT, may obstruct the substrate accessibility of these proteins which leads to their subsequent inhibition as shown in

(Fig. 3C, 4C, 5C & 6C) where the binding energies and inhibition constants are −5.7 kcal/mol, −4.9 kcal/mol, −5.3 kcal/mol, −6.1 kcal/mol, and 4.19 μM, 3.60 μM, 3.90 μM, 4.49 μM respectively (Table 2). The favorable interactions shown by 9,12-Octadecadienoic acid with SMT through a hydrogen bond with Lys198, TR via a hydrogen bond with Gly376, PTR11 by eighteen pi-alkyl bonds with Phe86, Lys87, Ala90, Ala94, Lys156, Ala157, His160, Arg161 and APRT through a hydrogen bond with Arg82. (Fig. 3D, 4D, 4D, 5D & 6D). The binding pattern of 6-Octadecenoic acid with SMT, TR, PTR11 and APRT may lead to their subsequent inhibition by obstructing their substrate accessibility as shown in (Fig. 3E, 4E, 5E & 6E) where the binding energies and inhibition constants are −5.7 kcal/mol, −4.9 kcal/mol, −4.4 kcal/mol, −5.9 kcal/mol, and 4.19 μM, 3.60 μM, 3.24 μM, 4.34 μM, respectively (Table 2). The important and favorable interactions shown by 6-Octadecenoic acid with SMT through two pi-alkyl bonds with Lys198, TR via a hydrogen bond with Gly376, PTR11 by a hydrogen bond with Leu92 and APRT through two hydrogen bonds with Arg37 and Arg82. (Fig. 3F, 4F, 5F & 6F).

The pattern of interaction of 13-Octadecenoic acid with SMT, TR, PTR11 and APRT may lead to their subsequent inhibition by obstructing their substrate accessibility as shown in (Fig. 3G, 4G, 5G & 6G) where the binding energies and inhibition constants are -5.6 kcal/mol, -4.7 kcal/mol, -5.4 kcal/mol, -5.9 kcal/mol, and 4.12 μM, 3.46 μM, 3.97 μM, 4.34 μM respectively (Table 2). It shows favorable interactions with SMT through two hydrogen bonds with Lys241 and Gln263, TR via a hydrogen bond with Gly376, PTR11 by twenty pi-alkyl bonds with Val83, Phe86, Lys87, Ala90, Ala94, Lys156, Ala157, His160 and APRT through a hydrogen bond with Arg37 and Ar82. (Fig. 3H, 4H, 5H & 6H).

3.6 Pharmacokinetics studies of *A. nilotica* bark-methanolic extract constituents:

The pharmacological studies was done for the selected ligands against Adenine phosphoribosyl transferase, Pteridine reductase, trypanothione reductase and sterol 24-c-methyltransferase proteins for a good oral administration established through the Lipinski rule of five[38], which was evaluated by Molsoft L.L.C.: Drug-Likeness and molecular property prediction. Lipinski's “rule of five” is ananalytical approach for predicting drug-likeness stating that molecules had Molecular weight (M.W. ≤ 500 Da), high lipophilicity expressed as LogP (LogP ≤ 5), hydrogen bond donors (HBD ≤ 5) and hydrogen bond acceptors (HBA ≤ 10) have good absorption or permeation across the cell membrane. Lupeol, 9,12-Octadecadienoic acid, 6-Octadecenoic acid, and 13-Docosenoic acid followed all the parameters of Lipinski rule of five, except low lipophilicity as observed from Table 2. As per the Lipinski rule of five, violation of one parameter is acceptable for an orally active drug. The absorption percentage (AB%) was calculated using the formula [39].

$$AB\% = [109 - (0.345 \times TPSA)]$$

It is important to look into the pharmacokinetic properties of the compounds, prior to animal and clinical studies. In order to evaluate the bio-chemical behaviour of these compounds inside an organism in respect of absorption, distribution, metabolism, and excretion (ADME), SwissADME database [37] was used to explore the drug likeness and pharmacokinetics properties of these compounds. The lipophilicity of Lupeol, 9,12-Octadecadienoic acid, 6-Octadecenoic acid, and 13-Docosenoic acid showed LogPo/w value of 4.76, 4.61, 4.73 and 5.65 that indicates high sublingual absorption respectively. Lupeol and 13-Docosenoic acid possess low gastrointestinal absorption and poor water soluble capability whereas 9,12-Octadecadienoic acid and 6-Octadecenoic acid shows high gastrointestinal absorption as well as moderate water soluble capability. None of the compounds are permeable to the blood–brain barrier. 9,12-

Octadecadienoic acid, 6-Octadecenoic acid, and 13-Docosenoic acid are CYP1A2 inhibitors, which likely to increase the half-life of these compounds and also prevent serious drug interactions. The drug-likeness criteria are qualified by all the ligands with one violation and possess a significant bioavailability score. The results are summarized in Table 3.

The bioactivity prediction of the major constituents of *A. nilotica* bark-methanolic extract was analysis through molinspiration. The activity was calculated against G-protein coupled receptor-ligand, ion channel modulator, a kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor [40]. The interpreted values for bioactivity were as: active (bioactivity score ≥ 0), moderately active (bioactivity score: between -5.0 to 0.0) and inactive (bioactivity score ≤ -5.0)[41]. Lupeol, 9,12-Octadecadienoic acid, 6-Octadecenoic acid, and 13-Docosenoic acid were evaluated as active enzyme inhibitors with values 0.52, 0.23, 0.12 and 0.10, respectively. Lupeol and 9,12-Octadecadienoic acid were evaluated as active protease inhibitor as well as ion channel modulator. (Table 4)

The principal aim of predicting the acute toxicity is to evaluate undesirable side effects of a compound after single or multiple exposures to an organism via a known administration route (oral, inhalation, subcutaneous, intravenous or intraperitoneal). GUSAR was used to determine the acute toxicity of the successfully docked compounds. The parameters used by GUSAR to probe compounds based on the prediction of activity spectra for substances algorithm and quantitative neighborhoods of atoms descriptors. The obtained results were compared with SYMYX MDL Toxicity Database to further categories them on the basis of Organisation for Economic Co-operation and Development (OECD) chemical classification manual[36]. The criteria used for these compounds to elicit toxicity based upon the administration route when the compound dose is more than 7000 mg/kg for intravenous route, more than 500,000 mg/kg in

case of the oral route, and more than 20,000 mg/kg for intraperitoneal route and subcutaneous database as shown in Table 5. As per the OECD chemical classification 9,12- Octadecadienoic acid, 6-Octadecenoic acid, 13-Docosenoic acid found to be non-toxic and Lupeol is a Class 5 chemical.

