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In silico Evaluation of Guaiane Sesquiterpenes as Multi-Target Inhibitors of Key Proteins in Breast Cancer

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ABSTRACT

Breast cancer is one of the most prevalent cancers among women worldwide, and despite advancements in treatment, the current therapeutic options often come with severe side effects and the risk of drug resistance. This highlights the need for identifying new compounds with better efficacy and safety profiles. In this study, twenty guaiane sesquiterpenes were investigated for their potential as inhibitors of key proteins associated with breast cancer progression, including Progesterone Receptor (PR), Epidermal Growth Factor Receptor (EGFR), p53 Inducible Ribonucleotide Reductase (P53R2), Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4), and Cyclin-Dependent Kinase 8 (CDK8).The compounds were first evaluated for drug-likeness using the SwissADME web server based on Lipinski's Rule of Five, followed by molecular docking to determine their binding affinities with each target protein. The binding energies of the compounds were compared with those of standard breast cancer drugs to assess their inhibitory potentials. Bioavailability and toxicity predictions were also conducted using the SwissADME and ADMETlab 2.0 web servers, respectively. All the compounds met Lipinski's criteria for drug-likeness. Five compounds (4, 8, 13, 16, and 20) demonstrated superior or comparable binding affinities to standard drugs across the five target proteins, suggesting their potential as lead candidates. Further analysis indicated that these compounds have favorable bioavailability profiles; however, one of them (Compound 8) exhibited a potential risk of carcinogenicity. In conclusion, the findings suggest that guaiane sesquiterpenes hold promise as a source of novel lead compounds for the treatment of breast cancer, with a need for further *in vitro* and *in vivo* studies to validate their efficacy and safety. This study contributes to the ongoing search for more effective and safer therapeutic options for breast cancer management.

Keywords: Cancer, guaiane, drug-likeness, docking, bioavailability, toxicity

INTRODUCTION

Cancer arises from uncontrolled cell division and the proliferation of abnormal cells in different body organs (Alzahrani *et al.*, 2021). Cancer is a major contributor to mortality worldwide, and its incidence has continued to rise. Deaths from cancer in 2020 totaled 10 million; this represents 1 out of every 6 reported deaths (WHO, 2022). Breast cancer is a commonly diagnosed cancer in women. According to the World Health Organization, 7.8 million women lived with breast cancer in 2020 (WHO, 2021). Cancer can develop in the ducts and the lobules, and the connective tissues of the breast (Feng *et al.*, 2018). The risk factors related to breast cancer development include alcohol use, presence of benign tumors in the breast, obesity, childbirth after 30 years of age, lack of physical exercise, vitamin D deficiency, and hereditary and reproductive factors (Cohen *et al.*, 2023). The treatment methods for cancer include chemotherapy, surgery, and radiotherapy. However, these methods are associated with high costs and severe side effects, and cancer continues to be associated with high mortality (Storme, 2023; Malik *et al.*, 2022). Furthermore, drug resistance arising from cancer-related mutations has reduced the effectiveness of drugs used for cancer treatment (Malik *et al.*, 2022). These factors have made the development of an affordable, effective, and safe drug for cancer treatment imperative.

Plants are used for treating various diseases in ethnomedicine. Phytochemicals are responsible for the broad range of biological activities exhibited by plants. Previous studies show that plant chemicals can suppress or reverse cancer in early stages, and limit invasion potential of premalignant cells. In addition, they regulate the apoptosis pathways and proliferation of cancerous cells (George *et al.*, 2021). Natural product researchers have put several efforts into isolating chemotherapeutic agents from plants. Significant successes have been achieved in this regard as some anticancer drugs, including Paclitaxel, Epipodophyllotoxin, Vincristine, and Vinblastine, are natural products or their derivatives (Greenwell and Rahman, 2015). However, the traditional approach to drug discovery involving preliminary *in vitro* and *in vivo* studies is costly and time-consuming, lasting approximately 12 years at an estimated cost of 2.7 billion USD (Li *et al.*, 2020). Computational techniques have reduced the cost and time required for drug discovery by enabling the prediction of the anticancer potentials of several molecules using computational tools. The data obtained from computational studies can subsequently improve the success rate of in vitro and in vivo studies (Iwaloye *et al.*, 2023). Molecular docking is used to investigate the behaviour of molecules on binding to target proteins.

