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Ligand-protein interactions of plant-isolated (9z,12z)-octadeca-9,12-dienoic acid with B-ketoacyl-Acp synthase (KasA) in potential anti-tubercular drug designing

Andrew G. Mtewa[a,b,*], Jonathan T. Bvunzawabaya[c,d], Kennedy J. Ngwira[e], Fanuel Lampiao[c,d], Reuben Maghembe[f,g], Hedmon Okella[b], Anke weisheit[b], Casim U. Tolo[b], Patrick E. Ogwang[b], Duncan C. Sesaazi[b]

[a] Chemistry Section, Department of Applied Studies, Institute of Technology, Malawi University of Science and Technology, Malawi
[b] Pharmbiotechnology and Traditional Medicine Centre (PHARMBIOTRAC), Mbarara University of Science and Technology, Mbarara, Uganda
[c] Department of Biomedical Sciences, Kamuzu University of Health Sciences, Blantyre, Malawi
[d] Africa Centre of Excellence in Public Health and Herbal Medicine (ACEPHEM), Kamuzu University of Health Sciences, Malawi
[e] School of Chemistry, Institute of Molecular Science, University of Witwatersrand, South Africa
[f] Biotechnology Section, Centre for Chemistry and Chemical Engineering, Lund University, Lund, Sweden
[g] Marian University College, Department of Biological and Marine Sciences, Bagamoyo, Tanzania

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ABSTRACT

Mycobacterium tuberculosis remains one of the world's contributors to mortality. With the emergence of SARS-CoV-2 coinfections, patients with TB are predisposed to being more heavily weighed down by COVID-19 disease and its opportunistic coinfections. The severity of the disease coupled with drug resistance on the currently used drugs warrants for the search for alternative remedies from synthetic agents, semisynthetics and natural products that include plants. Africa is rich in plant diversity with a promise as sources of drug agents, one of which is *Eichhornia crassipes*. This work aimed at isolating a fatty acid and dock it to β-ketoacyl-ACP synthase for possible anti-TB drug development prospects using computational tools. (9z,12z)-Octadeca-9,12-dienoic acid was isolated from *Eichhornia crassipes* for the first time using chromatographic techniques and identified using 1D and 2D NMR spectroscopic methods (^{1}H NMR, COSY, HSQC, HMBC and ^{13}C NMR). The compound was then docked to β-ketoacyl-ACP synthase (KasA), an essential member of the b-ketoacyl synthases encoded in the *M. tuberculosis* genome in comparison with its co-crystallized ligand JSF-3285, also for the first time. (9z,12z)-Octadeca-9,12-dienoic acid interacted with only phenylalanine239 and proline201 while JSF-3285 interacted with

* Corresponding author at: Chemistry Section, Department of Applied Sciences, Institute of Technology, Malawi University of Science and Technology, Malawi.
E-mail address: amtewa@must.ac.mw (A.G. Mtewa).

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proline201, glutamine120, alanine119, leucine116, glutamine199, histadine345, phenylalanine239, glycine240 and glycine200. (9z,12z)-Octadeca-9,12-dienoic acid had a ligand efficiency of 0.24, compared to the co-crystallized ligand's 0.36. The compound was too flexible and elongated with -4.72 KCalmol^{-1} binding energy. Despite some unfavourable physico-chemical properties, the compound still provides reliable interactions that only require logical structural modifications by the addition of polar regions amongst others to increase interactions and ligand efficiency, which can consequently stand to be a better potential drug lead. For the first time, plant-based (9z,12z)-Octadeca-9,12-dienoic acid isolated from *Eichhornia crassipes* was shown to interact fairly well with β-ketoacyl-ACP synthase and proved to be a potential starting material from which anti-tubercular drugs can be designed.

Introduction

By the year 2017, there were about 10 million new tuberculosis cases worldwide [1]. About a third of the world population is reported to be suffering from this infection [2]. *Mycobacterium tuberculosis* (Mtb) is known to be a major infectious factor leading to highest human mortality by its means of co-infecting patients with HIV/AIDS [3]. As if this challenge wasn't enough for researchers and health care providers, the emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019, another reported killer virus [4], brought more complications as possibilities of coinfection with tuberculosis in TB patients and those with incomplete recovery were reported [5]. It is suggested that people with previous lung diseases such as *Mycobacterium tuberculosis*, treated or not, were at a higher risk of being predisposed to getting COVID-19 disease [1,5–8]. This and the history of the disease makes Mtb one of the most perilous infectious bacteria in the world. Various pulmonary complications and sequelae such as broncholithiasis, tracheobronchial stenosis and bronchiectasis are likely to occur in *Mycobacterium tuberculosis* infected patients, whether on treatment or not [9].

