Supplementary materials

Molecular Docking, MMGBSA, and ADMET Studies of Phytoconstituents of *Ocimum gratissimum* on Multiple Breast Cancer Targets

Basiru Olaitan Ajiboye[a]*, Toluwase Hezekiah Fatoki[b], Precious Ayorinde Akinnusi[b], Olawale Rasaq Ajuwon[c], Babatunji Emmanuel Oyinloye[d,e], Temitope Olawale Jeje[f], Olutunmise Victoria Owolabi[g], Oluwatosin O Ogedengbe[h] and Claudia Genovese[i]*

[a]*Phytomedicine and Molecular Toxicology Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Ekiti State, Nigeria;* [b]*Bioinformatics and Enzymology Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Ekiti State, Nigeria;* [c]*Redox Biology Research Unit, Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti, Nigeria;* [d]*Phytomedicine, Biochemical Toxicology and Biotechnology Research Laboratories, Department of Biochemistry, College of Sciences, Afe Babalola University, Ado-Ekiti, Nigeria;* [e]*Biotechnology and Structural Biology (BSB) Group, Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, South Africa;* [f]*Biochemical Immunology and Phytomedicine Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti, Ekiti State, Nigeria;* [g]*Medical Biochemistry Unit, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria;* [h]*Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Oye-Ekiti, Ekiti State, Nigeria;* [i]*Institute for Agriculture and Forestry Systems in the Mediterranean, Catania, Italy*

*Corresponding author: bash1428@yahoo.co.uk and claudia.genovese@cnr.it

Abstract

O. gratissimum is one of the most common medicinal plants in every community in Nigeria. This plant has been presumed to be useful in the management of diseases including breast cancer, which is one the commonest cancers affecting women globally. Hence, this study aimed to computationally investigate the phytochemicals present in O. gratissimum by elucidate their binding dynamics against five selected molecular targets of breast cancer and predict their pharmacokinetics properties. Molecular docking, MMGBSA calculation and ADMET prediction were used. The results showed that isovitexin has the highest binding affinity of -9.11 kcal/mol and -9.80 kcal/mol for Human Epidermal Growth Factor Receptor 2 (HER2) and Epidermal Growth Factor Receptor (EGFR) respectively. Rosmarinic acid has the highest binding affinity of -12.15 kcal/mol for Phosphatidylinositol 3-kinase (PI3K), Nepetoidin A has the highest binding affinity of -9.14 kcal/mol for estrogen receptor (ER), and Vitexin has the highest binding affinity of -12.90 kcal/mol for Progesterone receptor (PR). MMGBSA provided total binding energy that confirmed the stability of the complexes under physiological conditions. The ADMET profiles showed that O. gratissimum top phytochemicals identified would be safe for oral administration with no hepatoxicity. Overall, this study identified isovitexin, vitexin, rosmarinic acid, nepetoidin A and luteolin among others, as compounds that exhibit strong anti-cancer properties against breast cancer cells.

Keywords: *Ocimum gratissimum*; breast cancer; EGFR; HER2; PI3K; binding affinity; pharmacokinetics

Experimental

Ligands and Protein targets

The phytoconstituents of Ocimum gratissimum were obtained from literature (Prabhu et al. 2009; Nassazi et al. 2020; Nganteng et al. 2022; Hasan et al. 2023) and mined from PubChem in 2-dimensional SDF formats. Concurrently, the 3D crystallographic structures of Epidermal Growth Factor Receptor (PDB ID: 3POZ), Estrogen receptor (PDB ID: 3ERT), Human Epidermal Growth Factor Receptor 2 (PDB ID: 3PP0), Phosphatidylinositol 3-kinase (PDB ID: 4JPS), and Progesterone receptor (PDB ID: 4OAR) were obtained from the protein data bank, as described by Ajiboye et al. (2023).