4. Discussion:

Plant extracts have promising medicinal properties and extensively used in the traditional system of medicine due to the presence of many active and leading medicinal [42]. From the previous studies, it has been revealed that many medicinal plants-extracts and their secondary metabolite contents have proven to be efficient and low toxic antileishmanial drug candidates[43,44]. *A. nilotica* which has been identified as the potential medicinal plants, are rich in secondary metabolites. Studies based on GC-MS analysis of *A. nilotica* showed presence of different types of secondary plant metabolites including polyphenols, mainly composed of condensed tannin and phlobatannin in addition to gallic acid, ellagic acid, catechin, epigallocatechin-7-gallate, flavonoids and gum [13].Different solvent extracts of the *A. nilotica* had been shown to have antimicrobial activities including anti-bacterial, anti-fungal, anti-viral and anti-amebic[18,45,46].We evaluated the antileishmanial potential of *A. nilotica* and identified its secondary metabolite constituents by GC-MS analysis.*A. nilotica* bark methanolic extract repressed the growth of *L. donovani* promastigotes in a time and dose dependent manner. It induced the morphological changes and cytocidal mode of parasite killing. The cytocidal mode of killing of *A. nilotica* may be because of its richness in phenolic compounds (31) and phenolic acids which cause irreversible changes to cell membrane (32). Methanolic extract of fruit of *A. nilotica* has been reported to have antileishmanial activity with IC_{50} value of 89.38 µg/ml on the *L. major* promastigotes (33). Here, we for the first time studied the role of *A. nilotica* bark-methanolic

extract against the growth and proliferation of *L. donovani* promastigotes and intra-macrophagic amastigotes. IC_{50} value of *A. nilotica* was determined as 19.6 ± 0.9037 µg/ml, which is higher as compared to the IC_{50} value of positive control miltefosine as 3.118 ± 0.2395 µg/ml. But the CC_{50} value of *A. nilotica on* macrophages was 432.7 ± 7.71 µg/ml, while that of the miltefosine was 8.219 ± 0.6337 µg/ml. The plant extract significantly inhibited the growth of intra-macrophagic form of the parasites. The IC_{50} value of the extract on amastigote form was calculated as 77.52 ±5.167 µg/ml. *A. nilotica* has found to have low cytotoxic even after having higher IC_{50} value as compare to the miltefosine. So, the higher concentration of *A. nilotica* can be used to inhibit the growth of amastigotes inside macrophages. The major constituents identified through GC-MS analysis were 13-docosenoic acid (34.06%), lupeol (20.15 %), 9,12-octadecadienoic acid (9.92 %) and 6-octadecanoic acid (8.43 %). To dissect the mechanism of antileishmanial activity of *A. nilotica*, we further performed the molecular docking study of major constituents of extract identified by GC-MS, with essential enzymes of *Leishmania* including SMT, TR, PTR1 and APRT. These enzymes play essential role in parasite growth, survival, virulence and its transmission inside host. SMT is required for the biosynthesis of ergosterol, the major membrane sterol in *L. donovani*[47]. The enzyme TR follows thiol-redox metabolism to keep trypanothione in reduced form. This antioxidant property of TR is essential for survival of *L. donovani* [48]. PTR1 catalyze the reduction of conjugated and non-conjugated pterins such as reduced biopterin to dihydrobiopterin. [49]. APRT plays vital role in purine metabolism by converting 6-aminopurines into 6-oxypurines [50]. Molecular docking results prove that lupeol and 9,12-Octadecadienoic acid possesses higher binding affinity with SMT, TR, PTR1 and APRT as shown in Table 2. Pharmacological studies of these selected inhibitors for Lipinski rule of 5 indicated violation of only one Lipinski parameter, as shown in Table 3.The pharmacokinetic properties and acute

toxicity of lupeol; 9,12-Octadecadienoic acid;6-Octadecenoic acid and 13-Docosenoic acidhave shown relatively low toxicity profile, which meansrequire high doses to evoke toxic response. The majority of the compounds are non-toxic chemicals whereas lupeol is a Class 5 chemical with very low toxic effects (31, 54). The pharmacokinetic attributes are in favour of these compounds to be exploited as promising antileishmanial drug candidates. It had already been reported that lupeol activates the PI3K/Akt pathway which triggers mechanism responsible for influencing various cell types, including keratinocytes, stimulated cytotoxicity in fibroblasts and the regulation of various diseases [51]. The lupeol treatment had shown high imbalance between Th1/Th2 cytokines production and initiation of pro-inflammatory cytokine response as well as generation of NO in *L. donovani* infected macrophages [52]. It further supports the antileishmanial activity of *A. nilotica* extract. Thus, *in vitro*, molecular docking, pharmacokinetics studies, bioactivity scores, and acute toxicity studies support possible mechanism of antileishmanial activity of the extract through inhibition of key *Leishmania* enzymes.

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Conflict of interest

The author showed no conflict of interest.

Author Contribution

Study was conceptualized by AR, SB and AABD, ASA; data acquisition and data analysis were performed by ST, RA and FR; manuscript preparation and manuscript editing were performed by AR, SB, AABD, FR, ASA, BMA, FAA, MAA and ST; the final manuscript was checked by AR and AABD; fund was obtained by SB, ASA, FAA, MAA, AABD and AR

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Figures legends:

Figure 1: *A. nilotica* bark methanolic extract inhibits the growth and proliferation of *L. donovani* promastigotes (A) 2 X 10^6 stationary phase *L donovani* promastigotes were treated with different concentration of *A nilotica* methanolic plants extracts, with miltefosine as standard drug and control (without any treatment). Each point is statistically significant as compare to control. **(B)**2 X 10^6 stationary phase *L donovani* promastigotes were treated with different concentration of *A nilotica* methanolic plant extract fraction and miltefosine, IC_{50} was determined as described in the methods. Each point represents the mean $\pm$ SE of the samples in triplicate.**(C)** Image of the promastigote morphological changes in the treated samples at different concentration of *A nilotica* fraction and miltefosine treated samples**.(D)** Stationary phase *L donovani* promastigotes were incubated with different concentration of *A nilotica*, with miltefosine and DMSO (solvent control). And the growth reversal was analyzed as described in methods. $^{***}P < 0.001$ with respect to parasite control. Anti-promastigote efficacy of *A nilotica* fractions.

Figure 2: *A. nilotica* bark methanolic extract decreases the intra-macrophagic parasites (A). Anti-amastigote efficacy determination.THP-1 differentiated macrophages was parasitized in 1:10 ratio with stationary phase promastigotes and then treated with different concentration of *A nilotica* fraction. Percent reduction in the parasite load was determined as described in the method. $^{***}P < 0.001$, value was statistically significant as compare to control.**(B).**THP-1 differentiated macrophages were treated with different concentration of *A nilotica* and miltefosine (0 to 1000 μg/ml) and cell viability was ascertained by MTT assay**. (C).** Microscopic *L donovani*infected macrophage images depict parasitized, treated THP-1 differentiated macrophages stained with modified Giemsa stained. The images were captured at 100X under oil immersion. The arrow indicates internalized parasites

Figure 3: *A. nilotica* major chemical constituents inhibit SMT of *L. donovani in silico*

(A) Lupeol blocking the binding pocket of SMT enzyme. **(B)** 2D plot showing interactions between receptor and ligand.**(C)** 9,12-Octadecadienoic acid blocking the binding pocket of SMT enzyme. **(D)** 2D plot showing interactions between receptor and ligand. **(E)** 6-Octadecenoic acid blocking the binding pocket of SMT enzyme. **(F)** 2D plot showing interactions between receptor and ligand. **(G)** 13-Docosenoic acid blocking the binding pocket of SMT enzyme. **(H)** 2D plot showing interactions between receptor and ligand.