The fight against tuberculosis has been staggeringly long and still continues. By the 1990s, various works on the development of potential vaccines against the disease were already underway [10–13]. The failure of the directly observed treatment, short course (DOTS) was reported to be amongst the leading causes of the emergence of multi drug resistant (MDR), extensively drug resistant (XDR), extremely drug resistant (XXDR) and totally drug resistant (TDR) strains of Mtb [14–16]. The emergence of strains that are resistant to various TB drug interventions and co-infections with HIV and SARS-COV-2 challenge current efforts in developing a universally acceptable effective drug against TB [16]. Rifampicin and isoniazid, the first-line drugs against TB, were reported to have become significantly obsolete in the management of the disease due to chromosomal mutations [16] among other factors.

β-ketoacyl-ACP synthase (KasA) is the only essential member of three b-ketoacyl synthases encoded in the *M. tuberculosis* genome. KasA catalyzes the 2-carbon elongation of growing fatty acyl chains in the FAS-II pathway, critical to the biosynthesis of mycolic acids and consequently, the bacterial cell wall [17,18]. Amino acid–altering mutations in the KasA protein have been previously identified in isonicotinic acid hydrazide (INH)-resistant patient isolates that lacked other mutations associated with resistance to isonicotinic acid hydrazide (INH) [19], also known as isoniazid. KasA has emerged as an interesting target in the treatment and management of *M. tuberculosis*. The isolation of several natural product inhibitors of the KAS enzymes, including thiolactomycin (TLM), platensimycin, and cerulenin has underscored the importance of KasA as an antibacterial drug target.

Since the elucidation of KasA and its deposition into the protein database, more studies have been conducted to identify KasA inhibitors through molecular docking. (9Z,12Z)-octadeca-9,12-dienoic acid had never been subjected to such studies with the KasA, therefore in this study, (9Z,12Z)-octadeca-9,12-dienoic acid, isolated from *Eichhornia crassipes* (Water hyacinth, *Namasipuni*) for the first time, was docked into the binding pocket of KasA using Autodock 4.2, to find out if it could be a KasA inhibitor and as a result, a potential TB drug hit [20]. According to Fileto-Pérez et al. [34], *Eichhornia crassipes* contains many active secondary metabolites including tepernoids, phenolic acids, sterols, stilbenes and alkaloids which are applied both ethno-medicinally and in conventional drug development as anti-inflammatories, anti-mcrobials, cardiac protective agents, agents reducing plasmatic cholesterol and inhibit cancers amongst other uses. Mtewa et al. [27] reported that the plant also contains compounds that have hepatoprotective, antiarthritic, diuretic, antimicrobial, nematicidal, antiasthmatic, antiacne, antiandrogenic, antieczemic, haemolytic, and antihistaminic activities.

According to literature, statistics from clinical trials have shown that there still is a need for anti-tuberculosis drugs that should be well tolerated [16]. As such, more research in looking for potential agents that can be used in managing or treating or in conjugating with other compounds to make drugs against TB is still warranted. Plants have provided promises in getting such compounds for some time now [21–26]. One plant that was yet to be explored as a potential source of such compounds is *Eichhornia crassipes* which is largely regarded as a useless water weed. This work aimed at isolating a fatty

acid from the leaves of *Eichhornia crassipes* that could be considered for exploration in nutraceutical, nutritional and drug discovery research in the management and/or treatment of TB by interacting it with β-ketoacyl-ACP synthase, an important protein in TB for the first time.

Methods

Collection of plant material

Whole plants of *Eichhornia crassipes* were collected from Liwonde, Malawi, in the Shire river at the following GPS coordinates: 15°03′11.3″S 35°31′12.5″E and 15°03′07.5″S 35°13′15.4″E between February and March of 2019. The identification of the plant was done by a Mr. Hassam Patel of the Malawi Herbarium and Botanic Garden (MHBG). The plant can be accessed from the MHBG under accession number 3596.

Preparation of plant material, chromatographic and spectroscopic analyses

The plant was prepared as described by Mtewa et al. [27], briefly, washed plant material was air-dried in a ventilated room at room temperature for 5 weeks. It was then ground and stored at −10 °C for subsequent use. Methanol (2.5 L) was used to macerate the powder (500 g) over a day. Extracts from the maceration were dried in a rotor evaporator at 40 °C in reduced pressure. Hexane : ethyl acetate at various gradient ratios:10 : 0, 9.5 : 0.5, 9.0 : 1.0, 8.5: : 1.5, 8.0 : 2.0, 7.5 : 2.5, 7.0 : 3.0, 6.0 : 4.0, and 5.0 : 5.0 and dichloromethane : methanol at the following ratios: 10.0 : 0, 9.5 : 0.5, 9.0 : 1.0, and 8.5 : 1.5 were used to isolate and purify compounds from the dried extracts. A liquid chromatography-mass chromatography (LCMS, Agilent; 5–95% acetonitrile/water and +1% formic acid) from Agilent, mounted with a UV detector was used to confirm purity (single peaks) and the molar masses of the isolated pure compounds. The MS scanned in a range of m/z 200 to 1400.

