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Molecular docking and ADMET properties of *Citrus sinensis* phytochemicals on insecticide resistance *Anopheles gambiae*: An *in-silico* analysis

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Abstract
Mosquitoes are one of the ancient insects that have survived with humans for millions of years. They are responsible for the transmission of malaria which persists in many parts of the world today. The most common method of controlling mosquitoes in Nigeria is the use of synthetic insecticides. Synthetic insecticides-based intervention for the control of mosquitos have disrupted natural biological settings and led to outbreaks of insect resistance as well as killing non-target organisms. Many plants produce secondary components that have insect growth inhibitory activities, which are also safer and less toxic to non-target organisms. In this study, we targeted agERG sodium channel transporter involved in the insecticide resistance of pyrethroid against *Anopheles gambiae*, which caused malaria disease. C-linker or CNBHD of agERG channel is responsible for inhibition of pyrethroid insecticide activity in *Anopheles gambiae.* The compounds present in *Citrus sinensis* were docked against the agERG sodium channel transporter protein involved in the inhibition of pyrethroid insecticide of *Anopheles gambiae*. PyRx-Python prescription 0.8. was used to identify binding affinities of compounds against the protein. Fourteen compounds show good binding potential to agERG protein. Going by the ADME/T analysis, only (Molecule 2), (Molecule 4), (Molecule 5), (Molecule 6), (Molecule 7) and (Molecule 8) follow the Lipinski rule of five, Ghose and Muegge criteria (ADME/T). We may conclude that compounds isolated from *Citrus sinensis* have a high potential for application as control agents for *Anopheles gambiae* and they will be less persistent in the environment.

Keywords: *Anopheles gambiae, Citrus sinensis, in-silico,* sodium channel transporter agERG

Introduction
Malaria occurs primarily in tropical and some subtropical regions of Africa, Central and South America, Asia, and Oceania [1]. Malaria is typically transmitted by the bite of an infective female *Anopheles* mosquito. In Nigeria, Control programmes are based on the application of chemical insecticides by the use of insecticide-treated bed nets or by indoor spraying. Insecticides used include, the WHO recommended insecticide for insecticide-treated bed nets (Pyrethroids) [2], DDT and carbamate for indoor spraying [3]. Both DDT and Pyrethroids target the Voltage-gated sodium channel (VDSC) in the mosquito's central nervous system [4], while carbamates inhibit acetylcholinesterase which block the degradation of the neuromediator acetylcholine [5]. Cyclodiene and fipronil insecticides target the γ-aminobutyric acid (GABA) receptors [6, 7]. (Figure 1)
Africa record the highest incidence rate of malaria (93%) [8]. Among the factors contributing to this scenario, it is possible to highlight the absence of an effective antimalarial vaccine, the development of insecticide resistance in vector mosquitoes [9]. In Nigeria there has been a great concern about the resistance of mosquitoes to insecticide which is seen as the major means of the vector control. Due to the fact that there is limited development of insecticides, as such only few insecticides are used in public health [10]. These few that are used can also impose threat to human health and environment. As such developing management strategies using natural extract will minimize the challenge of developing resistance by the insect vector. Nigeria have 83.33% resistance of gambiae resistance against Pyrethroids between 2010-2017 [11].
Constant and extensive use of chemical insecticides has created a selection pressure and favored resistance development in many insect species worldwide. One of the most important

pyrethroid resistance mechanisms is classified as target site insensitivity, due to conformational changes in the target site that impair a proper binding of the insecticide molecule. The voltage-gated sodium channel ERG (agERG) channel CNBHD) [12] is the target of pyrethroids and DDT insecticides, used to control insects of medical, agricultural and veterinary importance, such as anophelines [13]. After binding to the sodium channels, they cause the insect's nervous system to repetitively discharge and its nerve membranes to depolarize [14]. It has been reported that the presence of a few non-silent point mutations in the NaV gene are associated with pyrethroid resistance, termed as 'kdr' (knockdown resistance) for preventing the knockdown effect of these insecticides [13].