Figure 4: *A. nilotica* major chemical constituents inhibit TR of *L. donovani in silico*

(A) Lupeol blocking the binding pocket of TR enzyme. **(B)** 2D plot showing interactions between receptor and ligand. **(C)** 9,12-Octadecadienoic acid blocking the binding pocket of TR enzyme. **(D)** 2D plot showing interactions between receptor and ligand. **(E)** 6-Octadecenoic acid blocking the binding pocket of TR enzyme. **(F)** 2D plot showing interactions between receptor

and ligand. **(G)** 13-Docosenoic acid blocking the binding pocket of TR enzyme. **(H)** 2D plot showing interactions between receptor and ligand.

Figure 5: *A. nilotica* major chemical constituents inhibit PTR1 of *L. donovani in silico* (A) Lupeol blocking the binding pocket of PTR1 (PDB ID: 2XOX) enzyme. **(B)** 2D plot showing interactions between receptor and ligand. **(C)** 9,12-Octadecadienoic acid blocking the binding pocket of PTR1 (PDB ID: 2XOX) enzyme. **(D)** 2D plot showing interactions between receptor and ligand. **(E)** 6-Octadecenoic acid blocking the binding pocket of PTR1 (PDB ID: 2XOX) enzyme. **(F)** 2D plot showing interactions between receptor and ligand. **(G)** 13-Docosenoic acid blocking the binding pocket of PTR1 (PDB ID: 2XOX) enzyme. **(H)** 2D plot showing interactions between receptor and ligand.

Figure 6: *A. nilotica* major chemical constituents inhibit APRT of *L. donovani in silico*

(A) Lupeol blocking the binding pocket of APRT (PDB ID: 1QB7) enzyme. **(B)** 2D plot showing interactions between receptor and ligand. **(C)** 9,12-Octadecadienoic acid blocking the binding pocket of APRT (PDB ID: 1QB7) enzyme. **(D)** 2D plot showing interactions between receptor and ligand. **(E)** 6-Octadecenoic acid blocking the binding pocket of APRT (PDB ID: 1QB7) enzyme. **(F)** 2D plot showing interactions between receptor and ligand. **(G)** 13-Docosenoic acid blocking the binding pocket of APRT (PDB ID: 1QB7) enzyme. **(H)** 2D plot showing interactions between receptor and ligand.

Tables legends:

Table 1: Table 1: TLC-bioautography identification and GC-MS analysis of *A. nilotica* bark methanolic extractdepictedkey chemical constituents of extract

Table 2: Molecular docking interaction of abundant medicinal constituents of *A. nilotica* bark methanolic extract shows significant inhibition of *L. donovani* target proteins

Table 3: Evaluation of physicochemical and ADMET properties show feasibility of usage of the chemical constituents for the treatment of disease

Table 4: Bioactivity prediction of the selected ligands against *L. donovani* by molinspiration

Table 5: Acute toxicity shows the lethal dose and extent of toxicity of chemical constituents on rodent models

Tables

Table 1: TLC-bioautography identification and GC-MS analysis of *A. nilotica* bark methanolic extract depicted key chemical constituents of extract

S. NO.	RETENTION TIME	% AREA	COMPOUND IDENTIFIED
1.	13.606	0.45	1H-3A,7-METHANOAZULEN-6-OL
2.	14.220	0.79	1H-BENZOCYCLOHEPTENE
3.	14.290	0.32	PHENOL, 3,5-BIS(1,1-DIMETHYLETHYL)
4.	15.277	1.49	DIETHYL PHTHALATE
5.	16.139	0.54	1-(4-ISOPROPYLPHENYL)-2-METHYLPROPYL ACETATE
6.	18.855	5.42	HEXADECANOIC ACID, METHYL ESTER
7.	19.328	1.71	N-HEXADECANOIC ACID
8.	20.258	0.30	13-HEXYL-OXA-CYCLOTRIDEC-10-EN-2-ONE
9.	**20.465**	**9.92**	**9,12-OCTADECADIENOIC ACID**
10.	20.523	8.43	6-OCTADECENOIC ACID, METHYL ESTER
11.	20.761	2.81	METHYL STEARATE
12.	20.961	1.44	E,E,Z-1,3,12-NONADECATRIENE-5,14-DIOL
13.	22.267	0.17	HEXAHYDRO-3-BUTYLPHTHALIDE
14.	22.311	2.86	CIS-11-EICOSENOIC ACID, METHYL ESTER
15.	22.368	0.53	CIS-13-EICOSENOIC ACID, METHYL ESTER
16.	22.527	1.14	EICOSANOIC ACID, METHYL ESTER
17.	**23.965**	**34.06**	**13-DOCOSENOIC ACID**
18.	24.161	0.84	DOCOSANOIC ACID
19.	25.532	1.52	CIS-15-TETRACOSENSAEURE
20.	25.721	0.70	TETRACOSANOIC ACID
21.	25.897	0.85	CYCLOPENTADECANONE
22.	27.325	0.34	OCTACOSANE
23.	27.885	0.83	9-OCTADECENAL
24.	33.099	2.36	STIGMASTEROL
25.	**36.679**	**20.15**	**LUPEOL**

Table 2: Molecular docking interaction of abundant medicinal constituents of *A. nilotica* bark methanolic extract shows significant inhibition of *L. donovani* target proteins

S. No.	Proteins	Ligands	Binding energy (Kcal/mol)	p*Ki*$_{pred}$ (µM)	Interacting Residues
1.	Sterol 24-c-methytransferase	Lupeol	-8.5	6.25	Lys198, Tyr206, Met210, Asn215, Pro216, Asn217, Cys240, Gln242, Leu322, Ile344, Arg347, Lys348, Lys351
		9,12-Octadecadienoic acid	-5.7	4.19	**Lys198**, Cys202, Phe203, Tyr206, Met210, Asn215, Asn217, Cys240, Lys241, Phe259, Gln263, Leu322, Ile344, Arg347, Lys348, Lys351
		6-Octadecenoic acid	-5.7	4.19	**Lys198**, Cys202, Phe203, Tyr206, Met210, Asn215, Asn217, Cys240, Lys241, Ala257, Phe259, Ile261, Gln263, Leu322, Ile344, Arg347, Lys348, Lys351
		13-Docosenoic acid	-5.6	4.12	Lys198, Gly200, Cys202, Phe203, Tyr206, Met210, Asn215, Pro216, Asn217, **Lys241**, Phe259, **Gln263**, Leu322, Ile344, Arg347, Lys348, Lys351
2.	Trypanothione reductase	Lupeol	-8.4	6.12	Gly197, Tyr198, Phe230, Val332, Met333, Leu334, His359, Val362, Cys364, Gly374
		9,12-Octadecadienoic acid	-4.9	3.60	Tyr198, Phe230, Val332, Met333, Cys364, **Gly376**
		6-Octadecenoic acid,	-4.9	3.60	Gly197, Tyr198, Gly229, Phe230, Val332, Met333, Leu334, Cys362, Cys364, Gly374, Cys375, **Gly376**
		13-Docosenoic acid	-4.7	3.46	Gly197, Tyr198, Phe230, Gly286, Val332, Met333, Leu334, Lys361, Cys362, Cys364, Gly374, Cys375, **Gly376**
					His38, Gln63, Ala64,