The structure of the compound was determined using a Bruker (400 MHz) Nuclear Magnetic Resonance (NMR) spectrometer using both 1D and 2D NMR techniques. The sample was prepared in deuterated dimethylsulfoxide (DMSO-*d*) and subjected to proton NMR, correlation spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlation and Carbon-13 spectroscopic experiments as done by Mtewa and others [27]. TopSpin software (V4.0.6) from Bruker was used to analyse the obtained NMR spectra.

Ligand docking on β-ketoacyl-ACP synthase

This study was carried out on a personal laptop, Intel (R) Core(TM) i5-5200 U CPU, 2.20 GHz processor, and Windows 10 Pro were used as Operating system. The following molecular sets of software were used: Autodock tools (MGLTools 1.5.7 RC 1), Autodock suite (Autodock 4.2.6 and Autogrid 4.2.6), Discovery studio visualizer (DS) v12.2.16349 and Pymol Molecular Graphics System (V2.3.1).

Target selection and preparation

A recently reported crystal structure of *Mycobacterium tuberculosis* KasA in complex with JFX was retrieved from the RCSB PDB (http://www.rcsb.org/structure/6P9L). Using Discovery studio visualizer, water molecules and other small ligands were deleted from the PDB file. The co-crystalised ligand (JSF-3285) was used to identify the interacting residues in the binding sites before it was removed and isolated for redocking purposes.

Ligand preparation

3D structure of (9Z,12Z)-octadeca-9,12-dienoic acid was retrieved from the PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov/compound/5280450) in SDF format. DS visualizer was used to visualize and inspect the structure before it was converted to PBD format. Polar hydrogens were added to the structure and the energy of the structure was minimised using MMFF in Avagadro. MGLTools software was used to calculate the Gasteiger charges and to save the file in pdbqt format [28].

Docking protocol

To examine the binding modes of (9Z,12Z)-octadeca-9,12-dienoic acid in the binding pockets of KasA, MGLTools and Autodock 4.2.6 were used in docking [29]. Co-crystallized ligand JSF-3285 was used in validating the docking protocol by redocking it into the binding sites of KasA. All calculations for protein-ligand flexible docking were performed using the Lamarckian Genetic Algorithm (LGA) method [29,30]. A grid box with central grid points of X: 46.932, Y: −5.797and Z: −17.674 Å, with a default grid spacing of 0.375 Å was used. The best conformation was chosen with the lowest docked energy after the docking search was completed. The interactions of KasA and ligand conformations, including hydrogen bonds and bond lengths, were analysed.

Analysis of docking results

DS visualizer and Pymol tools [31,32] were used to generate receptor-ligand interactions from the ligand-receptor complexes; MGLTools software was used to retrieve binding energies, Ligand efficiencies, RefRMS and Ki values for different conformations of each ligand. The results were used in comparative studies for (9Z,12Z)-octadeca-9,12-dienoic acid.

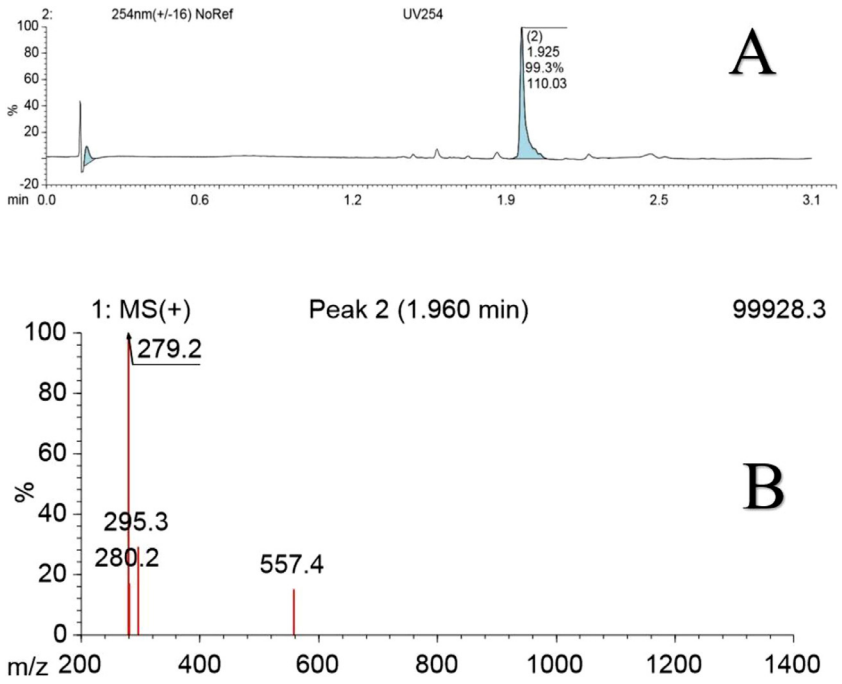

Fig. 1. UV spectrum of the compound (A) and *M*/*Z* profile of the compound (B)
1D and 2D Nuclear magnetic spectroscopy experiments gave the proton nuclear magnetic resonance (^{1}H NMR) spectrum in Fig. 2(A), the proton-proton correlated (COSY) spectrum in Fig. 2(B), the heteronuclear single quantum coherence (HSQC) spectrum in Fig. 2(C), the heteronuclear multiple bond correlation (HMBC) spectrum in Fig. 2(D) and the carbon 13 (^{13}C NMR) spectrum in Fig. 3(A).