The current anticancer drug discovery efforts have resulted in the identification of various proteins which regulate cell growth, motility, differentiation, and apoptosis. These proteins are potential drug targets; inhibiting them may arrest progression of cancer. Examples of such proteins include Epidermal growth factor receptor (EGFR), Progesterone receptor (PR), Ribonucleoside-diphosphatereductase (p53R2), Cytotoxic T-lymphocyte protein 4 (CTLA4) and Cyclin-dependent kinase 8 (CDK8). PR is an estrogen downstream effector and a lifetime risk factor in breast cancer (Trabet*et al.*, 2020; Daniel *et al.*, 2011). EGFR is a target is frequently overexpressed in inflammatory breast cancer and triple-negative breast cancer (Bernstein *et al.*, 2002). CTLA-4 limits anti-tumor immune responses and is an essential mediator of immune evasion (Sobhani*et al.*, 2021). CDK8 is upregulated in breast cancer and is associated with a shorter relapse-free survival of breast cancer patients (Broude*et al.*, 2015; Crown, 2017). In the current study, guaiane-type sesquiterpenes were screened for activity against breast cancer, *in silico*. Guaiane-type sesquiterpenes are reported in 70 genera of 30 plant families. These compounds possess a wide variety of pharmacological properties, including antimicrobial, anti-inflammatory, and antitumor activities. Notably, Mipsagargin (a prodrug of thapsigargin), a Guaiane-type sesquiterpene, has completed phase II clinical trials for treating glioblastomamultiforme and hepatocellular carcinoma (Ma *et al.*, 2019). The structures of the Guaianesesquiterpenes used for the current study are in Plate 1.

METHODOLOGY

Drug-Likeness Prediction

The guaiane sesquiterpenes were analyzed for drug-likeness using the SwissADME web server (https://www.swissadme.ch). This tool assesses compounds based on Lipinski's Rule of Five (ROF), which considers properties such as molecular weight, hydrogen bond donors and acceptors, and lipophilicity (Daina *et al.*, 2017). Only compounds that complied with these criteria were considered likely to possess good oral bioavailability.

Molecular Docking Analysis

Protein Retrieval

The 3D structures of the proteins targeted in this study, known to play crucial roles in breast cancer, were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). The selected proteins include the Progesterone Receptor (PR, PDB ID: 4OAR), Epidermal Growth Factor Receptor (EGFR, PDB ID: 2J6M), p53 Inducible Ribonucleotide Reductase (P53R2, PDB ID: 3HF1), Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4, PDB ID: 1DQT), and Cyclin-Dependent Kinase 8 (CDK8, PDB ID: 6T41).

Ligand Preparation

The chemical structures of the guaiane sesquiterpenes that satisfied the ROF were obtained from the PubChem database. Additionally, the structures of standard breast cancer drugs such as Anastrozole, Cyclophosphamide, Exemestane, Fluorouracil, Lapatinib, Methotrexate, and Tamoxifen were collected from the same database. The structures were downloaded in SDF format and subsequently converted to the mol2 format using Open Babel software (O'Boyle et al., 2011) to prepare them for the docking studies.

Molecular Docking

Molecular docking studies were carried out using the SwissDock server (http://www.swissdock.ch/) to investigate the interactions between the prepared ligands and target proteins (Grosdidier *et al.*, 2011). Both the proteins and ligands were uploaded to the server, which generated potential binding modes for each ligand. The binding mode with the lowest free energy was chosen as the optimal interaction. The UCSF Chimera software was used to visualize and analyze the binding interactions, identifying the specific residues and atoms involved (Wafa and Mohamed, 2020).

Bioavailability and Toxicity Prediction

The bioavailability profiles of the most promising compounds were predicted using the bioavailability radar tool on the SwissADME platform (http://www.swissadme.ch/). This tool evaluates key pharmacokinetic parameters such as flexibility, polarity, lipophilicity, solubility, and size. Toxicity assessments were performed using the ADMETlab 2.0 web server to predict toxicity endpoints like AMES toxicity, carcinogenicity, and oral acute toxicity, based on the chemical structures of the compounds (Xiong et al., 2021).

RESULTS

All the compounds satisfied Lipinski's conditions for drug-likeness. Full details on the various physicochemical parameters evaluated for the compounds are in Table 1. Other parameters evaluated include the gastrointestinal permeability of the compounds, their ability to cross the blood-brain barrier and their tendency to act as substrates for Permeability glycoprotein (Pg-p).