3.	Pteridine reductase	Lupeol	-7.9	5.81	Asp65, Lys71, Ala77, Val83, Lys87, Arg88, Asp91
		9,12-Octadecadienoic acid	-5.3	3.90	Phe86, Lys87, Ala90, Ala94, Lys156, Ala157, His160, Arg161
		6-Octadecenoic acid	-4.4	3.24	Lys71, Ala77, Val83, Lys87, Arg88, **Leu92**
		13-Docosenoic acid,	-5.4	3.97	Val83, Phe86, Lys87, Ala90, Ala94, Lys156, Ala157, His160, Arg161
4.	Adenine phosphoribosyl transferase	Lupeol	-6.2	4.56	Pro36, Arg37, Arg82, Lys103, Glu127, Asp146, Ala150, **Thr151**, Glu152, Gly153, Thr154
		9,12-Octadecadienoic acid	-6.1	4.49	Trp29, Arg37, Val39, Pro40, Arg41, Phe42, Ala43, **Arg82**, Val148, Ala150, Leu176, Ile178, Leu181, Asp206, Leu209
		6-Octadecenoic acid	-5.9	4.34	**Arg37**, Val39, Pro40, Arg41, Phe42, Ala43, **Arg82**, Val148, Ala150, Leu176, Ile178, Phe180, Leu181, Asp206
		13-Docosenoic acid	-5.9	4.34	**Arg37**, Val39, Pro40, Arg41, Phe42, Ala43, **Arg82**, Val148, Ala150, Leu176, Ile178, Phe180, Leu181, Asp206, Leu209

Table 3: **Evaluation of physico-chemical and ADMET properties show feasibility of usage of the chemical constituents for the treatment of disease**

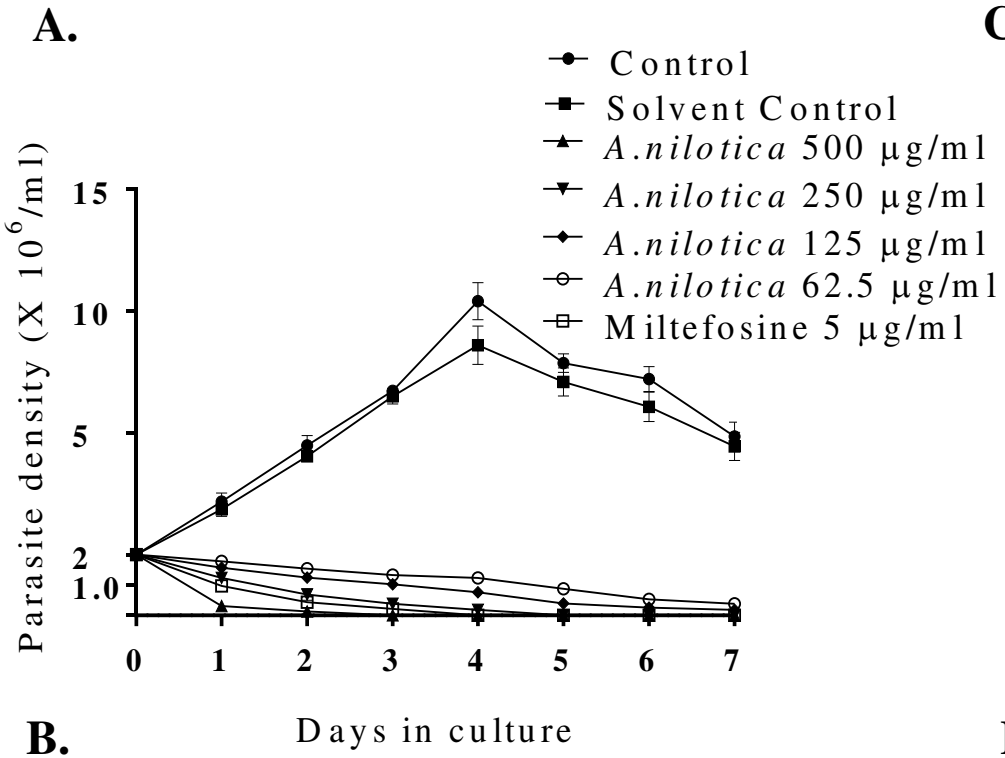
Ligands	**MW (<500)**	**HBD (<5)**	**HBA (<10)**	**Log Po/w (Lipophilicity)**	**TPSA (≤140)**	**Absorption percentage (AB%) (>50%)**	**Drugliness (Lipinski violations)**	**GI-absorption**	**BBB permeant**	**CYP1A2 inhibitor**	**Bioavailability score**	**Water solubility (Log S)**
Lupeol	426.39	1	1	4.76	20.23	102.02	Yes; 1 violation	Low	No	No	0.55	-8.64 (Poorly Soluble)
9,12-Octadecadienoic acid	294.48	0	2	4.61	26.30	99.93	Yes; 1 violation	High	No	Yes	0.55	-4.97 (Moderately Soluble)
6-Octadecenoic acid	296.50	0	2	4.73	26.30	99.93	Yes; 1 violation	High	No	Yes	0.55	-5.13 (Moderately Soluble)
13-Docosenoic acid	352.60	0	2	5.65	26.30	99.93	Yes; 1 violation	Low	No	Yes	0.55	-6.58 (Poorly Soluble)

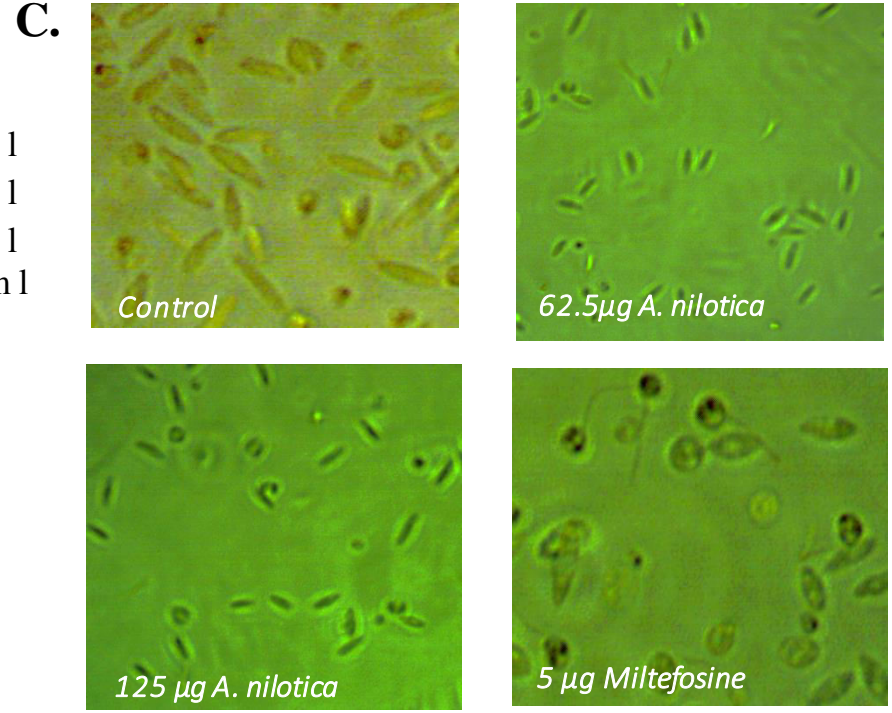
Table 4: Bioactivity prediction of the selected ligands against *L. donovani* by molinspiration

