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In Silico Identification of Canthin-6-one as a Pancreatic Lipase Inhibitory Anti-Obesity Drug Lead from *Hibiscus Sabdariffa*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The therapeutic use of the only Pancreatic Lipase (PL) - inhibiting anti-obesity drug available in clinical practice, orlistat, is bedevilled with unbearable side effects, necessitating the discovery of new and better-tolerated ones. *Hibiscus sabdariffa*, a folkloric anti-obesity plant is a plausible repertoire from which such agents could be sought.

Objective: The main objective of this work was to evaluate *in silico* the phytochemicals of *Hibiscus sabdariffa* for a possible identification of potential leads for PL inhibitory anti-obesity drug discovery..

Methods: Phytoligands from *H. sabdariffa* were subjected to a series of *in silico* evaluations including site directed docking, MM/GBSA calculations, SwissADME drug-likeness screening, Protox II-based toxicity evaluations and a 20 ns molecular dynamics (MD) simulation.

Results: MM/GBSA ranking of docked phytoligands and SwissADME evaluations produced three PL inhibitor hits. One of them, canthin-6-one, demonstrated minimal end-organ toxicity with a 1200 mg/kg LD_{50}; its PL complex generated stability-implying root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg) and solvent accessible surface area (SASA) plots, after the 20 ns MD simulation.

Conclusion: *Hibiscus sabdariffa*-based canthin-6-one has demonstrated, *in silico*, high human PL binding affinity, impressive drug-likeness/toxicity profiles and stability-implying MD simulation parameters. It is therefore, herein, recommended as lead for further *in vitro*, *in vivo* and molecular modification studies for possible development into a clinical PL inhibitory anti-obesity drug.

Keywords: Pancreatic Lipase inhibition, *In-silico* studies, Computer Assisted Drug Discovery, *Hibiscus sabdariffa*, Anti-obesity agents

INTRODUCTION

Obesity is a chronic disease involving excessive accumulation of fat. It is presently an epidemic, cutting across male-female and old-young divides of the world populace (Engin, 2017). Obesity, in addition to its social concerns, has health implications as it presents comorbid with a number of devastating diseases including cardiovascular disorders, type 2 diabetes mellitus, arthritis and a number of cancers like endometrial, breast, colon and prostate cancers (Afolabi *et al.*, 2022).

The molecular etiology of obesity is still a subject of intense investigation. However, an imbalance in energy metabolism is widely recognized as the fundamental underlining mechanism: Obesity occurs when the balance between the rather homeostatic energy storage as triglycerides in adipocytes and energy expenditure is skewed in favour of the former. This could be as a result of genetic, behavioural as well as environmental factors (Yasmin *et al.*, 2021). Though genetic constitution plays a pivotal role in the prevalence and severity of obesity, its rapid assumption of epidemic status within a very short period could not be accounted for on the platform of genetic mutation in the entire world population. Thus, emphasis has shifted on the environmental and behavioural underlining factors of obesity (Pozza and Isidori, *et al.*, 2018).

Behavioural and environmental treatment of obesity is usually by a combination of measures, notable amongst which are: calorie intake regulation (dieting), physical activity enhancement (physical exercise) and the use of anti-obesity medication (pharmacotherapy). Anti-obesity pharmacotherapy could be targeted at enhancing energy utilization or reducing calorie intake/storage or both (Wadden *et al.*, 2005; Bray, 2014). Manipulating the energy utilization arm of the energy balance in anti-obesity drug discovery has not been explored as much as the energy intake arm for three main reasons: One, there are more activities in the latter than the former; two, breakdown of fat is accompanied by thermogenic tendencies which may be unbearable and thirdly, the weight-loss benefits associated with lipolysis, in most cases, appear only marginal (Rodgers *et al.*, 2012). Nevertheless, tackling obesity and overweight using energy intake modifiers has not been without its own challenges as the rather best (i.e., anorexic) way of doing it would almost always require central activities eliciting unbearable psychiatric and cardiovascular side effects that have necessitated the withdrawal of a number of anorectic anti-obesity agents (Son and Kim, 2020). Anti-obesity drug research today therefore is more focused on the discovery of non-anorectic and/or peripheral metabolism modifiers (Son and Kim, 2020). This effort, however, is yet to yield as much success, given that only one medicament, orlistat, is currently the only peripherally-acting non-anorectic calorie in-take modifying anti-obesity agent approved for long-term obesity management in the United States of America (Henness and Perry, 2006). Other approved drugs with some form of anti-obesity affiliations include the rather anorectic phentermine/topiramate combination and agents having weight-loss as mere side activity, such as the antidiabetic liraglutide and the antidepressant naltrexone/bupriopion combination (Gadde and atkins, 2020).