Full details on the binding affinity of each compound with each target protein are provided in Table 2. Compound 8 (-7.26 kcal mol^{-1}) had better binding energy than Fluorouracil (-5.97 kcal mol^{-1}), Cyclophosphamide (-7.15 kcal mol^{-1}), Exemstane (-6.72 kcal mol^{-1}), and Anastrozole (-7.15 kcal mol^{-1}). The docking pose of Compound 8 in 1DQT is shown in Plate 2A. Compound 4 (-7.55 kcal mol^{-1}) had a better binding affinity than Fluorouracil (-6.42 kcal mol^{-1}) and a comparable binding affinity with Exemestane (-7.56 kcal mol^{-1}) against 2J6M. The docking pose of Compound 4 in 2J6M is shown in 2B. Compounds 13 and 16 had the best binding energy (-7.71 kcal mol^{-1}) among the compounds investigated against 3HF1. The docking poses of Compound 13 and 16 in 3HF1 are shown in Plate 2C and 2D, respectively. Compound 20 (-8.73 kcal mol^{-1}) had better binding energy than Fluorouracil (-6.20 kcal mol-1), Anastrozole (-8.41 kcal mol^{-1}), Cyclophospamide (-7.57 kcal mol^{-1}) and comparable binding energy with Exemestane (-8.73 kcal mol^{-1}) against 4OAR. The docking pose of Compound 20 in 4OAR is shown in Plate 2E. Furthermore, Compound 13 (-8.05 kcal mol^{-1}) had better binding energy than Fluorouracil (-6.57 kcal mol^{-1}), Tamoxifen (-7.85 kcal mol^{-1}), Cyclophosphamide (-7.41 kcal mol^{-1}), and Exemestane (-7.13 kcal mol^{-1}). The docking pose of Compound 13 in 6T41 is shown in Plate 2F.

Plate 3 (A-F) shows the bioavailability radar for Compounds 4, 8, 13, 16, and 20. The results show that the compounds would likely possess good bioavailability. Furthermore, all the Compounds tested negative to oral and AMES acute toxicity. All the compounds except Compound 8 are non-carcinogenic (Table 4).

DISCUSSION

In this study, an *in silico* investigation of guaiane sesquiterpenes as potential inhibitors of several proteins associated with breast cancer progression was carried out. Twenty (20) guaiane sesquiterpenes were randomly selected and assessed for drug-likeness. The results obtained in this study show that the compounds satisfied the Lipinski's conditions for drug-likeness (Malkia *et al.*, 2004). Furthermore, the results show the compounds showed good gastrointestinal permeability; eleven (11) are substrates for Permeability glycoprotein (Pg-p); while eight (8) could penetrate the blood-brain barrier. A good drug candidate should have excellent gastrointestinal permeability and should be a poor substrate for Pg-p. Pg-p transports drugs away from the cell membrane and cytoplasm and causes therapeutic failure when drug concentration is low (Geldenhuys*et al.*, 2015). A drug candidate should be able to cross the blood-brain barrier to effect therapeutic action in the brain.

The compounds, having satisfied Lipinski's Rule of Five for drug-likeness, were docked against the ligands to evaluate their binding affinities (Table 2). Molecular docking provides valuable information that are critical for lead optimization: the structure of the protein-ligand complex and the binding energy (Pinzi and Rastelli, 2019). By comparing the results to those of the standard drug, it was observed that the compounds possess good potentials as anticancer drugs. Compounds 4, 8, 13, 16 and 20, had better binding energy against the proteins than many of the standard drugs. The enhanced binding affinities exhibited by these compounds could be attributed to their optimal fit within the protein binding pockets, facilitated by hydrogen bonding and π-π interactions between the ligands and the protein residues. Plate 2 illustrates the binding poses of the ligands in the binding pockets of the proteins, while Table 2 provides detailed information on the hydrogen bond interactions between the ligands and the proteins. Results from previous studies indicate that secondary metabolites from plants exhibit strong binding affinities to several breast cancer-related proteins. For instance, several furanocoumarins exhibited excellent docking energy values against the breast cancer receptors such as the Estrogen receptor (ERα), Progesterone receptor (PR), and Epidermal Growth Factor Receptor (EGFR) (Acharya *et al.*, 2019). Similarly, α-pinene and D-limonene isolated from *Foeniculum vulgare* showed promising binding energies against the Estrogen Receptor α Y537S protein (Kaur *et al.*, 2022). Additionally, the compounds 7-hydroxyflavone, 2-(- 4-fluorophenyl)- 4-chromen-4-one, 3-hydroxyflavone, and 8-methylflavone exhibited comparable binding energy to Tamoxifen when evaluated against the Estrogen receptor (Shingal *et al.*, 2017).