Molecular Docking analyses

The 3D structures of all ligands were prepared using Schrödinger Maestro software. The ligands were prepared under physiological pH using Epik and the LigPrep module of Schrodinger's suite. The ligands were transformed to their 3D structures, and the OPLS3 force field was used to ionize and produce tautomeric states (Harder et al. 2016). The proteins were prepared using the Protein preparation wizard on maestro (Sastry 2013; Pattar et al. 2020). The Glide script on Maestro was employed to carry out the molecular docking procedure. Initially, the Standard Precision (SP) algorithm was employed to screen the compounds based on their favorable interaction with the ligand-binding sites of the protein targets. Subsequently, the top-scoring poses of the receptor-ligand complexes were rescored using the Extra Precision (XP) algorithm. Also, post-docking minimization was carried out to include only the best poses in the results generated Ajiboye et al. (2023).

MM-GBSA binding energy calculation

The mechanics and generalized born surface area (MM-GBSA) was implemented using Maestro's Prime functional tool, to calculate the binding free energy (ΔG bind) and forecast the spontaneity of the interactions (Hayes & Archontis 2012)

$\Delta G(bind) = \Delta G(solv) + \Delta E(MM) + \Delta G(SA)$

where: ΔG(solv) is the difference in GBSA solvation energy of the protein-ligand complex and the sum of the solvation energies for unliganded protein and ligand. ΔE(MM) is a difference in the minimized energies between protein-ligand complex and the sum of the energies of the free protein and ligand. ΔG(SA) is a difference in surface area energies of the complex and the sum of the surface area energies for the free protein and ligand (Pattar et al. 2020; Hart et al. 2022).

In silico ADMET prediction

The *in silico* ADMET (absorption, distribution, metabolism, excretion and toxicity) screening of the top compounds was done on pkCSM server, http://biosig.unimelb.edu.au/pkcsm/ (Pires et al. 2015).

References

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Table S1. *Ocimum gratissimum* top 8 phytochemicals with good binding affinity with breast cancer targets.

SN	Lead compounds	HER2 (PDB ID: 3PP0)		PI3K (PDB ID: 4JPS)		ER (PDB ID: 3ERT)		EFGR (PDB ID: 3POZ)		PR (PDB ID: 4OAR)	
		Binding affinity (kcal/mol)	MM/GBSA ΔGbind (kcal/mol)	Binding affinity (kcal/mol)	MM/GBSA ΔGbind (kcal/mol)	Binding affinity (kcal/mol)	MM/GBSA ΔGbind (kcal/mol)	Binding affinity (kcal/mol)	MM/GBSA ΔGbind (kcal/mol)	Binding affinity (kcal/mol)	MM/GBSA ΔGbind (kcal/mol)
1.	Isovitexin	**-9.11**	**-42.38**	-	-	-7.73	-35.50	**-9.80**	**-43.81**	**-11.55**	**-47.74**
2.	Luteolin	**-9.08**	**-45.35**	-9.69	-46.19	**-8.55**	**-42.04**	-9.42	-53.34	-7.79	-50.14
3.	Nepetoidin A	-8.81	-49.74	-9.00	-38.24	**-9.14**	**-48.30**	-8.29	-58.00	-8.27	-50.60
4.	Salvigenin	-8.71	-55.26	-9.17	-45.59	-	-	-8.82	-49.84	-	-
5.	Xanthomicrol	-8.37	-53.86	-8.94	-45.82	-	-	-	-	-8.48	-48.43
6	Apigenin	-7.91	-43.15	-	-	-	-	-8.74	-50.51	-7.76	-50.00
7	Nevadensin	-7.86	-61.30	-9.17	-42.41	-	-	-	-	-	-
8	Hymenoxin	-6.87	-46.99	-9.17	-46.41	-	-	-	-	-9.20	-55.11
9	Rosmarinic acid	-	-	**-12.15**	**-50.63**	-	-	-8.75	-42.13	-10.50	-46.32
10	Vitexin	-	-	**-11.45**	**-46.67**	-	-	**-9.55**	**-56.37**	**-12.90**	**-55.13**
11	Germacrene D	-	-	-	-	-8.42	-43.17	-	-	-	-
12	Alpha-humelene	-	-	-	-	-8.35	-46.06	-	-	-	-
13	Beta-caryophyllene	-	-	-	-	-8.23	-47.11	-	-	-	-
14	Alpha-copaene	-	-	-	-	-7.94	-36.59	-	-	-	-
15	Cubenene	-	-	-	-	-7.78	-43.03	-	-	-	-
16	Apigenin 7,4 - dimethyl ether	-	-	-	-	-	-	-8.72	-55.43	-	-

Table S2. The ADMET profile of *Ocimum gratissimum* phytochemicals with top binding affinities.