Orlistat is a Pancreatic Lipase (PL) inhibitor. PL is the most important of the alimentary lipases involved in the hydrolysis of dietary triglycerides required for the initial breakdown of dietary lipids (triglycerides, cholesteryl esters and phospholipids) into smaller absorbable fragments that are subsequently reassembled inside enterocytes (Kumar and Chauhan, 2021). It belongs to the hydrolase enzymes superfamily possessing an α/β hydrolase fold (made up of 8 β strands linked by α helices) surrounding a three-residue catalytic region (the catalytic triad) which may vary within the superfamily (Ollis *et al.*, 1992). PL is structurally divided into two domains, the N-terminal domain which is also the catalytic domain (comprising residues 1-336) and the C-terminal domain (comprising residues 337-449) involved the binding of co-lipase, the cofactor of PL (Winkler *et al.*, 1990). In modern societies, dietary fat contributes the most to the total body fat (Kumar and Chauhan, 2021). PL inhibition therefore remains a highly plausible anti-obesity mechanism. However, it is arguably largely underutilized given the paucity of PL-inhibitory anti-obesity drugs and the unbearable side effects (such as flatulence, steatorrhea, nephrotoxicity, kidney stones, and pancreatitis) of the only available clinical PL inhibitor (orlistat) (Harp, 1998). There is therefore a high need for the discovery of new PL inhibitors that could be ultimately deployed as anti-obesity agents.

Hibiscus sabdariffa is a medicinal plant with anti-obesity claims in West Africa and a number of Asian countries (Riaz and Chopra, 2018; Balarabe, 2019). These claims have been scientifically validated by investigations establishing the plant's ability both to prevent and eliminate body fats, i.e., lipogenesis inhibition and lipolysis respectively. For instance, Morales-Luna et al (2018) reported that 22.5mg/kg aqueous extract of *H. Sabdariffa* prevented body weight increase (lipogenesis inhibition) in rats fed with high-fat fructose diet. On another hand, inhibition of fat accumulation (lipogenesis inhibition) as well as adipose tissue atrophy (lipolysis) has been reported in obese C57BL/6NHsd mice after treating them with 33

mg/kg extract of *H. sabdariffa* three times a week for 8 weeks (Villalpando-Arteaga et al., 2013). A survey of phytochemical studies on the plant revealed a phenolic-dominated chemistry accounting largely for the plant's antioxidant properties (Anokwuru *et al.,* 2011). The presence of the polyphenolics quinic and caffeic acids, and their esters (chlorogenic acids) is particularly worthy of note (Da-Costa-Rocha *et al.,* 2014). Though most of previous anti-obesity investigations on *H. sabdariffa* implicated its phenolics to a great extent, the PL inhibitory actions mechanism remains largely speculative (Ojulari et al., 2019). In this investigation, we formed a library of thirty-two *Hibiscus sabradiffa* compounds and investigated them *in silico* for possible PL inhibitory anti-obesity activities.

METHODOLOGY

Materials and software

An X-ray crystal model of the human Pancreatic Lipase (PL)-colipase complex inhibited by a C11 alkyl phosphinate, (PDB1LPB, 2.46 Å) (Berman et al., 2000), was used as parent macromolecule from which colipase-free PL unit for docking was prepared. The main hardware for the work was an HP ProBook equipped with intel Core i5, 500GB Hard Disk, 8 GB RAM; Protein preparations were done using UCSF Chimera 1.14 (Pettersen et al., 2004); 2D and 3D ligand-macromolecule complex interactions were visualized using BIOVIA Discovery studio visualizer (Biovia, 2021); multiple ligands docking was carried out with PyRx (Dallakyan and Olson, 2015) molecular docking software equipped with AutoDock Vina and Open Babel plugins; MMGBSA calculations were done with Schrodinger Maestro; SwissADME (Daina et al, 2017) and Protox II (Banerjee, 2018) webservers were used for drug-likeness and toxicity profilings respectively; molecular dynamics simulations were performed using the University of Arkansas for Medical Sciences (UAMS) simlab WebGro webserver Abraham et al., 2015); other webservers visited in the course of this study included: RCSB Protein Databank (PDB) (Berman et al., 2000), Pubchem Kim et al., 2023), PRODRG (Schüttelkopf and van Aalten, 2004), CASTp (Tian et al., 2018)and Uniprot (The UniProt Consortium, 2023)

Protein preparation

The PL-colipase complex model (PDBID 1LPB; 2.46 Å) was uploaded into Chimera 1.14 workspace by direct fetch. The colipase unit was removed as were all non-standard residues including the C11 alkyl phosphinic acid inhibitor - Methoxyundecylphosphinic acid (MUP). Hydrogen atoms and amber charges were added and the structure subsequently minimized using 200 steepest descent and 10 conjugate gradient steps energy minimization algorithm of the software (Pettersen et al., 2004). The ensuing prepared protein structure was saved as a pdb file for subsequent uses.