It is essential to evaluate the bioavailability of a drug candidate; otherwise, it may fail to exhibit the desired therapeutic action in the body, though it might have shown significant activity in vitro. The *in silico* prediction of the bioavailability of a drug candidate can be studied using the bioavailability radar plot of the SwissADME tool (Aye *et al*., 2015). The results show that Compounds 4, 8, 13, 16 and 20 would exhibit good bioavailability. Toxicity prediction by the ADMETlab 2.0 web server showed that Compound 8 has the tendency to be carcinogenic. All the other compounds showed negative potential for AMES toxicity, oral acute toxicity, and carcinogenicity.

CONCLUSION

This study explored the potential of selected guaiane sesquiterpenes as therapeutic candidates for breast cancer through *in silico* analysis. The results indicated that all the tested compounds met Lipinski's criteria for drug-likeness, suggesting favorable pharmacokinetic properties. Notably, the compounds displayed significant binding affinities for key proteins implicated in breast cancer progression, including EGFR, PR, P53R2, CTLA4, and CDK8. Among the evaluated compounds, Compounds 4, 13, 16, and 20 emerged as the most promising based on their superior binding energies relative to standard anticancer drugs, which suggests they could effectively inhibit these target proteins.

However, while the in silico findings highlight their potential as lead compounds, further experimental validation is crucial. The observed binding affinities point to possible inhibitory mechanisms, but these interactions and their effects on cancer cell proliferation and progression require comprehensive in vitro and in vivo testing. Such studies would confirm the compounds' efficacy, bioavailability, and safety profiles. Overall, these findings provide a foundation for future research aimed at developing novel breast cancer therapeutics from guaiane sesquiterpenes.

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Table 1: Prediction of Drug-likeness of Compounds

Compound	M.W. (g/mol)	NRB	HBA	HBD	TPSA	iLogP	GI	P-gp	BBB	Drug-likeness (Lipinski)
1	208.38	1	0	0	0.00	3.50	Low	No	No	Yes
2	252.35	1	3	1	46.53	2.83	High	No	Yes	Yes
3	288.38	2	5	5	101.15	0.64	High	Yes	No	Yes
4	272.38	2	4	4	80.92	2.05	High	Yes	No	Yes
5	222.37	0	1	0	9.23	3.22	High	No	Yes	Yes
6	270.36	1	4	2	58.92	2.63	High	No	Yes	Yes
7	272.38	1	4	4	80.92	2.08	High	Yes	No	Yes
8	254.37	1	3	3	60.69	2.42	High	No	Yes	Yes
9.	240.38	1	2	2	40.46	2.84	High	No	Yes	Yes
10.	272.38	2	4	4	80.92	2.30	High	Yes	No	Yes
11.	272.38	2	4	4	80.92	2.51	High	Yes	No	Yes
12.	272.38	2	4	4	80.92	2.30	High	Yes	No	Yes
13.	288.38	2	5	5	101.15	1.95	High	Yes	No	Yes
14.	288.38	2	5	5	101.15	2.00	High	Yes	No	Yes
15.	272.38	1	4	4	80.92	2.11	High	Yes	No	Yes
16.	256.38	1	3	3	60.69	2.63	High	No	Yes	Yes
17.	256.38	1	3	3	60.69	4.05	High	No	Yes	Yes
18.	272.38	2	4	4	80.92	2.39	High	Yes	No	Yes
19.	272.38	2	3	4	80.92	2.39	High	Yes	No	Yes
20.	338.44	4	5	0	61.83	2.99	High	No	Yes	Yes

NRB- Number of rotatable bonds; HBA- Hydrogen Bond Acceptors; HBD – Hydrogen Bond Donors; PSA- Polar Surface Area ; iLogP - Po/w = octanol/water partition coefficient ; GI- Gastrointestinal absorption; Pgp-permeability glycoprotein, ; BBB- Blood-Brain Barrier

Table 2: Binding Energy of Selected GuaianeSesquiterpenesAgainst the Target Proteins