Determination of selected drug pharmacokinetic and pharmacodynamic (PKPD) properties for oral drug considerations

The method used by Mtewa et al. [27] was employed to determine PKPD properties of the compound to see prospects of developing an oral drug from it. Briefly, the structure of the compound was designed and its simplified molecular-input line-entry system (SMILES) was generated. The structure was run in StarDrop® (version 3.4; Optibrium Ltd.) and the SMILES string was run in SWISSADME descriptors algorithm protocol platform. The measures of lipophilicity, solubility, flexibility, polarity, size, ability to cross the blood brain barrier and saturation were determined.

Results

Upon isolation, purification and drying, the compound was obtained as a viscous oily colourless liquid (7.4 mg). Mass spectroscopic analyses showed the UV spectrum in Fig. 1A and the mass to charge ratios in Fig. 1B.

The ^{1}H NMR profile of the compound (Fig. 2A) was as follows:

δ 11.98, 1H, s; δ5.41, 4H,m; δ2.75, 2H, t; δ2.17, 2H, t; δ2.02, 4H, q, δ1.49, 2H, t; δ1.36-1.22, 14H, m; δ0.87, 3H, *t*.

COSY experimental data (Fig. 2B) shows that the singlet at δ 11.98 belonged to the proton on the carboxylic acid which made sense to be a singlet as it had no interactive neighbouring protein. As expected, the CH_2s on the chain had multiple interactions with their neighbouring protons. The absence of the carboxylic carbon in the HSQC (Fig. 2C) and HMBC (Fig. 2D) confirmed that it must be a quaternary carbon which appears in the 13-C NMR (Fig. 3A) around δ175.

The carbon peaks around δ130 indicated the chemical shifts of the two carbon-double bonds in the compound. Analysis of all the spectroscopic data suggested that the compound was (9Z,12Z)-octadeca-9,12-dienoic acid. The proposed structure was in agreement with the LCMS profile (Fig. 1B) on the overall identity of the compound by showing matching masses between the compound and the proposed structure. At *m*/*z*: 280.2 (Fig. 3B), there was expected to be 100.0% of the whole non-ionised compound [*M*] and the most abundant fragment was at *m*/*z* 279.2 which is the $[M+1]^{+}$ of the monoisotopic mass of the compound whose mass is 280 g/mol [33]. The peak at *m*/*z* 557.4 was a dimeric ion fragment of the compound. (Fig. 3B showed the proposed structure of the compound from the spectroscopic data presented.

Docking protocol validation and ligand-protein complex

The docking protocol was first validated before undertaking further experiments. Fig. 4 shows results obtained from docking experiments for both the co-crysatallised ligand and the (9Z,12Z)-octadeca-9,12-dienoic acid -KasA complex.

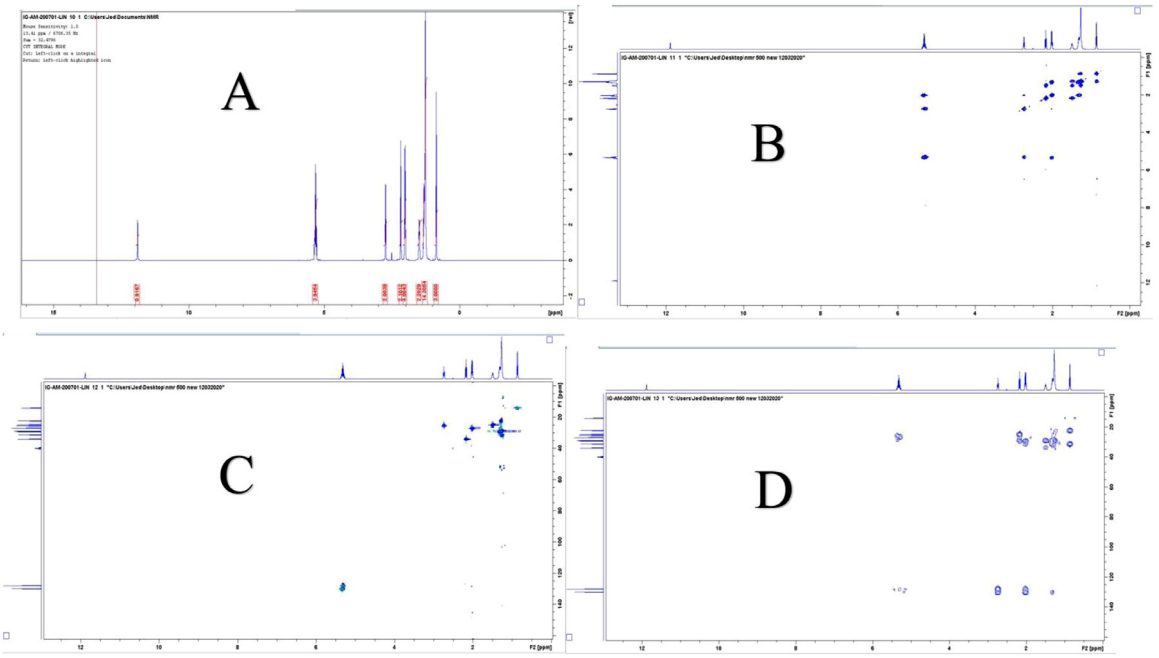

Fig. 2. H-NMR profile of the compound (A), COSY NMR profile of the compound (B), HSQC profile of the compound (C) and HMBC Profile of the compound (D).