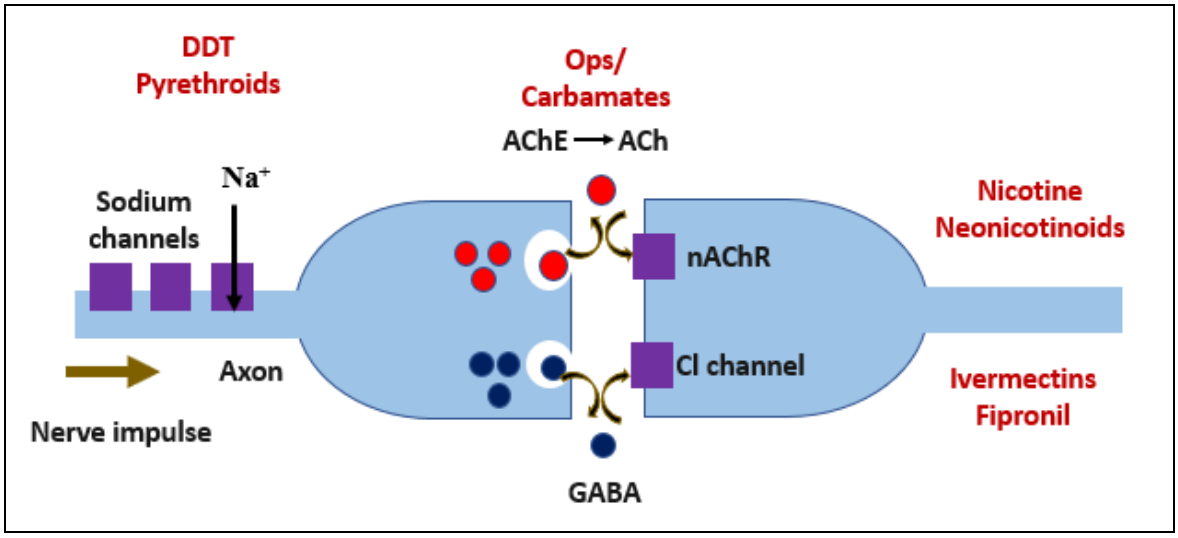

Fig 1: Diagrammatic representation of two neurons and an intervening synapse, showing the sites of action of the most commonly used classes of insecticide

Citrus is a commercial fruit which is globally grown for its sweet taste [15]. Citrus is among the largest grown fruit due to its diversified use and demand at all-time [16]. The recovery of by-product from the fruit wastes can improve the overall processing units of economy and drastically reduce environmental pollution. The citrus peels are rich in nutrients and contain many phytochemicals; they can be efficiently used as drugs or as food supplements too [17]. All-natural compounds found shows good efficacy on insect repellence, oviposition, fumigation as well as insecticidal activity. This study is aimed at targeting agERG sodium channel transporter involved in the insecticide resistance of pyrethroid against *Anopheles gambiae*, which caused malaria disease.

Materials and Methods

1. Protein Preparation

The protein structure of target protein was downloaded from RCSB Protein Data Bank in .pdb format. All the heteroatoms were removed leaving only the residues of the proteins using the PyMOL Molecular Graphics System (version 1.1).

2. Ligand Preparation

The occurrence of phytochemicals in *Citrus sinensis* was carried out by searching various literature databases. At the end of the literature survey, 96 phytochemicals were found. The literature-based 3D or 2D structure of phytochemicals of agERG Target protein were retrieved in .sdf format from National Centre for Biotechnology Information (NCBI) PubChem. Open Babel molecule format converter was used for the conversion of 2D to 3D conformation. Ligand's energy was minimized by relating the mmff94 force field and conjugate gradients optimization algorithm using PyRx-Python prescription 0.8 for 200 steps [18]

Standard inhibitor such as pyrethroids and DDT bind with the target protein on the heterodimer surface [19]. The literature-based 3D or 2D structure of Discovery Studio shows the amino acid of the target protein which is involved in the interaction with the ligand. GCMC analysis revealed 96 ligands, these ligands were subjected to first screening, that is, ligand with binding energy below standard ligand (-6.3 k.cal/mol) are eliminated. After the first screening, 43 ligands are retrieved. These ligands are subjected to second screening, that is, ligand with hydrogen bond above the standard. After the second screening, 14 ligands were retrieved.