Ligands	1DQT	2J6M	3HF1	4OAR	6T41
1	-6.59	-6.95	-6.96	-7.72	-6.89
2	-6.75	-7.23	-7.18	-7.59	-7.06
3	-6.82	-7.35	-7.56	-7.77	-7.47
4	-6.99	-7.55	-7.49	-8.15	-7.61
5	-6.53	-6.59	-6.95	-7.86	-6.90
6	-6.83	-7.32	-7.31	-7.93	-7.27
7	-6.75	-7.14	-7.45	-7.90	-7.16
8	-7.26	-7.45	-7.52	-7.71	-7.26
9	-6.68	-7.04	-7.25	-7.74	-7.04
10	-6.89	-7.41	-7.70	-8.43	-7.47
11	-6.82	-7.61	-7.54	-8.02	-7.35
12	-6.89	-7.51	-7.67	-8.02	-7.51
13	-6.83	-7.27	-7.71	-7.97	-8.05
14	-7.14	-7.49	-7.55	-7.97	-7.48
15	-6.87	-7.05	-7.70	-8.02	-7.71
16	-6.65	-7.15	-7.71	-7.75	-7.25
17	-6.57	-7.20	-7.50	-7.91	-7.38
18	-6.92	-7.21	-7.53	-7.96	-7.37
19	-6.85	-7.45	-7.36	-7.96	-7.40
20	-6.95	-7.35	-7.66	-8.73	-7.42
Fluorouracil	-5.97	-6.42	-6.24	-6.20	-6.57
Tamoxifen	-7.37	-8.12	-8.05	-9.06	-7.85
Methotrexate	-8.33	-9.59	-9.87	-9.46	-8.31
Lapatinib	-8.10	-9.04	-9.08	-10.12	-8.78
Anastrozole	-7.15	-8.66	-8.27	-8.41	-9.34
Cyclophosphamide	-6.86	-7.97	-7.84	-7.57	-7.41
Exemestane	-6.72	-7.56	-7.56	-8.73	-7.13

2J6M-Epidermal growth factor receptor (EGFR); 4OAR- Progesterone receptor (PR); 3HF1- Ribonucleoside-diphosphatereductase (p53R2); 1DQT - Cytotoxic T-lymphocyte protein 4 (CTLA4); and 6T41 - Cyclin-dependent kinase 8 (CDK8).

Table 3: Hydrogen bonding interactions of Compounds Showing the Best Affinity with the Target Proteins

Ligand	Protein	H-bonding interactions ligand…protein	Bond length
Compd 8	1DQT	H25…OT2 PRO 117	7.959
		O1…HN LEU 89	5.132
Compd 4	2J6M	O1…HN ILE 1018	6.138
		H26…O ASP 770	5.063
		H27…O ARG 831	7.154
		H19…O ILE 1018	6.571
		H19…O PRO 1019	5.297
		O…HN LEU 703	2.568
Compd 13	3HF1	H26…O VAL 43	2.611
		O3…HN VAL 43	2.229
Compd 16	3HF1	H20…O PRO 149	1.949
Compd20	4OAR	O4…HN LEU 763	8.878
		O2…HN THR894	9.224
		O3…HN LEU 721	9.022
Compd 13	6T41	H26…O GLU 229	6.298
		H26…O ILE 230	7.874
		H20…VAL 233	7.330
		H20…O ARG 185	8.047

2J6M-Epidermal growth factor receptor (EGFR); 4OAR- Progesterone receptor (PR); 3HF1- Ribonucleoside-diphosphatereductase (p53R2); 1DQT - Cytotoxic T-lymphocyte protein 4 (CTLA4); and 6T41 - Cyclin-dependent kinase 8 (CDK8).

Table 4: Bioavailability and Toxicity Predictions on GuaianeSesquiterpenes with the Best Binding Energy

Compound	AMES Toxicity	Oral Acute Toxicity	Carcinogenicity
4	Negative	Negative	Negative
8	Negative	Negative	Positive
13	Negative	Negative	Negative
16	Negative	Negative	Negative
20	Negative	Negative	Negative