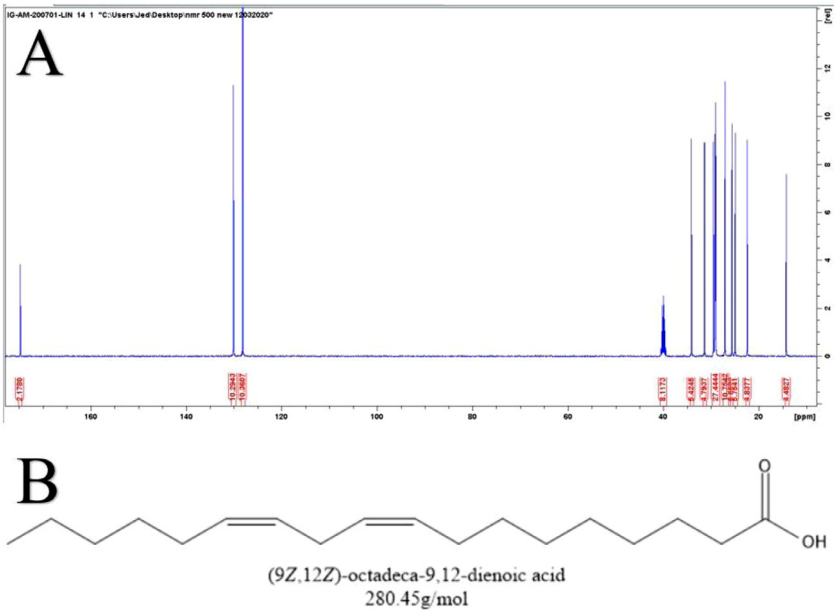

Fig. 3. Carbon-13 NMR profile of the compound (A) and the proposed structure of the compound (B).

Table 1
Binding affinities and physicochemical Properties of (9Z,12Z)-octadeca-9,12-dienoic acid and JSF-3285.

Ligand	Weight /gmol^{-1}	torsions	Binding Energy /KCalmol^{-1}	Ligand efficiency	Ki/uM	Interacting residues	Types of interactions
(9Z,12Z)-octadeca-9,12-dienoic acid	43.4	15	−4.72	0.24	345	PHE239, PRO201	Hydrogen bond, Pi-alkyl
JSF-3285	285	5	−6.98	0.36	10.5	PRO201, GLU120, ALA119, LEU116, GLU199, HIS345, PHE239, GLY240, GLY200	Pi-alkyl, hydrogen bond, Pi-Sigma, halogen bond