Type	Properties	Phytochemicals															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
	Molecular weight (g/mol)	204.35	204.35	298.29	270.24	204.35	100.12	204.35	374.3	432.4	286.24	314.29	344.3	360.3	328.3	432.4	344.3
Absorption	Water solubility (log mol/L)	-1.544	-0.122	-2.892	-3.329	-0.471	-0.851	-0.471	-3.681	-2.812	-3.094	-3.17	-3.505	-3.059	-3.897	-2.845	-3.377
	Caco-2 permeability (log Papp in 10 cm/s)	1.406	1.342	0.642	1.007	1.366	1.382	1.366	1.431	-0.618	0.096	-0.327	1.412	-0.937	1.087	-0.956	0.515
	Intestinal absorption (human) (% Absorbed)	100	100	87.216	93.25	100	100	100	87.248	64.729	81.13	76.909	94.581	32.516	95.869	46.695	96.278
	Skin Permeability (log Kp)	-2.422	-2.221	-2.735	-2.735	-2.24	-2.297	-2.24	-2.747	-2.735	-2.735	-2.736	-2.757	-2.735	-2.712	-2.735	-2.739
	P-glycoprotein substrate	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No
	P-glycoprotein II inhibitor	No	No	No	No	No	No	No	Yes	No	No	No	Yes	No	Yes	No	Yes
Distribution	VDss (human) (log L/kg)	-0.435	0.088	0.011	0.822	-0.106	-0.238	-0.106	-0.132	1.239	1.153	0.37	-0.155	0.393	-0.162	1.071	0.11
	Fraction unbound (human)	0.572	0.57	0.381	0.147	0.572	0.573	0.572	0.092	0.21	0.168	2.44	0.101	0.348	0.14	0.242	0.014
	BBB permeability (log BB)	0.187	0.264	-0.027	-0.734	0.235	0.216	0.235	-1.074	-1.375	-0.907	-1.031	-0.845	-1.378	-0.695	-1.449	-0.575
	CNS permeability (log PS)	-2.83	-2.83	-1.35	-2.061	-2.83	-2.83	-2.83	-3.176	-3.754	-2.251	-3.096	-3.088	-3.347	-2.309	-3.834	-3.295
Metabolism	CYP2D6 substrate	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No
	CYP3A4 substrate	No	No	No	No	No	No	No	Yes	No	No	Yes	Yes	No	Yes	No	Yes
	CYP1A2 inhibitor	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes
	CYP2C19 inhibitor	No	No	No	Yes	No	No	No	Yes	No	No	Yes	Yes	No	Yes	No	Yes
	CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	Yes
	CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No	No	No	No	Yes	No	No	No	Yes	No	Yes	No	Yes
Excretion	Total Clearance (log ml/min/kg)	0.094	0.834	-28.613	0.566	0.649	0.55	0.649	0.621	0.442	0.495	0.205	0.608	0.25	0.703	0.444	0.657
	Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	Yes	No	Yes	No	No
Toxicity	AMES toxicity	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No
	Max. tolerated dose (human) (log mg/kg/day)	0.731	0.915	0.438	0.328	0.855	0.808	0.855	0.435	0.649	0.499	-0.146	0.379	0.152	0.202	0.577	0.031

	hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	hERG II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.482	2.482	2.482	2.45	2.482	2.482	2.482	2.264	2.558	2.455	2.824	2.226	2.811	2.229	2.595	2.341
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	0.034	4.462	12.287	2.298	0.456	0.247	0.456	1.89	5.37	2.409	2.894	1.841	2.907	1.307	4.635	1.515
	Hepatotoxicity	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Skin Sensitisation	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	T. Pyriformis toxicity (log ug/L)	1.215	-0.248	0.285	0.38	0.573	0.977	0.573	0.308	0.285	0.326	0.548	0.352	0.302	0.481	0.285	0.337
	Minnow toxicity (log mM)	4.806	4.806	7.25	2.432	4.806	4.806	4.806	2.233	5.18	3.169	1.4	1.691	2.698	0.856	4.897	1.647