Ligands preparation

Thirty-two (32) compounds of *Hibiscus sabdariffa* identified from literature (Da-Costa-Rocha et al., 2014; Riaz and Chopra, 2018; Izquierdo-Vega et al., 2020) and MUP were retrieved from Pubchem database as structure data files and built into a one-file library. The library file was uploaded into the Open Babel workspace of PyRx for energy minimization and subsequent conversion into pdbqt (or autodock-compliant) ligands (Dallakyan and Olson, 2015).

Multiple ligands docking

The prepared colipase-free Pancreatic lipase from the ternary 1LPB complex (see above) was uploaded into the PyRx docking workspace and made macromolecule. The native pose of the co-crystallized ligand was used to define the grid walls of the binding site as follows: center_x = 9.17176184368; center_y = 22.8113716245; center_z = 42.213734134; size x = 25.0; size_y = 25.0; size z = 25.4203963487 (all in angstroms). The *H. sabdariffa* phytoconstituents library file was imported into the docking workspace and the 33 compounds (MUP inclusive) therein selected as ligands before the autodock vina algorithm was run (Dallakyan and Olson, 2015).

Molecular Mechanics-Generalized Born Surface Area Calculations

The PyRx-derived least-energy pose of each ligand was reset at it's binding position in the prepared colipase-free PL (1PLB) protein using Biovia Discovery Studio visualizer (Biovia, 2021). The ensuing complexes were saved as pdb files and uploaded into the Schrodinger masetro's Prime module for Molecular Mechanics-Generalized Born Surface Area (MM/GBSA) free binding energy calculations (Schrodinger, 2017). The equation for free binding energy used was: $\Delta G_{bind} = \Delta G_{complex} - (\Delta G_{protein} + \Delta G_{ligand})$, with lower (or more negative) scores indicative of stronger binding affinities (Gilson et al., 1997).

Docking Protocol validation

Coordinates of native and best-pose docked MUP were superimposed, calculating RMSD with BIOVIA Discovery Studio visualizer.

Drug-likeness and Toxicity Profiling

Top 15 of the MM/GBSA-ranked docked ligands were particularly of very high MM/GBSA scores compared to the rest (Table 1). They were, on this account, selected for screening against the five (i.e., Lipinski, Verber, Ghose, Muegge and Egan) drug-likeness filters of the SwissADME webserver, setting violation of not more than one stipulation of any of the five filters as criterion for drug-likeness selection. Three drug-like compounds ensuing fromt this screening were subsequently subjected to toxicity profiling using Protox II webserver. Canonical SMILES (O'Boyle, 2012) of the compounds were the inputs of both screenings.

Molecular dynamics simulations

Webgro, the University of Arkansas for Medical Sciences (UAMS) webserver for molecular dynamics simulation, was used to carry out molecular dynamics simulation studies on the colipase-free Pancreatic lipase complex of the selected lead, thereby validating its docking score. Independent variable parameters were set as follows: Box type was triclinic with SPC water model; GROMOS9643a1 was selected as force field; equilibrium temperature was 300 K, while simulation time was set at 20 ns. Ligand - macromolecule complexes were prepared as pdb files with BIOVIA Discovery Studio; Ligand topology files were prepared with PRODRG webserver, using coordinates extracted from the text formats of the complexes (Abraham et al., 2015).

RESULTS AND DISCUSSION

Molecular docking and MMGBSA ranking

The docking scores of the 32 phytoligands ranged between -5.9 to -9.7 Kcal/mol and were higher than that of the co-crystallized ligand (-5.2 Kcal/mol). The more accurate MM/GBSA free binding energy scoring correlated pooly with the docking scores (R^2 = 0.37), ranking the co-crystallized ligand above a number of the docked phytoligands in binding affinity (Table 1, Fig. 1).

Docking validation

The coordinates of the docked MUP in its best-pose conformation superimposed well on those of its native counterpart with a calculated 1.79 Å RMSD.

Drug-likeness and toxicity potentials screenings

Three compounds, 1-caffeoylquinic acid, canthin-6-one and pelentanic acid, were selected as drug-like based on the set criterion after screening throught the five drug-likeness filters of the SwissADME webserver (Table 2).

Only one (canthin-6-one) of the three selected drug-like compounds demonstrated a good safety profile, with an LD_{50} of 1200 mg/Kg and minimal organ toxicity/ toxicity endpoint tendencies. The remaining two had LD_{50} values less than 250 mg/Kg (Table 3).