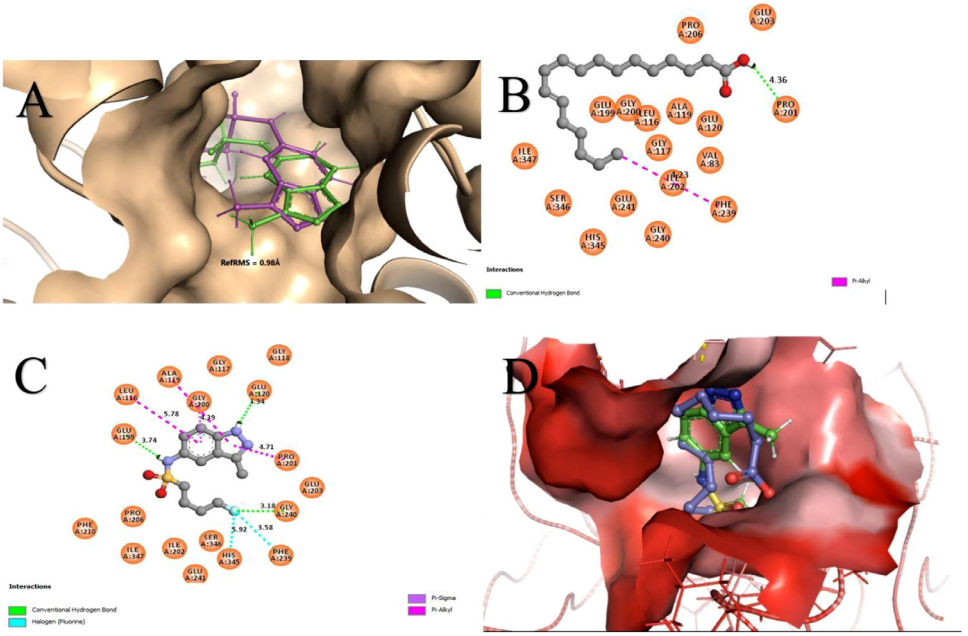

Fig. 4. (A) JSF-3285 co-crystallized and docked confirmations in the binding pocket of the KasA enzyme. JSF-3285-docked (green) and JSF-3285-co-crystallized (purple) have RefRMS of 0.98 Å in conformational poses. (B) (9Z,12Z)-octadeca-9,12-dienoic acid interacting with PRO201 in a hydrogen bond (green dots) and forming a Pi-alkyl bond with PHE239 (pink dots). (C) (9Z,12Z)-octadeca-9,12-dienoic acid and JSF-3285 interacting with KasA binding pocket residues; JSF-3285 interacting with KasA binding pocket residues through 3 hydrogen bond (green dots), 2 halogen bonds (light blue), a Pi-sigma bond and a Pi-alkyl bond (pink). (D) 3D picture (9Z,12Z)-octadeca-9,12-dienoic acid (green) and JSF-3285 (purple) in the binding pocket of KasA. The gradient of redness on KasA depicts the hydrophobicity of the pocket (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1 presents a summary of important observations in the interactions of the isolated compound and the co-crystallized compound for comparisons.

Medicinal chemistry and cheminformatic properties of the compound

Run on SWISSADME, the compound was found to be safe. It is not mutagenic, tumorigenic, an irritant and does not have adverse reproductive effects. The compound had a BBB ratio of 0.6503 and synthetic accessibility of 3.10. StarDrop helped in showing specific regions of the compound that are essential in influencing some physico-chemical properties of the compound. Fig. 5 presents some physico-chemical properties that are essential in Medicinal chemistry in determining the potential of a compound into being developed as an oral drug.

Discussion

The UV chromatogram in Fig. 1(A) shows that the compound was isolated in a pure form (99.3%) and the one taken further into subsequent experiments was a single pure compound. This compound, (9Z,12Z)-octadeca-9,12-dienoic acid depicted in Fig. 3(B), has previously only been detected in *Eichhornia crassipes* using GC/MS [34] but this is the first time that it has actually been isolated and purified from the plant.

Since the focus of this work was only on isolation, characterisation and docking, bioassays were out of scope at this moment as appropriate scaffolds need to be determined first from this exercise before going further with bioassays. The co-crystallized ligand, JSF-3285 was re-docked into the binding side of KasA to find out if Autodock 4.2 could reproduce the conformation of JSF-3285 in the crystal structure complex. Out of the 15 poses generated, 4 poses had RefRMS of less than 1.50 Å and of the 4, one had binding energy of −6.79 Kcal/mol and RefRMS of 0.98 Å. A docking program and protocol is considered as reliable if the RefRMS of redocked co-crystallized ligand pose is less than 2.0 Å [8]. These results validated the used docking protocol in Autodock 4.2.6 and showed the protocol was reliable in this docking studies. An overlay of two conformations of JSF-3285(docked and crystallized) showed that they have a little difference in position (Fig. 4A).

(9Z,12Z)-octadeca-9,12-dienoic acid docked very well into the binding pocket of KasA at the position where JSF-3285 was situated and JSF-3285 was used as a fitting reference for (9Z,12Z)-octadeca-9,12-dienoic acid affinity to KasA protein. The conformation with the lowest energy had binding energy of −4.72 Kcal/mol and an inhibition constant of 345 uM. These values were higher than the ones calculated for JSF-3285 that had binding energy of −6.79 Kcal/mol and Ki of 10.5 uM

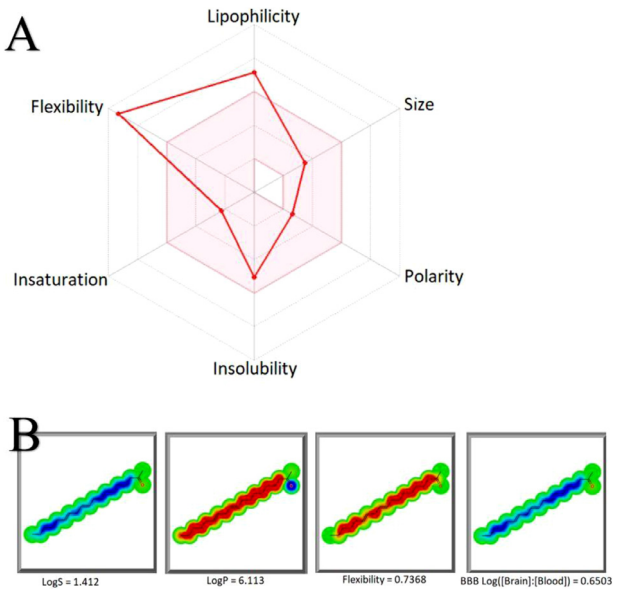

Fig. 5. (A) Supervector machine Web diagram of some physicochemical properties of the compounds and (B), Property effector regions of the compound.

(Table 1). This comparison showed than (9Z,12Z)-octadeca-9,12-dienoic acid was a weaker binder of KasA when compared to JSF-3285.