Legend: 1. alpha-copaene (PubChem CID: 19725). 2. Alpha-humulene (CID: 5281520). 3. Apigenin 7,4-dimethyl ether (CID: 5281601). 4. Apigenin (CID: 5280443). 5. Beta-caryophyllene (CID: 5281515). 6. Cubenene (CID: 57357909). 7. Germacrene D (CID: 5317570). 8. Hymenoxin (CID: 171488). 9. Isovitexin (CID: 162350). 10. Luteolin (CID: 5280445). 11. Nepetoidin A (CID: 5316820). 12. Nevadensin (CID: 160921). 13. Rosmarinic acid (CID: 5281792). 14. Salvigenin (CID: 161271). 15. Vitexin (CID: 5280441). 16. Xanthomicrol (CID: 73207). Based on pkCSM ADMET predictive model (Pires et al., 2015), a compound is said to have high Caco-2 permeability at a value of >0.90; poor GIA at less than 30% absorption; low skin permeability (logKp > -2.5); VDss is low at <0.71 L/kg (log VDss < -0.15) and high at >2.81 L/kg (log VDss > 0.45); BBB permeant at a logBB > 0.33 and non-permeant at logBB < -1; CNS permeant at a logPS > -2 and non permenat at a logPS < -3; *Tetrahymena pyriformis* toxicity (pIGC50) at a value > - 0.5 log µg/L is considered toxic; minnow toxicity (LC50) at a value <0.5 mM (logLC50 < -0.3) is regarded as high acute toxicity; maximum recommended tolerated doses (MRTD) of ≤ 0.477 log(mg/kg/day) is considered low, and high if > 0.477 log(mg/kg/day).