Table 1: MM/GBSA binding free energy ranking of MUP and 32 docked phytoligands of *H. sabdariffa*

Compounds	Name	MMGBSA (Kcal/mol)	Docking score (Kcal/mol)
Ref.*	Methylundecylphosphinic acid.	-40.12	-5.9
1	3,4,5-tricaffeoylquinic acid	-65.07	-9.7
2	Ellagic acid	-62.11	-9.6
3	1-caffeoylquinic acid	-60.34	-9.5
4	Hibiscetin	-58.82	-9.2
5	Canthin-6-one	-55.97	-9.1
6	chlorogenic acid	-55.11	-8.7
7	Pelentanic acid	-46.31	-8.6
8	4-o-galloylchlorogenic acid	-46.14	-8.5
9	Neochlorogenic acid	-45.11	-8.4

10	2-chloropentanedioc acid	-44.02	-5.4
11	Hydroxycitric acid	-42.23	-5.8
12	Caffeic acid hexoside	-40.03	-7.7
13	Fluorescein-digalactoside	-40.00	-8.2
14	Myricetin-3-o-galactoside	-38.37	-7.3
15	5-o-caffeoylshikimic acid	-35.54	-8.2
16	3,4-dihydroxybenzoic acid	-18.28	-5.8
17	Cis-5-caffeoylquinic acid	-18.22	-7.7
18	Quercetin-7-olate	-17.10	-8.2
19	Malic acid	-16.45	-5.2
20	Hexamethylquercetagetin	-16.29	-7.7
21	Cynarin	-16.20	-7.8
22	Caffeic acid	-15.67	-6.6
23	Ascorbic acid	-15.01	-6.3
24	5,6-dichloronicotinic acid	-12.99	-5.7
25	Caffeidine acid	-12.45	-6.1
26	Methyl-4-caffeoylquinate	-11.66	-7.7
27	Phenylgalactoside	-10.50	-8.1
28	Caffeic acid-3-glucoside	-10.10	-7.8
29	Sinapic acid	-9.55	-6.2
30	Hirsutic acid C	-9.10	-8.1
31	D-tartaric acid	-8.40	-5.3
32	4-hydroxycinnamic acid	-8.28	-6.0

Cocrystallized ligand, Methylundecylphosphinic acid (MUP).

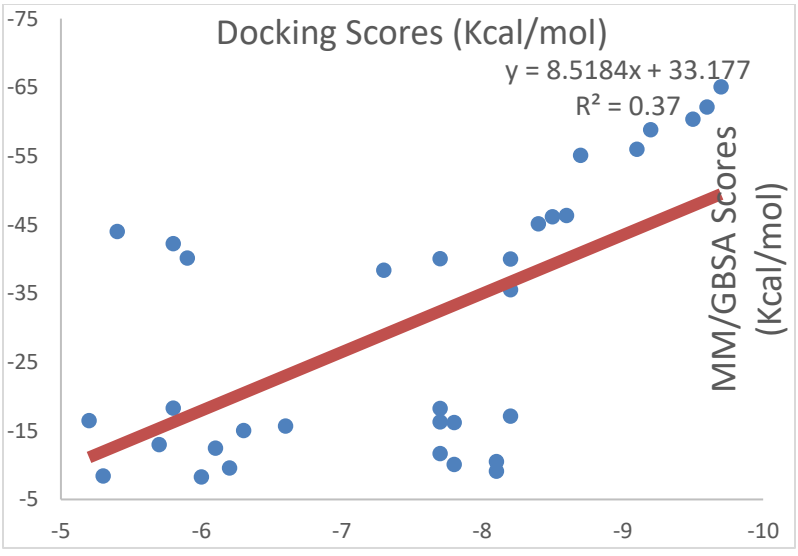

Fig. 1. Regression of docking scores on MM/GBSA scores of 32 phytoligands of *H. sabdariffa*

Table 2: Violations of the five (Lipinski, Ghose, Veber, Egan and Muegge) drug-likeness filters of SwissADME webserver by the top 15 of MM/GBSA-ranked 32 phytoligands of *H. sabdariffa*.

Compound	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score
MUP	0	0	1	0	0	0.55
1	3	3	2	1	4	0.11
2	0	0	1	1	0	0.55
3*	0	0	0	1	0	0.56
4	1	0	1	1	2	0.55
5*	0	0	0	0	0	0.55
6	1	1	1	1	2	0.11
7*	0	0	0	1	0	0.56
8	3	1	1	1	3	0.11
9	1	1	1	1	2	0.11
10	0	2	0	0	1	0.85
11	0	2	1	1	2	0.11
12	3	4	1	1	4	0.17
13	2	2	1	1	1	0.17
14	1	1	1	1	2	0.55
15	1	1	0	1	1	0.56

Table 3: LD50 values and organ toxicity/toxicity endpoint tendencies of three drug-like compounds ensuing from SwissADME screening