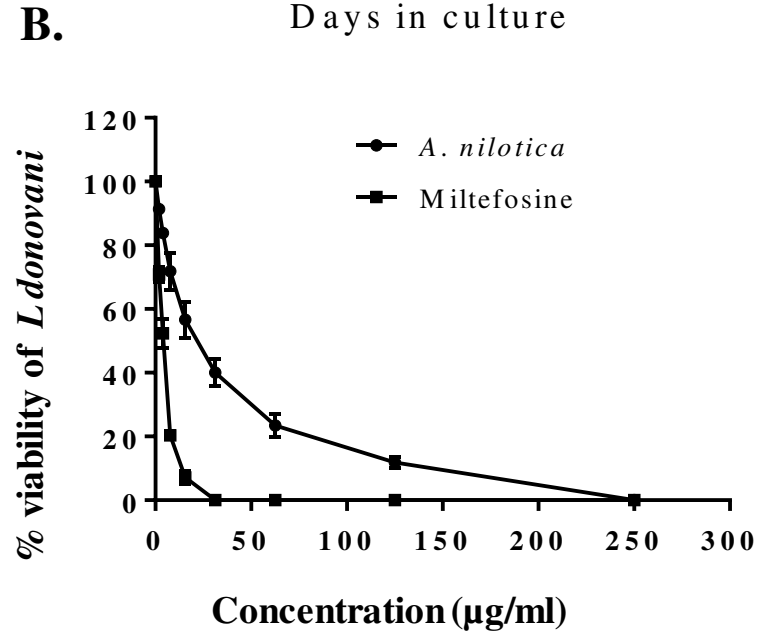
Ligands	GPCR ligand	Ion channel modulator	Kinase Inhibitor	Nuclear receptor ligand	Protease Inhibitor	Enzyme Inhibitor
Lupeol	0.27	0.11	-0.42	0.85	0.15	0.52
9,12- Octadecadienoic acid	0.15	0.07	-0.20	0.14	0.03	0.23
6-Octadecenoic acid	0.03	-0.03	-0.25	0.06	-0.02	0.12
13-Docosenoic acid	0.07	-0.02	-0.17	0.10	0.07	0.10

Table 5: Acute toxicity shows the lethal dose and extent of toxicity of chemical constituents on rodent models

S. No.	Ligands	Rat Oral LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat SC LD50 (mg/kg)	Rat IP LD50 (mg/kg)	OECD chemical classification
1.	Lupeol	2888,000	5,867	786,900	1684,000	Class 5
2.	9,12-Octadecadienoic acid	8747,000	309,300	9261,000	4673,000	Non-Toxic
3.	6-Octadecenoic acid	7813,000	381,700	7007,000	3028,000	Non-Toxic
4.	13-Docosenoic acid	9279,000	428,600	1,1160000	5206,000	Non-Toxic


A.
Control
Solvent Control
A.nilotica 500 µg/ml
A.nilotica 250 µg/ml
A.nilotica 125 µg/ml
A.nilotica 62.5 µg/ml
Miltefosine 5 µg/ml
Parasite density (X 10^6/ml)
15
10
5
2
1.0
0
1
2
3
4
5
6
7
Days in culture


C.
Control
62.5µg A. nilotica
125 µg A. nilotica
5 µg Miltefosine


B.
A. nilotica
Miltefosine
% viability of L donovani
120
100
80
60
40
20
0
0
50
100
150
200
250
300
Concentration (µg/ml)

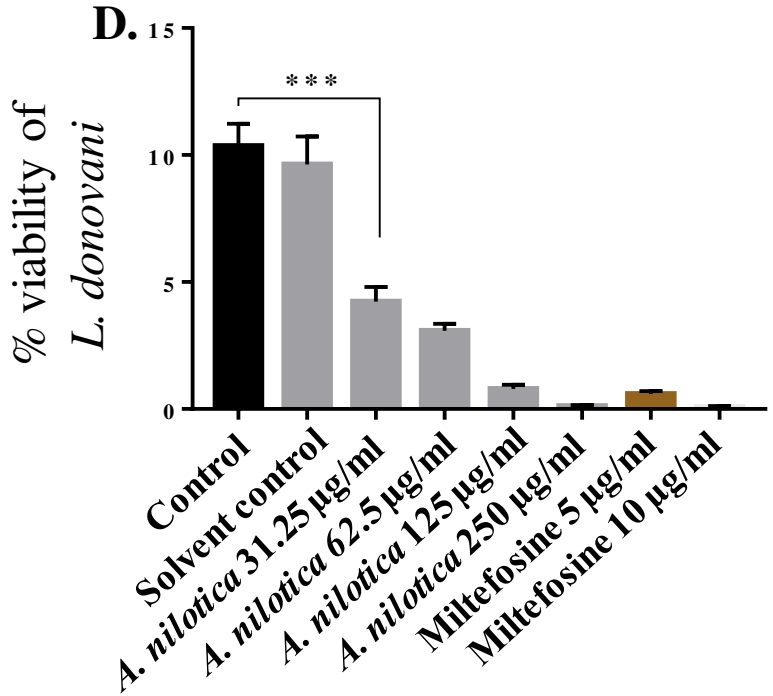

D.
% viability of L. donovani
15
10
5
0
***
Control
Solvent control
A. nilotica 31.25 µg/ml
A. nilotica 62.5 µg/ml
A. nilotica 125 µg/ml
A. nilotica 250 µg/ml
Miltefosine 5 µg/ml
Miltefosine 10 µg/ml