(9Z,12Z)-octadeca-9,12-dienoic acid was only able to form 2 notable interactions with KasA; a hydrogen bond and a Pi-alkyl bond (Fig. 4B). Out of all the amino acids that made up the binding pocket, only two residues where involved in these interactions; PRO201 and PHE239. On the other hand, JSF-3285 interacted with 8 residues in the binding pocket of KasA and 3 of them formed hydrogen bonds and the rest were hydrophobic interactions (Fig. 4C). Both ligands fitted perfectly into the buried pocket of the enzyme with JSF-3285 having a greater affinity than (9Z,12Z)-octadeca-9,12-dienoic acid (Fig. 4D). Although this was the case, (9Z,12Z)-octadeca-9,12-dienoic acid still showed a great promise of a drug scaffold against the protein.

Cell wall biosynthesis inhibitors have proven highly effective for treating tuberculosis (TB). Members of the indazole sulfonamide class of small molecules have been previously validated as inhibitors of *Mycobacterium tuberculosis* KasA [35]. Several natural products have also been shown to be active inhibitors of KasA. JSF-3285 was an important ligand to compare with as it is a preclinical inhibitor of KasA which was designed from optimising DG167, a lead compound that showed synergistic activity against TB in combination with NIH(Isoniazid) [20]. From the docking results, it is evident that both ligands could fit into the binding pocket of the KasA enzyme but the atomic group differences between ligands contributed much to the difference in the interaction of the ligands with the KasA protein. (9Z,12Z)-octadeca-9,12-dienoic acid is a long-chain aliphatic carboxylic acid that constitutes three types of atoms; oxygen, carbon and hydrogen. The two oxygens that form the carboxyl group interacted with PRO201 through a hydrogen bond. Although the hydrogen bond is significant enough to contribute to the overall binding energy, it is, however, a weak one since the distance of the bond is 4.36A. The same residue, PRO201 forms a hydrophobic Pi-sigma bond with the indazole ring of JSF-3285. The last carbon in the fatty acid interacted with PHE239 in a weaker hydrophobic Pi-alkyl bond while the same residue contributed to the halogen bond with JSF-3285. The absence of rings and few heteroatoms in the fatty acid possibly explains the big differences in the binding energy of the two ligands. The indazole ring of JSF-3285 interacted with 4 binding pocket residues, the fluorine atom interacted strongly with 3 residues and the amine bond acted as a hydrogen bond donor. This study confirms other studies that have shown the importance of the indazole group in the designing of KasA inhibitors. While this molecular docking study shows that (9Z,12Z)-octadeca-9,12-dienoic acid binds weakly to KasA this does not mean that it does not have any effect on TB since some studies report that some fatty acids have antimicrobial TB activity [36,37]. This calls for more in vitro studies on (9Z,12Z)-octadeca-9,12-dienoic acid to validate its expected activity against TB on the bench.

The observations made from the docking studies shows the good position that the compound occupies as a potential hit in the development of drugs against TB. Various fatty acids and the compound's derivatives have been instrumental in TB drug designing. Previous studies conducted on guinea pigs indicated that dietary polysaturated fatty acids were capable of modulating enough resistance against Mtb [36–39] and one novel inhibitor to tyrosine phosphatase A, a mycobacterium tuberculosis Protein and a fatty acid-thiadiazole [38] were obtained from fatty acids. Sometimes, these fatty acids are not administered alone but are either chemically complexed with already existing drugs [16] or other phytocompounds. A complex of fatty acids with carotenoids was also reported as an effective agent against TB [40].

The activity of (9Z,12Z)-octadeca-9,12-dienoic acid, a fatty acid, is not new in the management and control of Mtb [39]. Both the α- and the γ- forms of (9Z,12Z)-octadeca-9,12-dienoic acid and its various conjugated forms were reported to be active against mycobacterium tuberculosis (Mtb) [3] but the current findings show the potential that the compound can have as it is or as a scaffold for the development of better drugs. When the anti-Mtb activity was determined using the resazurin microtiter assay (REMA) for five days and the Mycobacterium growth indicator tube (MGIT 960) system assay for 21 days, the (9Z,12Z)-octadeca-9,12-dienoic acid and its other conjugates were reported to be able to inhibit growth and proliferation of Mtb dose dependently with an MIC of atleast 200 μg/ml [3]. Swain and other researchers [16] suggested that the designing of conjugates of the 'obsolete' tuberculosis drugs with viable phytocompounds through structural modifications and semi-synthesis could be a better and faster way to develop safe and effective tuberculosis drugs. In their work, they realised that some characteristic pharmacophores of a drugs known to contribute to its potency can be maintained whilst various modifications are carried out on the rest of the molecule to be conjugated tactfully with medicinally interesting phytocompounds.