Compound	Name	LD_{50} (mg/Kg)	Organ toxicity/Toxicity endpoints				
			Hepato Toxicity	Carcino Genicity	Immuno Toxicity	Cyto toxicity	Muta genicity
3	1-caffeoylquinic acid	159	-	Active	-	-	-
5	Cantin-6-one	1200	-	-	-	-	Active
7	Pelentanic acid	233	-	-	-	-	Active

Molecular dynamics of canthin-6-one – PL complex

Radius of gyration (Rg) of the protein model (1LPB) was as high as 22.5 Å (Fig.1A). Its root mean square fluctuation (RMSF) plot showed significant fluctuations in the equilibrium positions of most of the residues at the binding site (Fig. 2). However, the root mean square deviations (RMSD) plot of its complex with canthin-6-one showed early convergence around 2.5 ns and minimal deviations maintained largely below 3 Å from the initial complex structure throughout the 20 ns simulation period (Fig. 3).

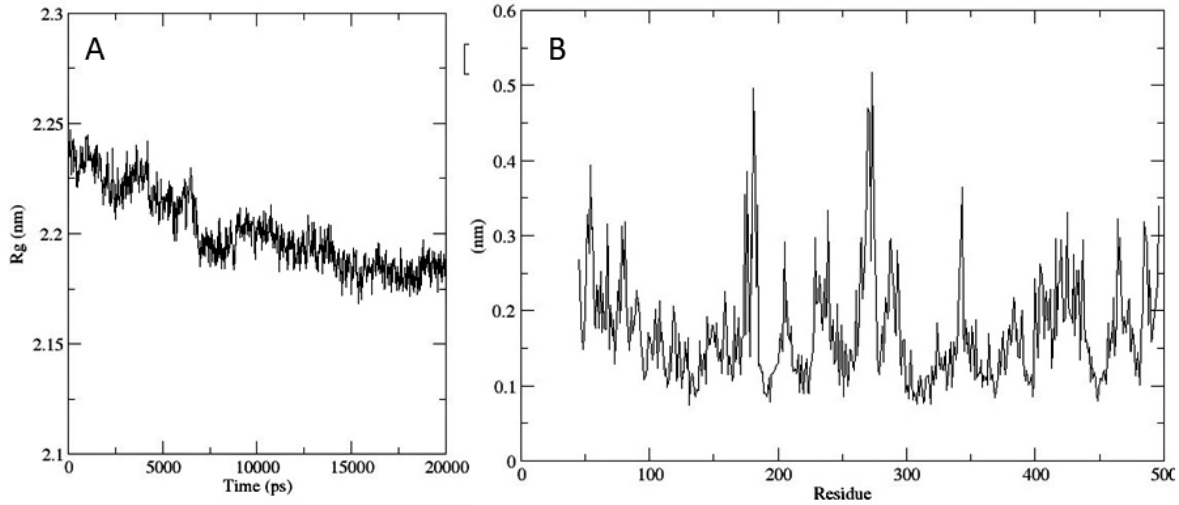

Fig. 2: A – Radius of gyration (Rg) plot and B –Root Mean Square Fluctuations (RMSF) of the colipase-free chain of the human Pancreatic lipase model (1LPB) in a 20 ns simulation Molecular Dynamics simulation of its complex with canthin-6-one.