3. Generation of receptor Grid and Molecular docking

Molecular docking is a computational technique used to recognize the structures which bind well to the enzyme pocket [20]. Computer-aided virtual screening was carried out with PyRx virtual screening tool on fourteen ligand compounds against the binding site of agERG protein [21] through flexible docking option. PyRx software is open version software with an intuitive user interface that runs on all major operating systems (Linux, Windows, and Mac OS) [22]. Each of these phytochemicals was docked into target protein therefore with positions, orientations, and conformations of the ligand in the receptor-binding site, and the docking structure keeping the lowest energy was preferred [23]. Compound with highest binding affinities were then analyzed and considered as possible template for further optimization. Data of Grid- box of the three coordinates X, Y, Z were 19.6337, -10.454, 13.0550 respectively were obtained after full maximization of protein area. The grid box includes the whole binding site of the proteins line and provides sufficient space for the ligands translational and rotational walk. After that, PyMOL Molecular Graphics System (version 1.1) was used for visualization of the interaction pattern in the protein-ligand complex [23].

4. Protein Ligand Interaction

The binding of the modified ligands with the active sets of target proteins was the determination of protein and ligand

binding [19]. The surface structure of the protein and ligand is determined through PyMOL Molecular Graphics System (version 1.1). The binding of the modified ligands with active sites of targeted protein is determined and visualized by surface structure determination of protein and ligand binding. It is a molecular modelling technique whereby the interaction between the protein and the ligands is determined by the position of orientation of the ligand when bound to protein. The surface structure of the protein and ligand is determined through PyMOL Molecular Graphics System (version 1.1).

5. Protein Ligand Visualization

The 2D and 3D molecular interaction models of the docked compounds- agERG complex involving H-bonds, Pi-Pi and Pi-sigma interactions are displayed using Accelrys Discovery Studio Visualizer software version 3.5 [24]. The protein-ligand complexes in the .pdb format are displayed via the software Accelrys Discovery Studio Visualizer software version 3.5. The protein-ligand interactions along with the hydrophobic interactions and hydrogen bonding with the complex binding residues are given for the lead phytochemicals. The amino acid of the target protein which is involved in the interaction with the ligand and the distance between the amino acids and the ligands are displayed via Accelrys Discovery Studio Visualizer software version 3.5. The interaction profile of lead phytochemicals and the interface among heterodimers of a protein can be determined. The amino acid involved in hydrophobic interaction and hydrogen bonding with the ligands can also be easily determined.

6. ADME/T Properties

ADME/T properties were analysed through swissADME (http://www.swissadme.ch) used for the estimation of the ADMET properties (Physiochemical properties, Lipophilicity, Water solubility, pharmacokinetics and Druglikeness) of the docked molecules [25].

Results and Discussion

Molecular Docking

Ligands were docked in contrast to the target proteins for prediction of the binding score. The molecular docking interaction models of the fourteen ligand molecules and the target protein were illustrated in (Table 1). The molecular interaction results like H-bonds, π-π and Pi-sigma are represented in (Figure 2).