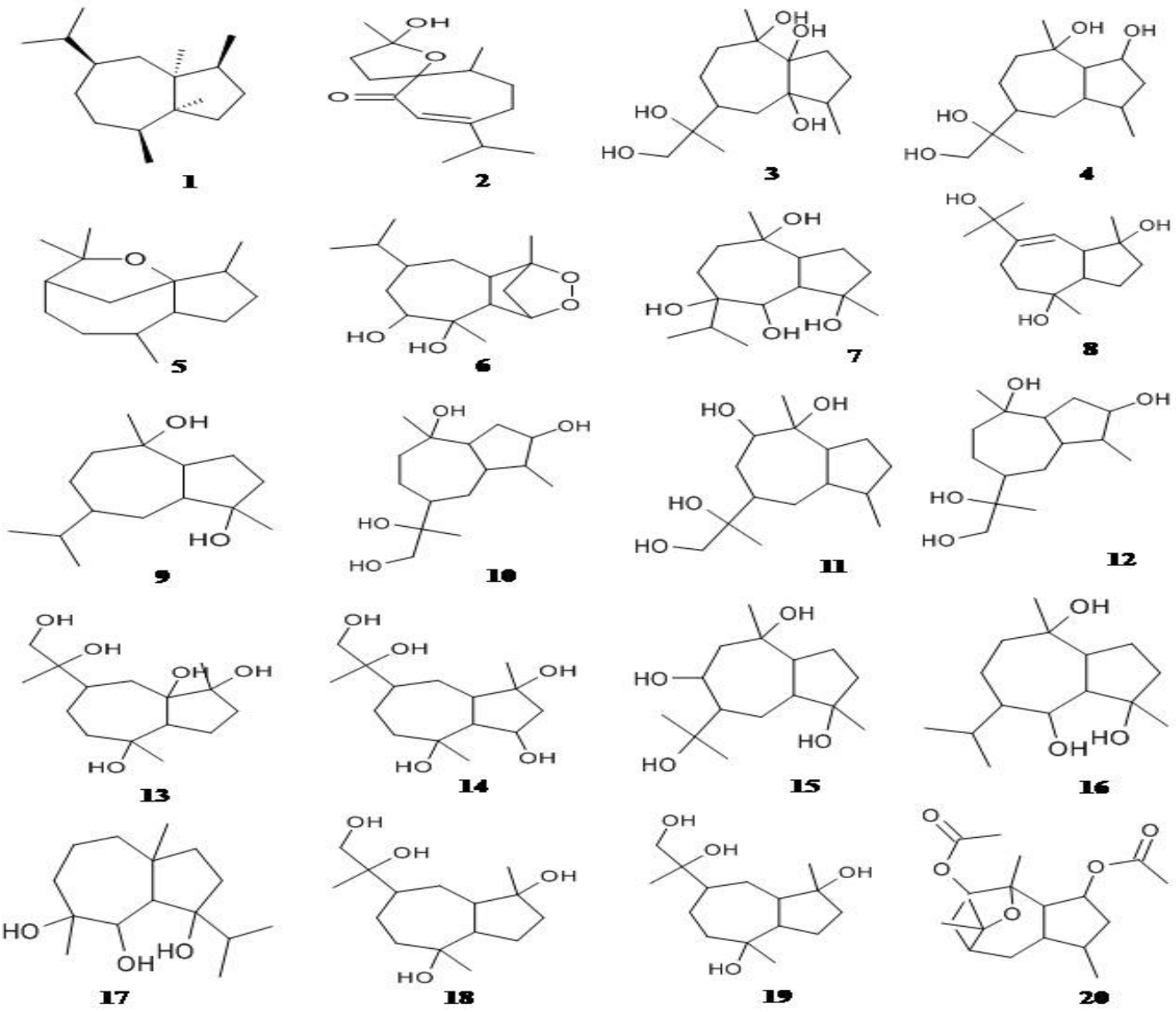

Plate1: GuaianeSesquiterpenes under investigation:Guaiane (**1**); 4,5-seco-Guaiane (**2**);(1R,4S,5R,7R,10R)-Guaiane-1,5,10,11,12-Pentaol (**3**); (1S,2S,4S,5S,7R,10R)-Guaiane-2,10,11,12-Tetraol (**4**); Guaiane Oxide (**5**); (1*R*,2*S*,3*R*,4*R*,6*S*,8*S*,9*S*)-3,9-dimethyl-6-propan-2-yl-10,11-dioxatricyclo[7.2.1.02,8]dodecane-3,4-diol (**6**); Alismorientol A (**7**); Alismorientol B (**8**); Guaiane-4,10-Diol (**9**); (1R,3R,4R,5S,7R,10R,11R)-guaiane-3,10,11,12-tetraol (**10**) (1R,4S,5S,7S,9R,10S,11R)-guaiane-9,10,11,12-tetraol (**11**); (1R,3S,4R,5S,7R,10R,11S)-guaiane-3,10,11,12-tetraol (**12**); (1S,4R,5S,7R,10R)-guaiane-4,5,10,11,12-pentaol (**13**); (1S,2S,4R,5R,7R,10R)-Guaiane-2,4,10,11,12-Pentaol (**14**); Saniculamoid C (**15**); Teuclatriol (**16**); Homalomenol E (**17**); Xylaguaianol A (**18**); Xylaguaianol B (**19**); Kessoglycoldiacetate (**20**)