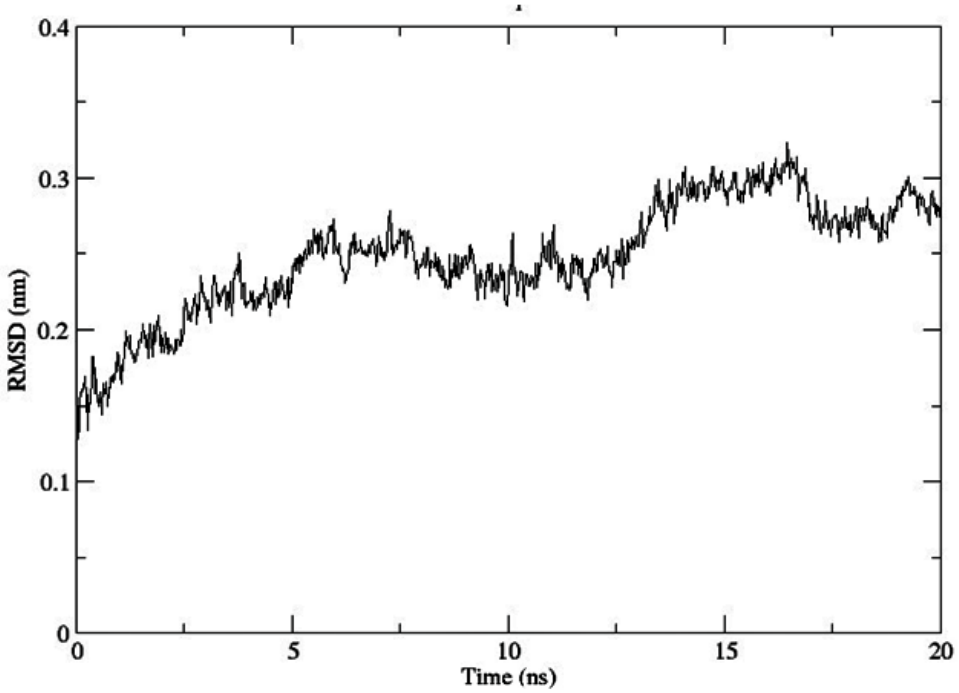

Fig. 3: Root Mean Square Deviations (RMSD) plots of colipase-free human Pancreatic Lipase in complex with canthin-6-one.

Active site interactions simulation for canthin-6-one and MUP

Both compounds showed one conventional hydrogen bond each but to different residues. MUP showed more alkyl/pi-alkyl interactions than canthin-6-one did. Interaction with residues ARG256, ALA259 and LEU264 were preserved but via different modes, van der waals with MUP and pi alkyl/alkyl with canthin-6-one (Figs. 4 and 5).

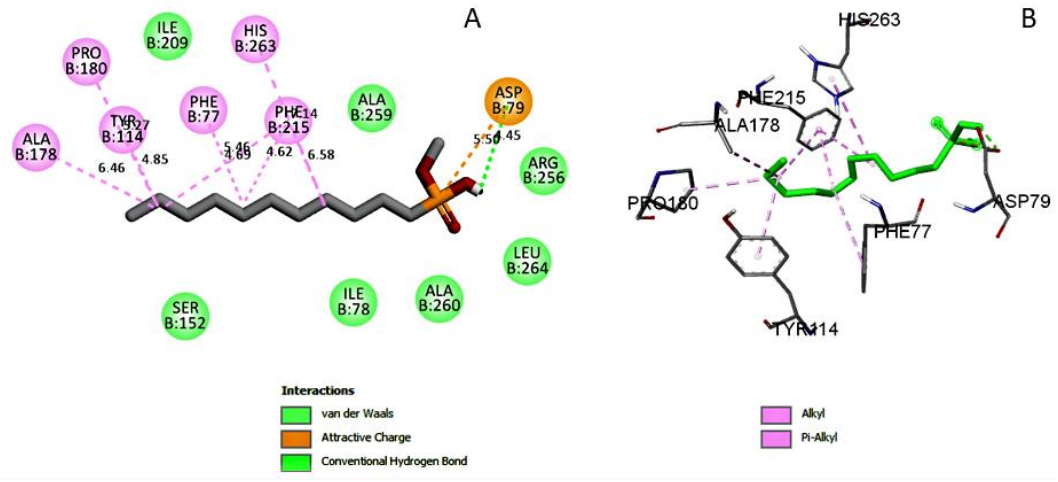

Fig.4: A – 2D and B – 3D simulations of supramolecular interactions at the active site of the colipase- free human Pancreatic Lipase in complex with Methoxyundecylphosphinic acid (MUP).

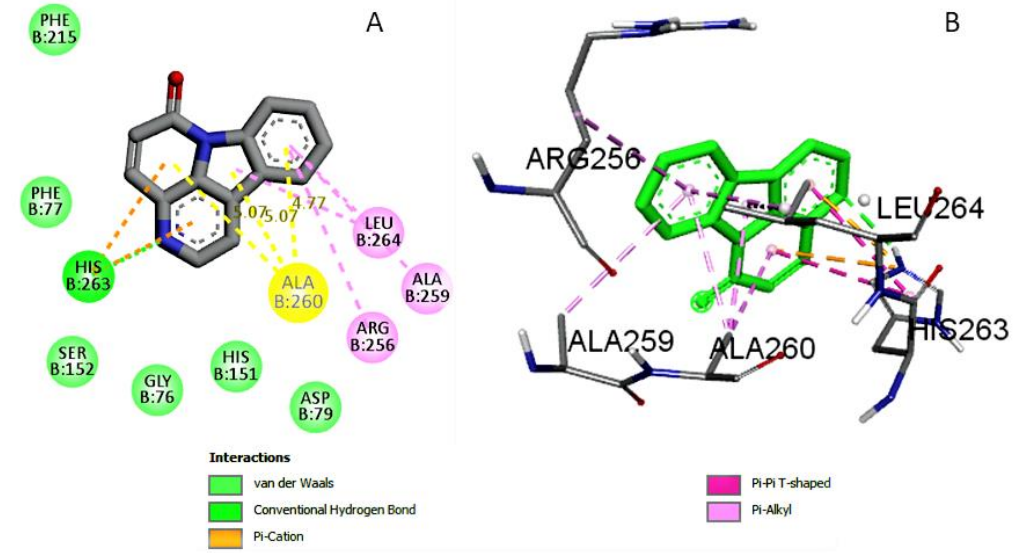

Fig. 5: A – 2D and B – 3D simulations of supramolecular interactions at the active site of the colipase- free human Pancreatic Lipase in complex with canthin-6-one.