Table 1: Docking score, Hydrophobic Bond and H-bond interaction of ligands against mosquito sodium channel protein (agERG)

SN	Ligand name	Pub Chem ID	Binding Affinity k.cal/mol	Docked complex (amino acid –ligand) interactions			
				Hydrogen Bond	Distance	Hydrophobic Bond (Alkyl and pi-Alkyl)	Distance
1	Hesperidin	10621	-8.6	Phe558(A), Glu549(A)	4.92 5.12	ARG546(A), ARG651(A), HIS698(A), LEU559(A), AUE630(A)	4.15 5.77 5.98 4.72 5.13
2	Perillaldehyde	16441	-7.3	Ile578(A)	4.29	LEU564(A), LEU584(A), ILE595(A), ILE551(A), VAL547(A)	4.98 4.77 5.74 4.61 4.24
3	Neohesperidin Dihydrochalcone	30231	-6.4	CYS596(A)	4.56	ALA630(A), TYR647(A), LEU636(A)	5.33 6.13 4.18
4	Citral ethylene glycol acetal	47922	-7.4	ILE578(A)	4.79	PHE587(A), LEU584(A), ILE595(A), ILE556(A), PHE550(A), VAL547(A)	5.84 4.45 4.61 5.56 4.61 4.88
5	Isosakuranetin	160481	-6.6	ARG546(A)	5.58	ARG651(A)	3.81
6	Limonin	179651	-7.6	GLU549(A)	6.26	ARG651(A) Tyr 694(A)	4.99 4.19
7	Neohesperidin	442439	-7.1	HIS629(A) LYS585(A) GLY592(A) THR628(A)	4.54 4.09 4.05 4.05	LYS626(A) LEU584(A)	3.94 6.84
8	Geraniol	637566	-7.1	MET580(A) ARG546(A)	3.86 4.01	ILE556(A) ILE551(A) ILE595(A) LEU564(A) LEU584(A) VAL547(A) PHE550(A) PHE587(A)	6.04 5.91 4.19 5.97 4.55 5.97 4.57 4.99
9	Quercitrin	5280459	-7.1	GLY667(A) ASP696(A) ASN602(A) HIS598(A)	3.93 4.63 4.30 4.93	HIS554(A)	4.39
10	Diosimin	5281613	-8.1	MET580(A) ASP579(A)	3.96 4.91	TYR694(A) LEU603(A)	5.50 4.74

				HIS698(A) ASN602(A) CYS695(A) ASP669(A)	5.54 3.90 4.52 6.12	ARG651(A)	5.56
11	Methylhesperidin	5284419	-8.1	ARG546 HIS698(A) ASN602(A) PHE558(A) GLU549(A) ASP669(A) ASP696(A)	5.29 5.65 4.13 5.63 5.36 5.80 5.44	LEU599(A) ARG651(A)	5.61 5.66
12	Apocarotenal	5478003	-7.0	ASP717(A)	5.73	LEU706(A) CYS677(A) LEU709(A) PRO713(A)	4.44 5.13 6.37 4.97
13	Hesperidin methylchalcone	6436550	-7.5	ASP696(A) HIS554(A) PHE553(A) GLU549(A)	4.33 5.20 5.44 6.39	TYR694(A) LEU599(A) ALA650(A) ARG546(A)	4.72 4.74 5.19 4.21
14	Didymin	16760075	-8.6	PHE558(A) GLU549(A)	5.41 5.22	LEU599(A) ALA650 HIS554(A) HIS698(A) ARG651(A) ARG546(A)	4.76 5.15 5.22 6.11 5.68 4.14

Protein Ligand Interaction

There is some interaction like hydrophobic interactions and hydrogen bonding between amino acids of proteins and selected compounds. Mostly it has been found that hydrophobic interaction plays a very important role in strongly binding to atoms of ligands and amino acids of proteins