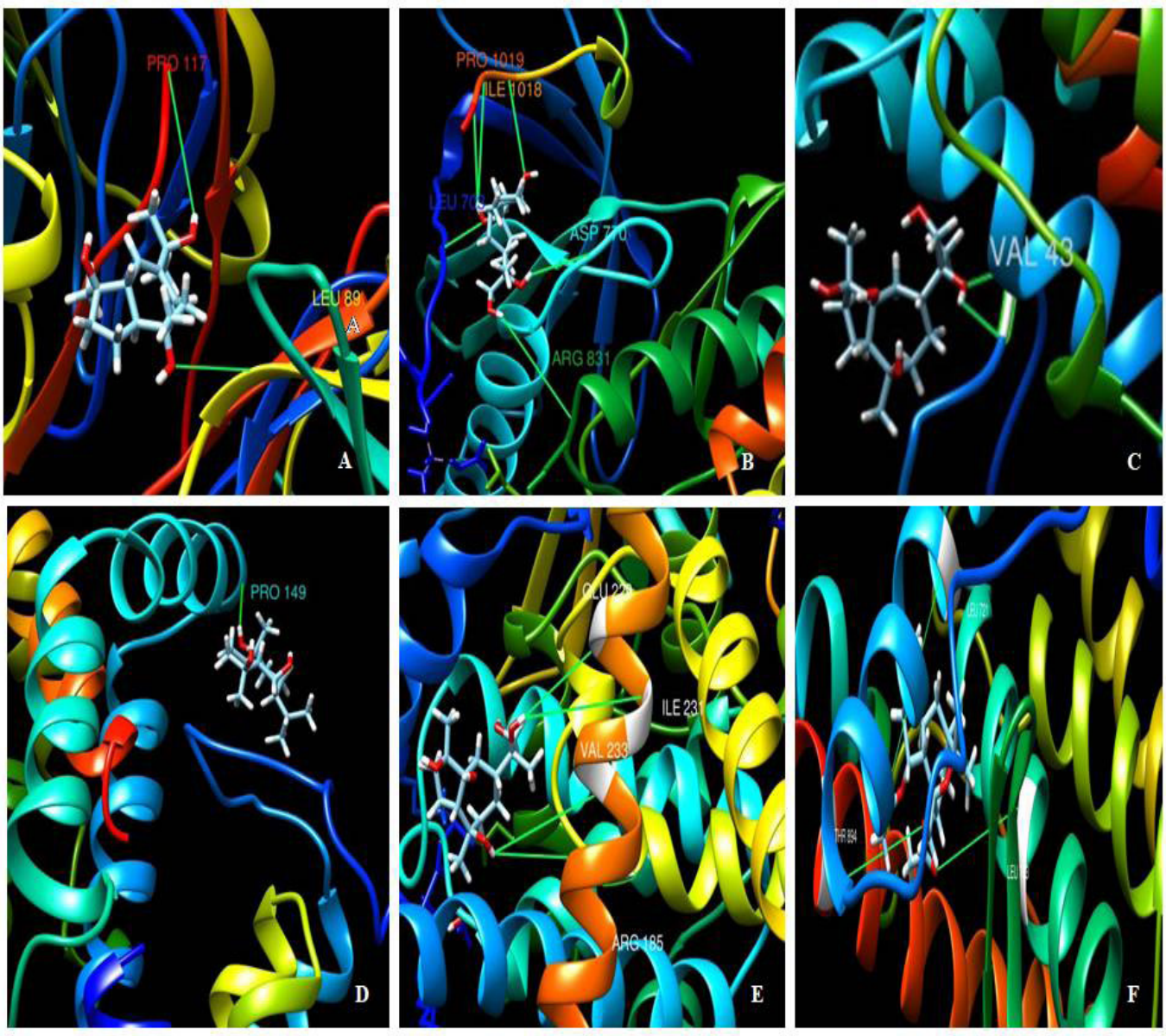

Plate 2: Docking Poses of Compound 8 in 1DQT (**A**);Compound 4 in 4J6M **(B)**; Compound 13 in 3HF1 (**C**); Compound 16 in 3HF1 (**D**); Compound 20 in 4OAR (**E**); and Compound 13 in 6t41 (**F**)