Looking at the medicinal chemistry prospects of the compound, it appeared poised to suffer from being too greasy to effectively cross biological membranes and also making it very non-specific to target binding, too flexible to effectively bind to particular protein binding sites and has poor solubility. This is one of the reasons the ligand efficiency in Table 1 is slightly lower, showing that binding is not as good as a good drug would need to have. The medicinal chemistry conclusion according to *in silico* data on SWISSADME (Fig. 5A) and in StarDrop (Fig. 5B) was that the molecule can hardly make a drug lead as it is. However, a synthetic accessibility of 3.10 for the compound indicates that the compound can easily be synthesized which can be so helpful in expanded experimentations including modification of the structure for improved properties.

StarDrop visualisation showed regions that enhance various properties on the molecule, information which may help a Medicinal Chemist to get an idea of how best the structure needs to be modified if to be improved of its physicochemical properties. The measure of solubility (Log *S*) for example is negatively affected by the blue region, taking note of the reduced blue colour on the pi-bonds due to the presence of high electron density. The measure of lipophilicity (Log *P*) is enhanced by the red colour and the blue dot region, the oxygen, has a lot of electron density hence no lipophilic contribution from that region. Flexibility of the compound emanates from the flexible C-H bonds which correlates to the number of rotatable bonds, 14 in this case according to findings on StarDrop. The rigid pi-bonds can be seen not participating in this property. The compound would be capable of crossing the BBB at the ratio of 0.6503 and the blue region contributes to the property [16]. However, this work did not focus on whether there would be adverse events or not if this would occur, which can be explored in further studies. By looking at these properties, a medicinal chemist can manage to do hit expansion on the compound and add more hydrophilic regions that should ultimately improve the interactions and ligand efficiency of the compound with the protein.

Conclusion

For the first time, (9z,12z)-Octadeca-9,12-dienoic Acid has been isolated from *Eichhornia crassipes* and docked to β-ketoacyl-ACP synthase (KasA) an essential member of b-ketoacyl synthases encoded in the *M. tuberculosis* genome in comparison with its co-crystallized ligand JSF-3285 for the first time as well. (9z,12z)-Octadeca-9,12-dienoic Acid was interacting with only two amino acids of the KasA (PHE239, PRO201) while JSF-3285 interacted with nine amino acids (PRO201, GLU120, ALA119, LEU116, GLU199, HIS345, PHE239, GLY240, GLY200). The binding affinity followed the same observation, (9z,12z)-Octadeca-9,12-dienoic Acid having a lower ligand efficiency of 0.24, compared to the co-crystallized ligand's 0.36. It was not surprising to note that the high flexibility and elongation of the fatty acid with its higher lipophilicity was having a binding energy of -6.98 KCalmol^{-1}. Although the physico-chemical properties of the compound aren't good enough for a stand-alone drug to interact with KasA in comparison to JSF-3285, the compound needs a strategic hit expansion that can help to improve the structure for better interaction with the protein. This work suggests an addition of more hydrophilic regions to improve on the interaction with polar regions of the proteins which should ultimately increase the ligand efficiency and the interactions with the protein [27]. Appropriate bench protein assays are also warranted upon every structural modification to ascertain the efficiency of the *in silico*-led interactions. This work stands as an important guide to such bioassays that can be carried out on the bench along with various other proteins that have not been worked on in this work. For the first time, *Eichhornia crassipes* has been shown to be a good source of this important fatty acid by compound isolation and also, potential agents that can be so useful in the treatment and management of TB, as part of a drug, a supplement or a carrier. Malawi and Africa in general needs to begin treating this plant not as a useless water-weed but an important species that should just be tamed and explored further for drug-like compounds against TB and other diseases burdening the continent. The limitation of this work was the use of a single docking tool but it did not have negative effects to the results as it managed to demonstrate the docking protocol and the understanding of the interactions between this ligand and the selected TB protein. The demonstration of computational work and medicinal chemistry aspects exhibited in this work shows that it is possible to explore ligands from various more plants on as many targets as possible. This work is the first of its kind to be done in Malawi and on Malawian plant products, it therefore shows the virgin area that the country has requiring exploration.

Declarations

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Ethics approval

Ethical approval was obtained from the Mbarara University of Science and Technology Research Ethics Committee with reference number MUREC 1/7.

Consent to participate

N/A.

Consent for publication

All contributors accepted to have this work published

Availability of data and material (data transparency)

All necessary data has been presented in this work, however, if more clarification on the data will be required, the corresponding author will be available to supply as required.

Declaration of Competing Interest

The authors declare no conflict of interests.

CRediT authorship contribution statement

Andrew G. Mtewa: Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Jonathan T. Bvunzawabaya:** Investigation, Writing - review & editing. **Kennedy J. Ngwira:** Supervision, Writing - review & editing. **Fanuel Lampiao:** Supervision, Writing - review & editing. **Reuben Maghembe:** Writing - review & editing. **Hedmon Okella:** Writing - review & editing. **Anke weisheit:** Writing - review & editing, Project administration. **Casim U. Tolo:** Writing - review & editing, Project administration. **Patrick E. Ogwang:** Project administration. **Duncan C. Sesaazi:** Supervision, Writing - review & editing.

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