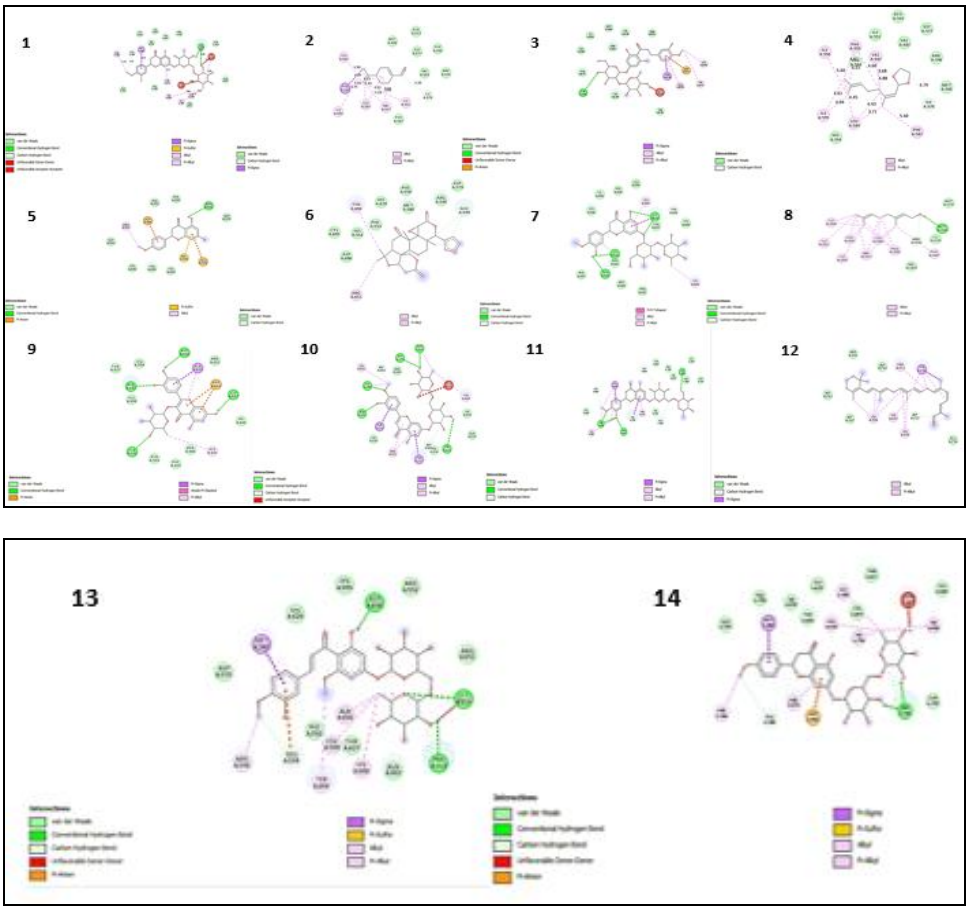

Fig 2: H-bonds, π-π and Pi-sigma interaction of ligands against mosquito sodium channel protein (agERG)

Protein Ligand Visualization

The protein-ligand complexes in the .pdb format are displayed, edited and run via the software Discovery Studio (version v.1.4.5) for the generation of Discovery Studio schematic diagrams. The protein-ligand interactions along with the hydrophobic interactions and hydrogen bonding with the complex binding residues are given for the lead phytochemicals (Figure 3).