Figure 1

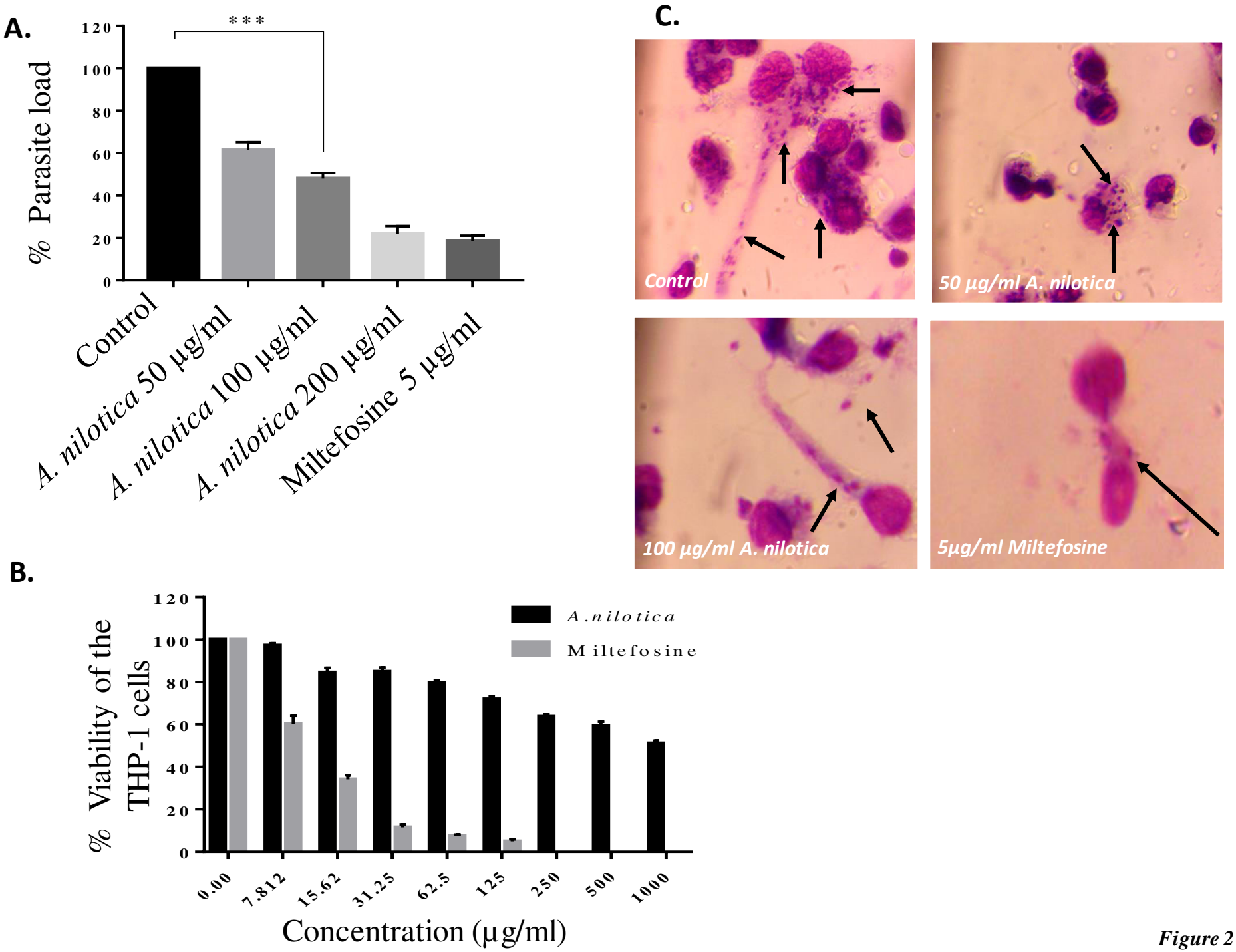

A.
% Parasite load
120
100
80
60
40
20
0
***
Control
A. nilotica 50 µg/ml
A. nilotica 100 µg/ml
A. nilotica 200 µg/ml
Miltefosine 5 µg/ml
C.
Control
50 µg/ml A. nilotica
100 µg/ml A. nilotica
5µg/ml Miltefosine
B.
% Viability of the THP-1 cells
120
100
80
60
40
20
0
A.nilotica
Miltefosine
0.00
7.812
15.62
31.25
62.5
125
250
500
1000
Concentration (µg/ml)

Figure 2

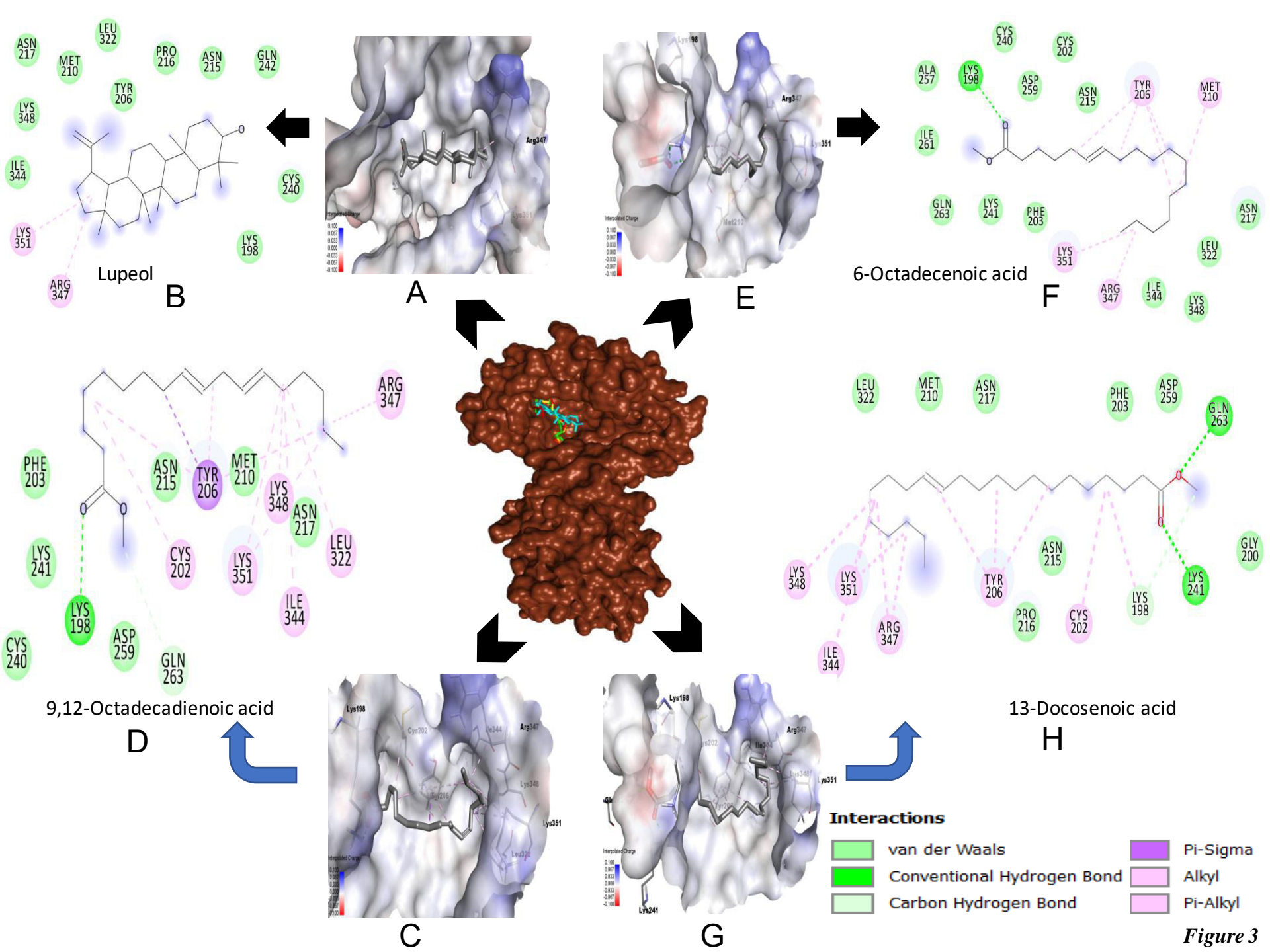

Lupeol
B
A
E
6-Octadecenoic acid
F
9,12-Octadecadienoic acid
D
13-Docosenoic acid
H
C
G
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl

Figure 3

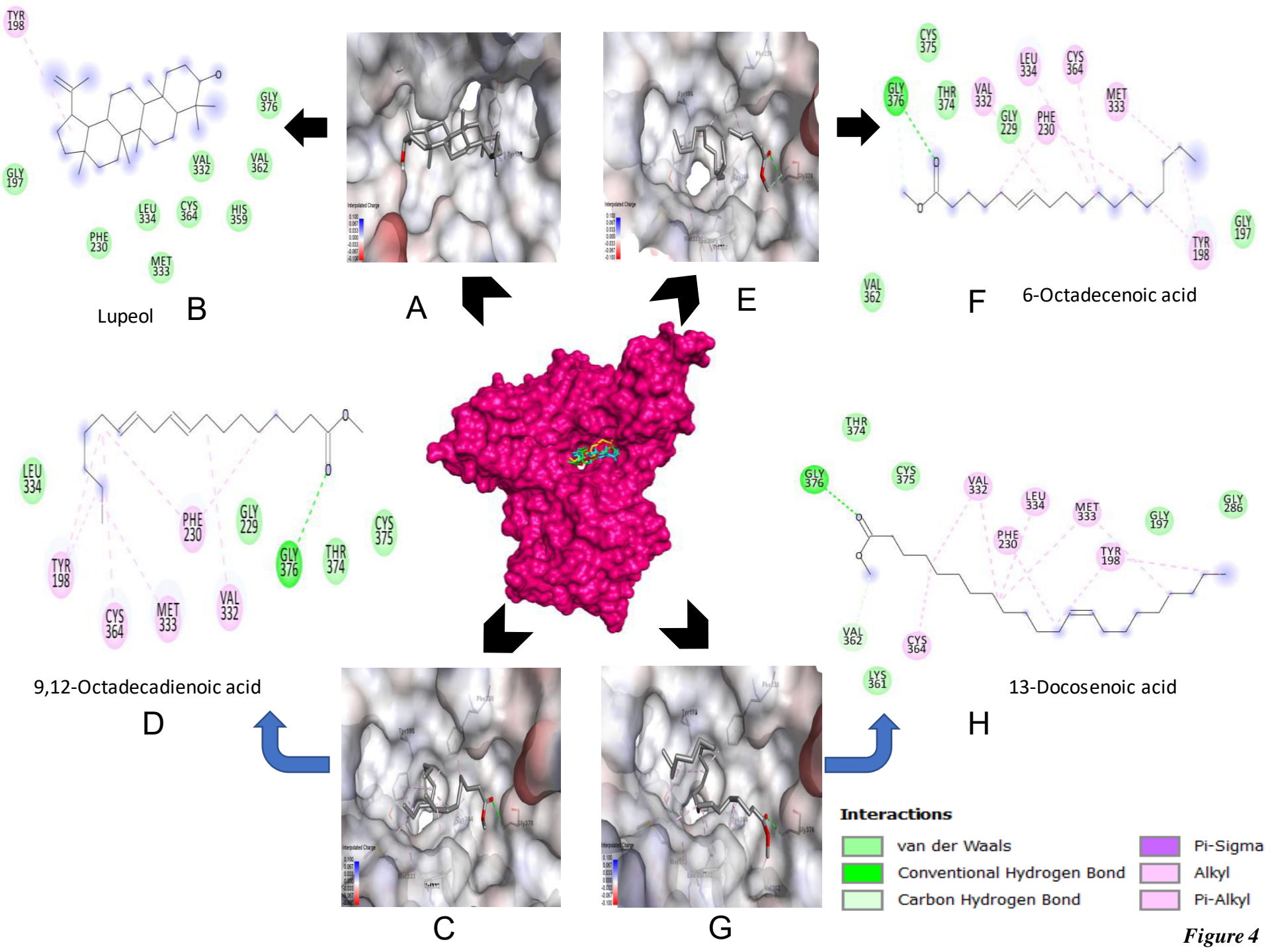
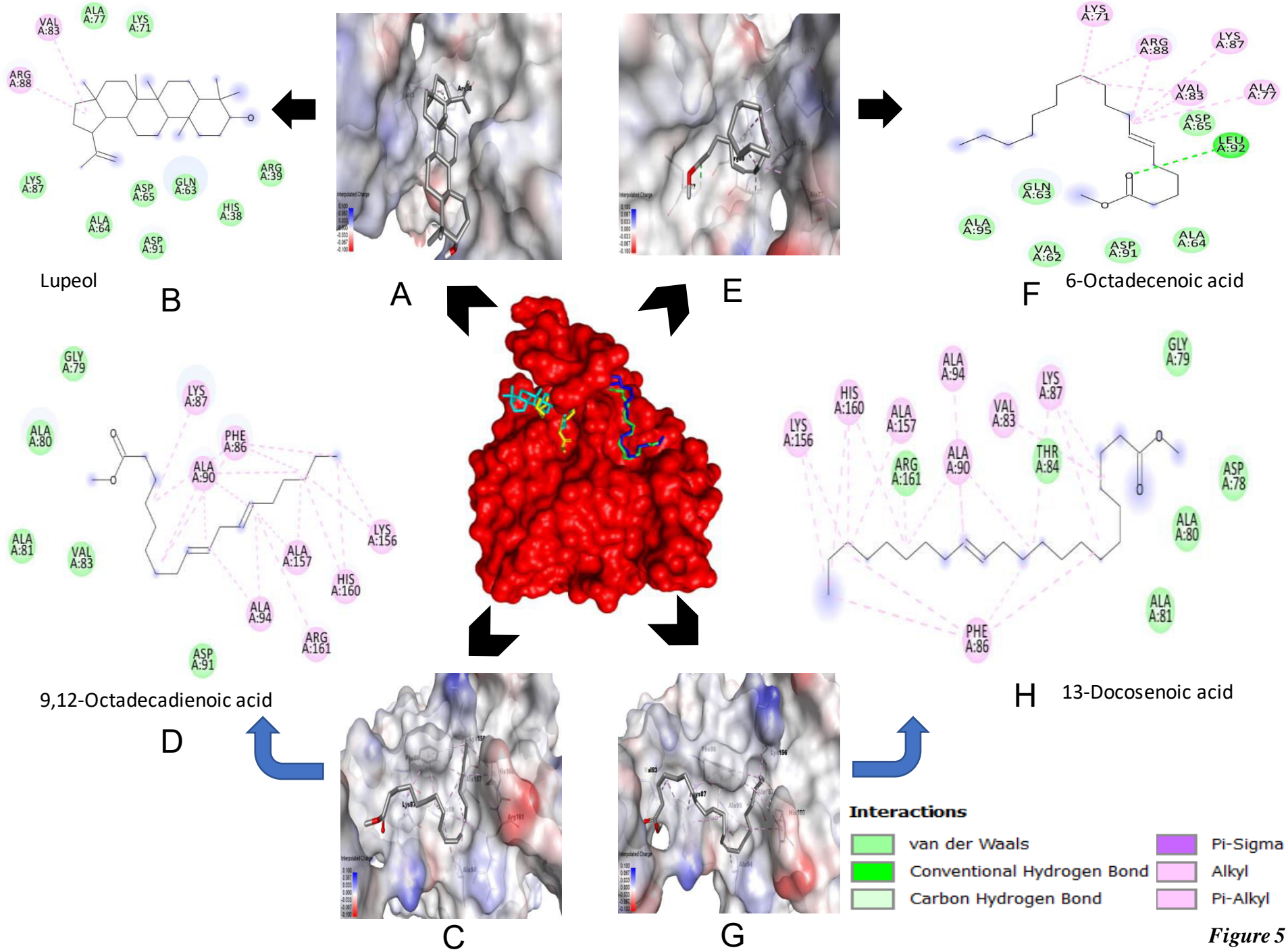