DISCUSSION

Though the docked 32 compounds of *H. sabdariffa* comprised compound groups of structural diversity, the dominance of the list by the caffeoyl subfamily of cinnamoyl esters of quinic acid, broadly referred to as chlorogenic acids (Clifford, 1999), is worthy of note. Of a fact, this is enough indication that the chemistry/biochemistry of the plant could be predicted as largely that of the chlorogenic acids superfamily, and makes the ultimate selection of canthin-6-one, an indole alkaloid (fig. 6) with no structural or biogenetic resemblance to the chlorogenic acids, a paradox. Nevertheless, this observation underscores the privilege nature of indole alkaloids showing inherent propensity of binding to diverse macromolecular entitities (Zhang *et al.*, 2014), the human PL inclusive. The fact that PL requires colipase binding for activation informed the search for a colipase-bound X-ray model of PL from the Protein Databank (PDB) thereby ensuring that the colipase-free PL used in the

in-silico studies was present in the active conformation, the only form in which it binds the triglyceride substrates (Winkler and D'Acry, 1998). The final selection of the PDB model 1LPB was based partly on this co-lipase activation requirements and partly on its good (2.46 Å) resolution, which is non-negotiable for reliable docking experiments (Sousa et al., 2006; Pujadas et al., 2008).

Given the plethora of assumptions associated with docking scoring function algorithms, docking scores could only, at best, be treated as mere estimates of docked ligands' binding affinities, regardless of the docking tool employed (Warren et al., 2006; Plewczynski et al., 2011). This inherent inaccuracy in docking algorithms could be deciphered from the poor correlation the obtained docking scores showed to the rather more accurate MM/GBSA free binding energy scores ($R^2 = 0.37$) (Fig. 1) (Sahakyan, 2021). Hence, binding affinity ranking of the docked ligands was according to the MM/GBSA binding free energies, lower (or more negative) values being indicative of high binding affinities (Table 1).

In modern drug discovery, high binding affinity is only suitable for selecting hits or chemical species capable of optimal binding interactions with the target of interest (Keserti and Makara, 2006). It is not necessarily indicative of a lead, which, in addition to high biniding affinity, is also expected to possess good prospects of *in-vivo* activity (Oprea, 2000; Lipinski, 2004). This is because binding affinity is merely a Pharmacodynamic indicator, giving average estimates of the strength of supramolecular interactive forces between a macromolecular target and a potential drug (Enyedi and Egan, 2008). It is, therefore, at best, an *in vitro* interaction predictor. However, drug action is not only about ligand-macromolecule interaction. In addition, it involves other crucial factors bordering on the ability of the drug candidate to reach the macromolecular action site (i.e., it's Pharmacokinetics) and restriction of its biological activity to the desired site (i.e., it's selectivity and, hence, safety). The docking and its subsequent binding affinity ranking excercises carried out could therefore, at best, only be used to select hit compounds. Selected hits were limited to the top 15 compounds on account of being conspicuously higher in binding free energy values than the rest (Table 1).

Screening the 15 compounds through the five (i.e., Lipinski, Verber, Ghose, Egan and Muegge) drug-likeness filters of the SwissADME webserver (Diana *et al.*, 2017) was aimed at selecting hits with great propensities of *in vivo* activity in addition to inherent receptor binding potentials predicted by their docking and MM/GBSA ranking. Drug-likeness, in medicinal chemistry parlance, is the propensity of a molecule to be optimally orally bioavailable (Ursu *et al.*, 2011; Allam *et al.*, 2011). It summarizes the pharmacokinetics data of a molecule. Seting only one criterion of not violating more than one stipulation of any of the aforementioned five SwissADME filters, three drug-like hits (1-caffeoylquinic acid, canthin-6-one and pelentanic acid) were selected from the screened 15. The structural diversity of these compounds is striking, 1-caffeoylquinic acid being a chlorogenic acid of the caffeoyl subfamily; canthin-6-one being basically an indole alkaloid and pentelanic acid a biscoumarin (Fig.6). Nevertheless, common pharmacophoric features upon which their similar biochemistry might be anchored are obvious. For instance, aromatic ring, extensive conjugation and oxygen electron donor groups are common pharmacophoric features to the three compounds (Fig.6).