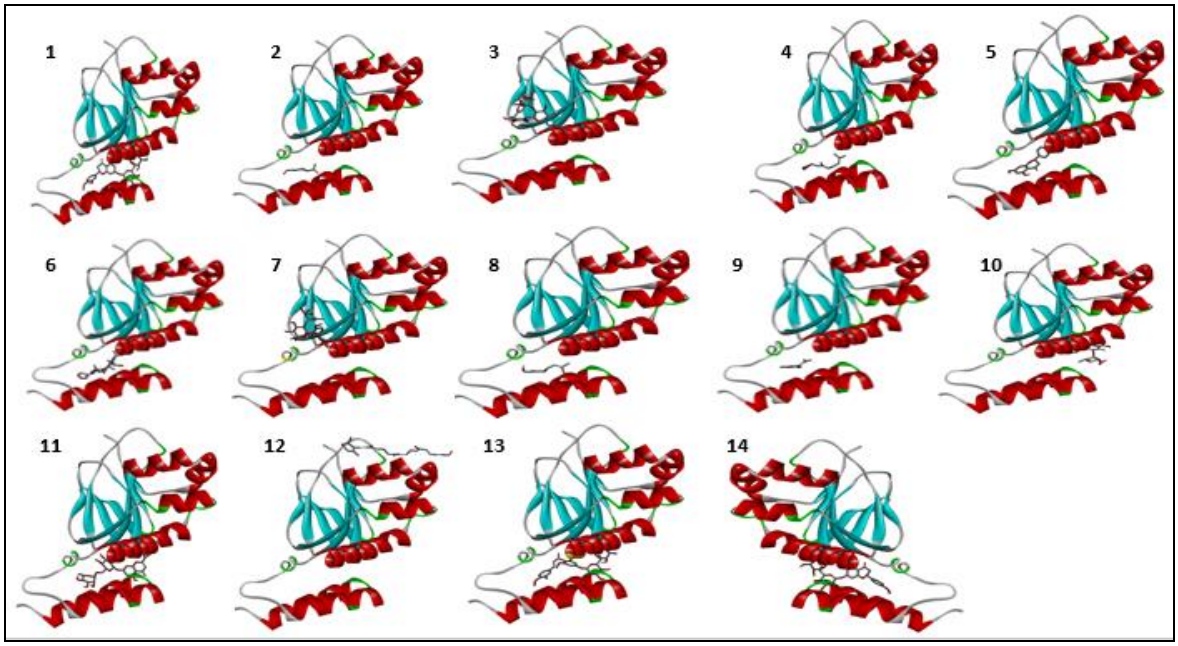

Fig 3: Visualization of protein-ligand interactions

ADMET Properties

The result of ADMET properties reveal that the all the fourteen compounds which were analyzed have good water solubility except molecule 12 (Apocarotenal). Perillaldehyde (Molecule 2), Citral ethylene glycol acetal (Molecule 4), Isosakuranetin (Molecule 5), Limonin (Molecule 6), Neohesperidin (Molecule 7) and Geraniol (Molecule 8) drug-likeness properties are represented by the red distorted hexagon within the pink shade (Figure 4) which shows that their drug-likeness fall within parameters of a bioavailable drug. Hesperidin (Molecule 1), Neohesperidin Dihydrochalcone (Molecule 3), Quercitrin (Molecule 9), Diosimin (Molecule 10), Methylhesperidin (Molecule 11), Apocarotenal (Molecule 12), Hesperidin methylchalcone (Molecule 13), Didymin (Molecule 14) have high unsaturation indicated by an off-shoot of one of the vertices which shows negative controls with no bioactivity. The drug-likeness of the compound was analyzed Lipinski [26], Ghose [27] and Muegge [28] Principles. Only (Molecule 2), (Molecule 4), (Molecule 5), (Molecule 6), (Molecule 7) and (Molecule 8) met the Lipinski, Ghose and Muegge qualifying criteria of drug-likeness and pharmacophore point. (Table 2).

For the toxicity analysis, only (Molecule 2), (Molecule 4), (Molecule 6), (Molecule 7) and (Molecule 8) returned "No" for P-gp substrate and "No" for CYP isoenzymes inhibition. (Molecule 5), returned "Yes" for CYP3A4 inhibition (Table 2). All the other molecules returned Yes for P-gp substrate. ADMET properties also reveal that only (Molecule 2), (Molecule 4), (Molecule 5), (Molecule 6), (Molecule 7) and (Molecule 8) have better Human Intestinal Absorption (HIA) score, good BloodBrain Barrier (BBB) values except (Molecule 7) (Figure 5).