Lupeol
B
A
E
F
6-Octadecenoic acid
9,12-Octadecadienoic acid
D
C
G
H
13-Docosenoic acid
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl

Figure 4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ALA A:77
LYS A:71
ARG A:88
LYS A:87
ASP A:65
GLN A:63
ARG A:39
HIS A:38
ALA A:64
ASP A:91
Lupeol
B
A
E
LYS A:71
ARG A:88
LYS A:87
VAL A:83
ALA A:77
ASP A:65
LEU A:92
GLN A:63
ALA A:95
VAL A:62
ASP A:91
ALA A:64
F
6-Octadecenoic acid
GLY A:79
LYS A:87
ALA A:80
PHE A:86
ALA A:90
ALA A:81
VAL A:83
ALA A:157
LYS A:156
HIS A:160
ALA A:94
ARG A:161
ASP A:91
9,12-Octadecadienoic acid
D
GLY A:79
ALA A:94
HIS A:160
LYS A:87
ALA A:157
VAL A:83
LYS A:156
ARG A:161
ALA A:90
THR A:84
ASP A:78
ALA A:80
ALA A:81
PHE A:86
H
13-Docosenoic acid
C
G
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl

Figure 5

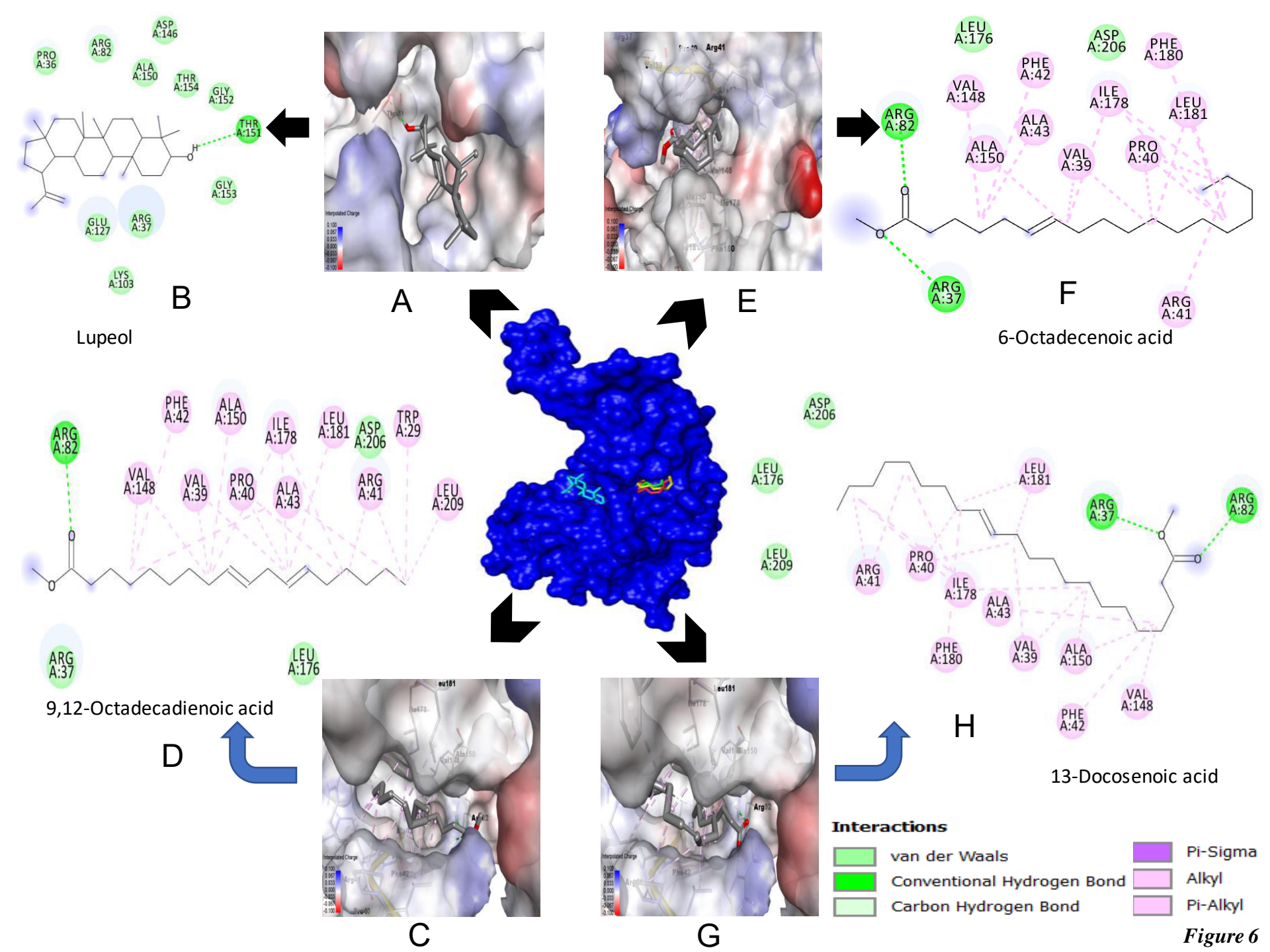
ASP A:146
ARG A:82
PRO A:36
ALA A:150
THR A:154
GLY A:152
THR A:151
GLY A:153
GLU A:127
ARG A:37
LYS A:103
B
Lupeol
A
E
LEU A:176
ASP A:206
PHE A:180
PHE A:42
VAL A:148
ILE A:178
LEU A:181
ARG A:82
ALA A:43
ALA A:150
VAL A:39
PRO A:40
ARG A:37
ARG A:41
F
6-Octadecenoic acid
PHE A:42
ALA A:150
ILE A:178
LEU A:181
ASP A:206
TRP A:29
ARG A:82
VAL A:148
VAL A:39
PRO A:40
ALA A:43
ARG A:41
LEU A:209
ARG A:37
LEU A:176
9,12-Octadecadienoic acid
D
C
G
ASP A:206
LEU A:176
LEU A:209
LEU A:181
ARG A:37
ARG A:82
ARG A:41
PRO A:40
ILE A:178
ALA A:43
PHE A:180
VAL A:39
ALA A:150
VAL A:148
PHE A:42
H
13-Docosenoic acid
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Alkyl
Pi-Alkyl

Figure 6