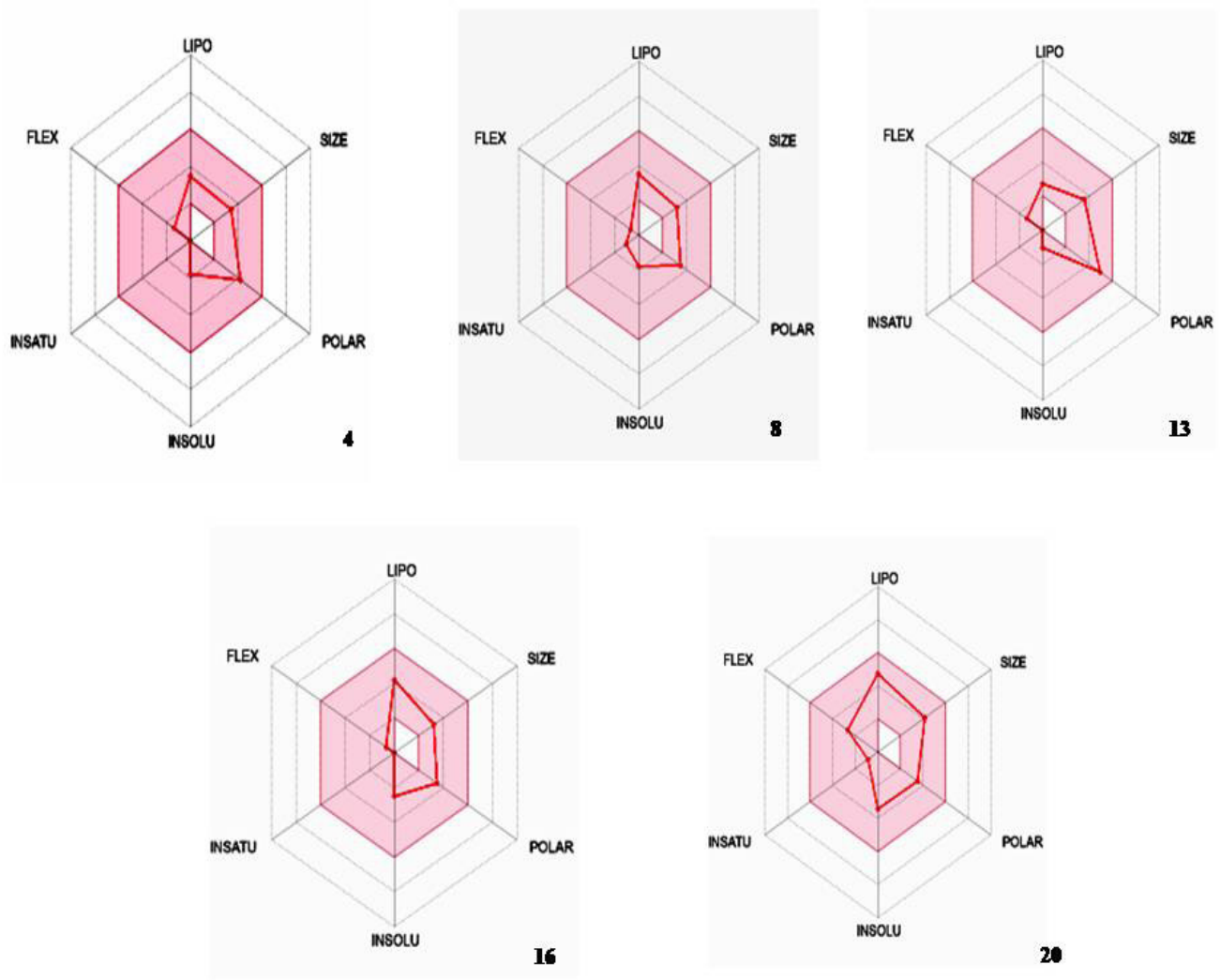

Plate 3: Bioavailability Radar for Compounds 1-20