Conclusion

In this study, out of the 96 compounds isolated from *Citrus sinensis*, 14 shows good binding potential to agERG protein. Going by the ADME/T analysis, only (Molecule 2), (Molecule 4), (Molecule 5), (Molecule 6), (Molecule 7) and (Molecule 8) follow the Lipinski rule of five, Ghose and Muegge criteria (ADME/T). A 'BOILEDegg evaluation', predicts that these compounds are not effluxed by P-glycoprotein (P-gp) and have higher gastrointestinal absorption (HIA) than the remaining 8 compounds. Additionally, K8A is lipophilic but does not penetrate the blood brain barrier (BBB) and is not a substrate of most CYP enzymes. So, we can conclude that the six selected compounds target the agERG protein and inhibit it and maybe useful for designing new and safe inhibitors against agERG sodium channel protein.

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Declaration of interests

There is no conflict of interest in any form of competing financial interests or personal relationships that could have appeared to influence the work reported in this review.

Table 2: Physiochemical properties, Lipophilicity, Water solubility, pharmacokinetics and Druglikeness of ligands

SN	PUB ID	M/Formula	MW (g/mol)	Atom Count	NRB	NHA	NHD	MR	TPSA	Log P_{aw}	Log *S* (ESOL)	P-gp	CYP3A4 Inhibitor
1	10621	$C_{28}H_{34}O_{15}$	610.56	43	7	15	8	141.41	234.29 Å^2	-1.06	-3.28 s	YES	NO
2	16441	$C_{10}H_{14}O$	150.22	11	2	1	0	47.32	17.07 Å^2	2.49	-2.61 s	NO	NO
3	30231	$C26H36O_{15}$	612.58	43	10	15	9	143.86	245.29 Å^2	-0.66	-3.00 s	YES	NO
4	47922	$C_{12}H_{20}O_{12}$	196.29	14	4	2	0	58.91	18.46 Å^2	2.91	-2.80 s	NO	NO
5	160481	$C_{16}H_{14}O_5$	286.28	21	2	5	2	76.04	75.99 Å^2	2.25	-3.70 s	NO	YES
6	179651	$C_{26}H_{30}O_8$	470.51	34	1	8	0	116.17	104.57 Å^2	2.54	-3.92 s	NO	NO
7	442439	$C_{28}H_{34}O_{15}$	610.56	43	7	15	8	141.41	234.29 Å^2	-1.02	-3.07 s	YES	NO

8	637566	$C_{10}H_{18}O$	154.25	11	4	1	1	50.40	20.23 Å^2	2.74	-2.78 s	NO	NO
9	5280459	$C_{21}H_{20}O_{11}$	448.38	32	3	11	7	109.00	190.28 Å^2	0.22	-3.33 S	NO	NO
10	5281613	$C_{28}H_{32}O_{15}$	608.54	43	7	15	8	143.82	238.20 Å^2	-0.52	-3.51 S	YES	NO
11	5284419	$C_{29}H_{36}O_{15}$	624.59	44	8	15	7	145.88	223.29 Å^2	-0.86	-2.95 S	YES	NO
12	5478003	C30H40O	416.64	31	9	1	0	139.88	17.07 Å^2	7.84	-7.74 P	YES	NO
13	6436550	C29H36O15	624.59	44	10	15	8	148.65	234.29 Å^2	-0.51	-3.05 S	YES	NO
14	16760075	C28H34O14	594.56	42	7	14	7	139.38	214.06 Å^2	-0.57	-2.86	YES	NO

NRB= Number of Rotatable Bonds
NHA= Number of Hydrogen Acceptors
NHD= Number of Hydrogen Donors
MR= Molar Refractivity