Analysis of Table 3 for the toxicity potentials of the three drug-like compounds showed each of canthin-6-one and pentelanic acid demonstrating mutagenic tendencies probably because of their planar structures which make them amenable to intercalating between strands of DNA double helix (Ferguson and Denny, 2007). It also showed 1-caffeoylquinic acid as demonstrating immunogenic tendencies. A consideration of the LD_{50} values of the compounds however showed that while the toxicity tendencies of 1-caffeoylquinic and pelentanic acids have very high propensities of clinical manifestation, given their rather low LD_{50} values, any toxicity inherent in canthin-6-one is not likely to manifest at reasonable human doses given its relatively high (1200 mg/Kg) LD_{50} value and, hence, its selection as the only compound for molecular dynamics (MD) simulation studies aimed at predicting the stability of its binding interactions in the rather dynamic *in vivo* environments (Hollingsworth, 2018).

In principles, MD simulations of receptor-ligand complexes validate the binding interactions predicted by molecular docking, the algorithm of which, in most cases, function in a semi-flexible manner, simulating macromolecular entities as rigid and their small-molecule interactives (or ligands) as somewhat momentarily flexible (Hanson *et al.*, 2002; Pagadala *et al.*, 2017). Analysing trajectories of atoms of a macromolecule-ligand complex can provide diverse vital information about it, depending on the order and/or duration of the simulation time period. At nanosecond time-scale, trajectory parameters like the Root Mean Square Deviations (RMSD), Root Mean Square Fluctuations (RMSF) and Radius of Gyration (Rg), could be analysed to assess the stability of the macromolecule-ligand complex, more or less validating facile binding interactions predicted by molecular docking.

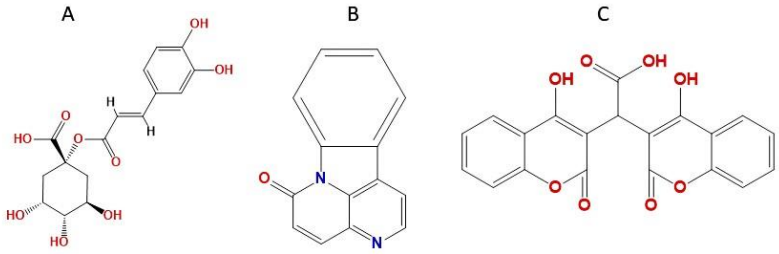

Fig.6: 2D structures of A – 1-caffeoylquinic acid (a chlorogenic acid); B – canthin-6-one (an indole alkaloid) and pentelanic acid (a biscoumarin)

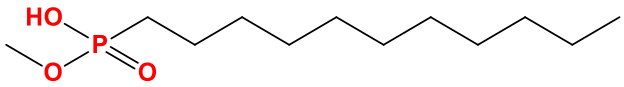

Fig. 7: 2D structure of methoxyundecylphosphinic acid (MUP)

Despite the huge fluctuations deciphered in the RMSF (Fig. 2), suggestive of conformational perturbations, and a relatively less compact complex-implying 22.5 Å Rg value (Justino *et al.*, 2021), analysis of the RMSD trajectory of the complex largely showed a stable complex on account of the convergence of its instantaneous and initial structures at around 2 ns and the maintenance of their deviations largely below 3 Å over a 20 ns simulation time period (fig. 2). This seemingly paradoxical observation is probably due to the compact topology of canthine-6-one as opposed to the rather diffused topologies of the substrate and the co-crystalized ligand, limiting the fluctuating and displaced residues it interacted with to just a few (Figs. 6 and 7). The observed contrasts between the active site interactions of canthin-6-one and the reference co-crystalized inhibitor MUP were certainly responsible for the observed differences in their binding affinities as shown in Table 1 which revealed the less binding affinity of MUP relative to that of canthin-6-one. These observations could be attributed to the different topologies of the two compounds: While MUP is a relatively flexible long-alkyl chain compound (fig. 7), canthin-6-one is largely a rigid flat compound with conjugation and aromaticity (fig. 6). The flexible alkyl topology of MUP accounted for its pronounced alkyl interactions. It is worthy of note that a few residues (specifically ARG256, ALA259 and LEU264) with alkyl features interacted with cantin-6-one via pi-alkyl interactions but were not involved in the diffused alkyl-alkyl interactions of PL with MUP. Their binding/catalytic involvements were however preserved in the PL-MUP complex via Van der Waals interactions (Figs. 4 and 5).

CONCLUSION

This investigation has led to the identification of three drug-like PL inhibitor hits (1-caffeoylquinic acid, canthin-6-one and pelentanic acid) from *Hibiscus sabdariffa*. It has also led to the identification of canthin-6-one as a plausible lead, on the accounts of high binding affinity; drug-likeness; good safety profiles and stable MD simulation parameters, for further in vitro, in vivo and molecular modification studies aimed at discovering new PL inhibitory anti-obesity drugs.

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