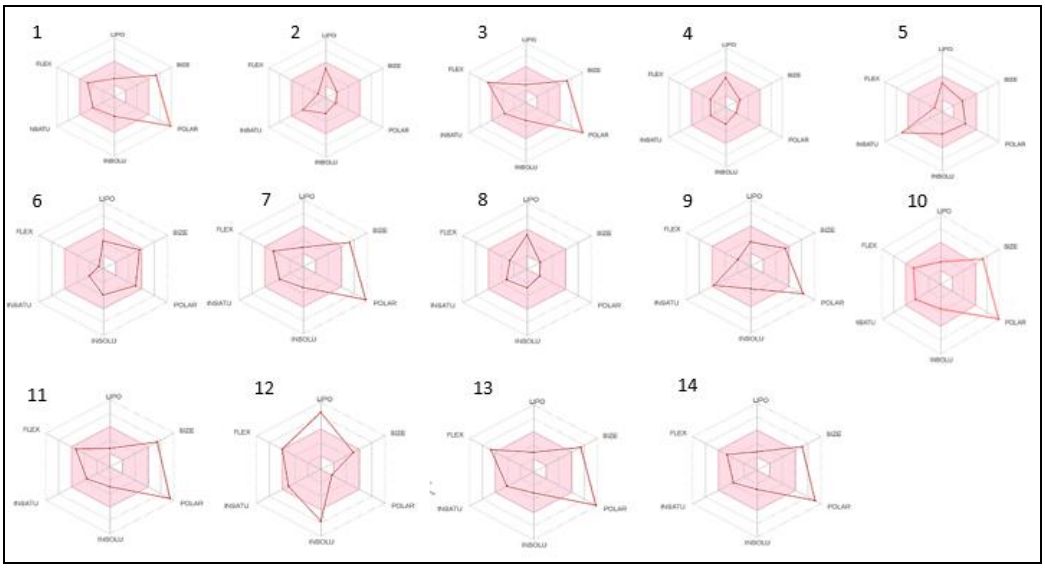

Fig 4: Shows that their drug-likeness fall within parameters of a bioavailable drug

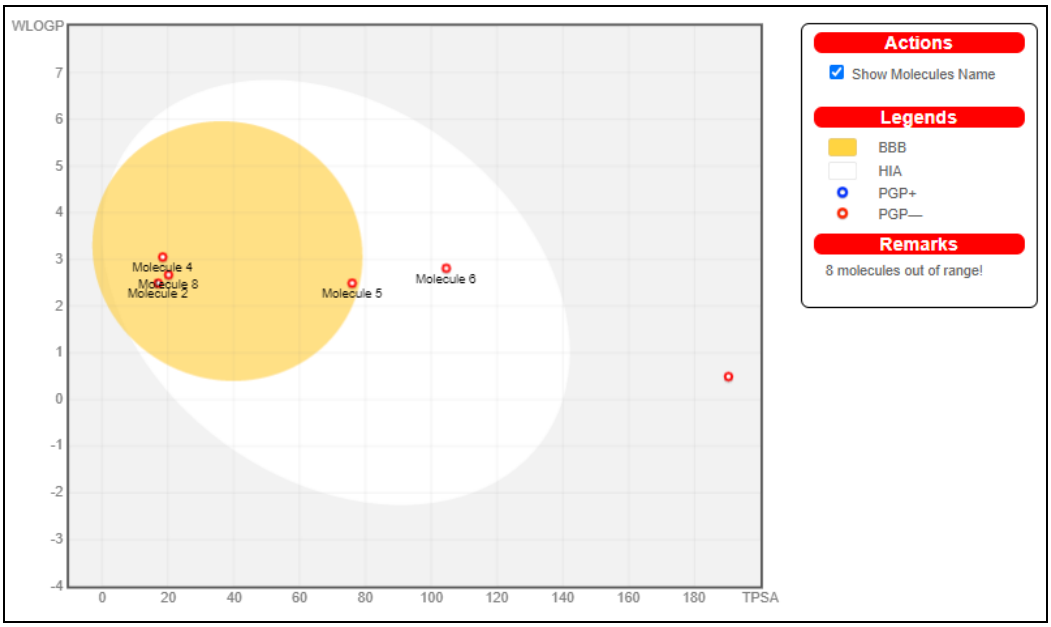

Fig 5: The BOILED-Egg showing the evaluation of passive gastrointestinal absorption (HIA), brain penetration (BBB) and P-glycoprotein activity in the compounds

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