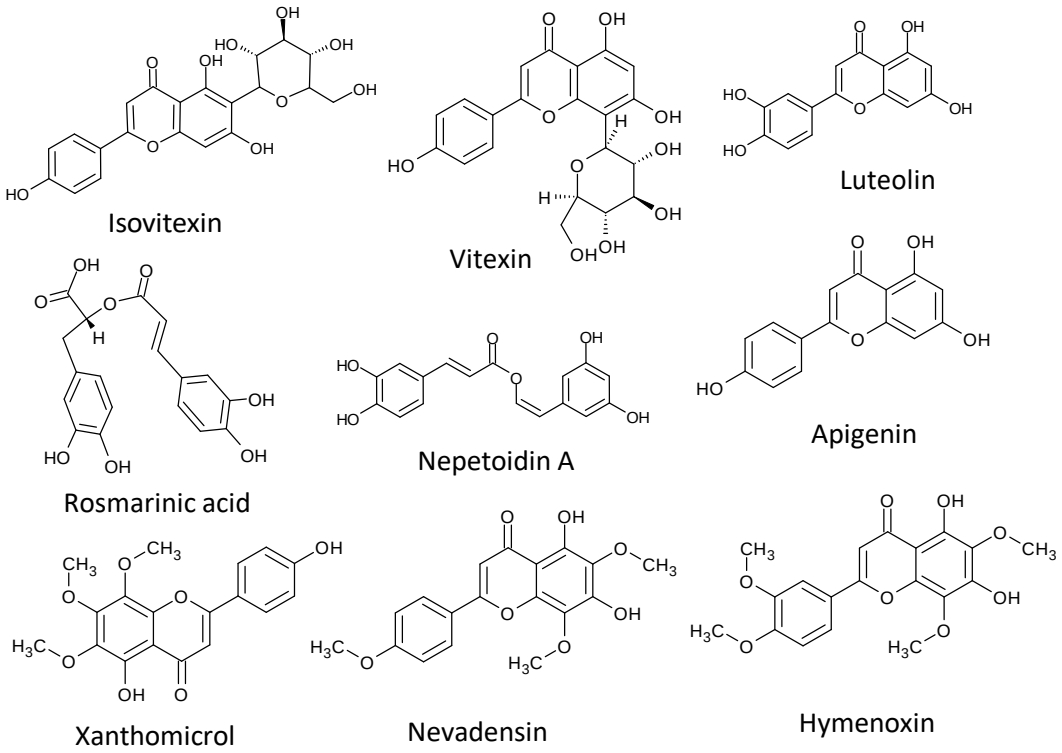
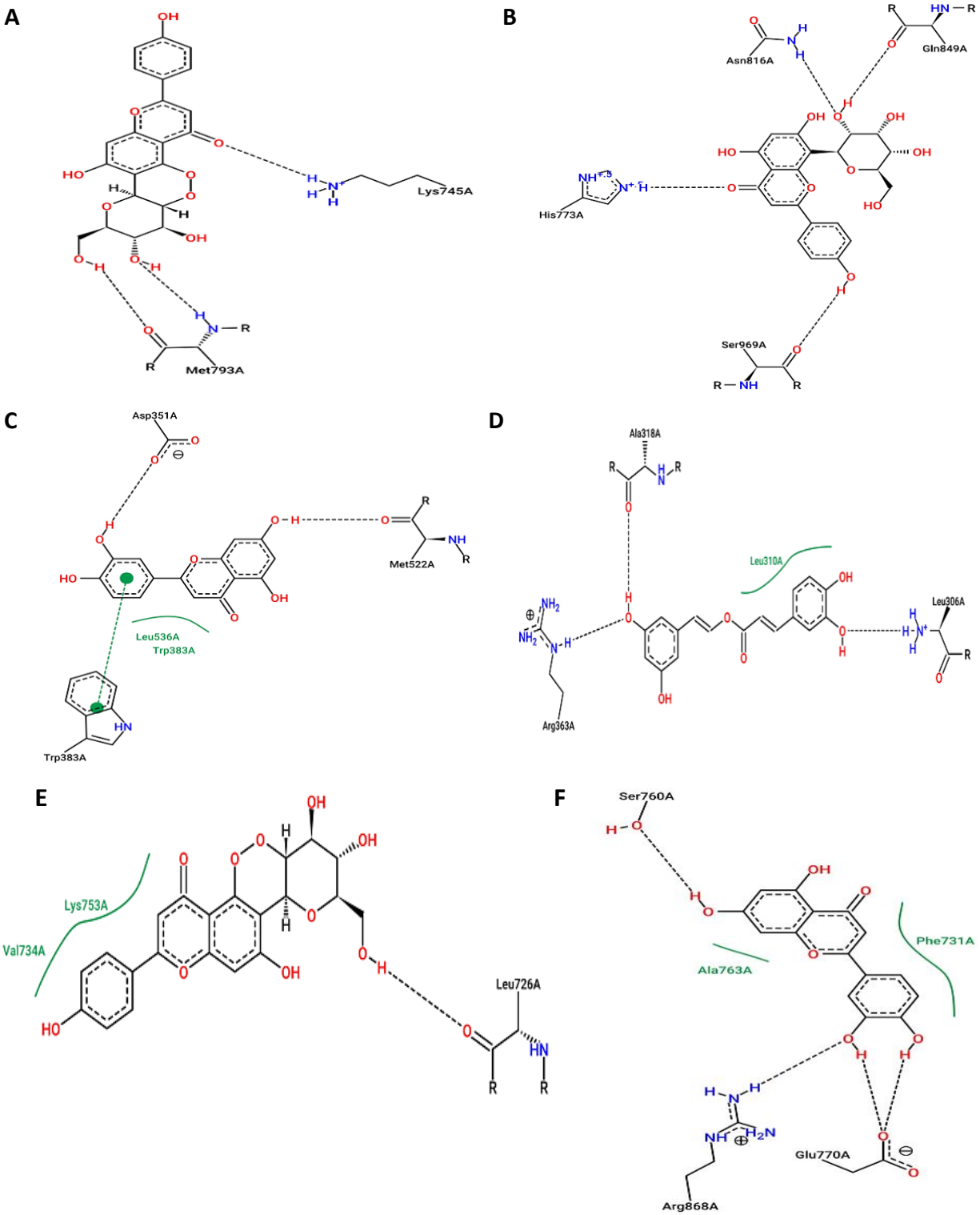


Figure S1. Chemical structures of some phytochemicals in *Ocimum gratissimum*.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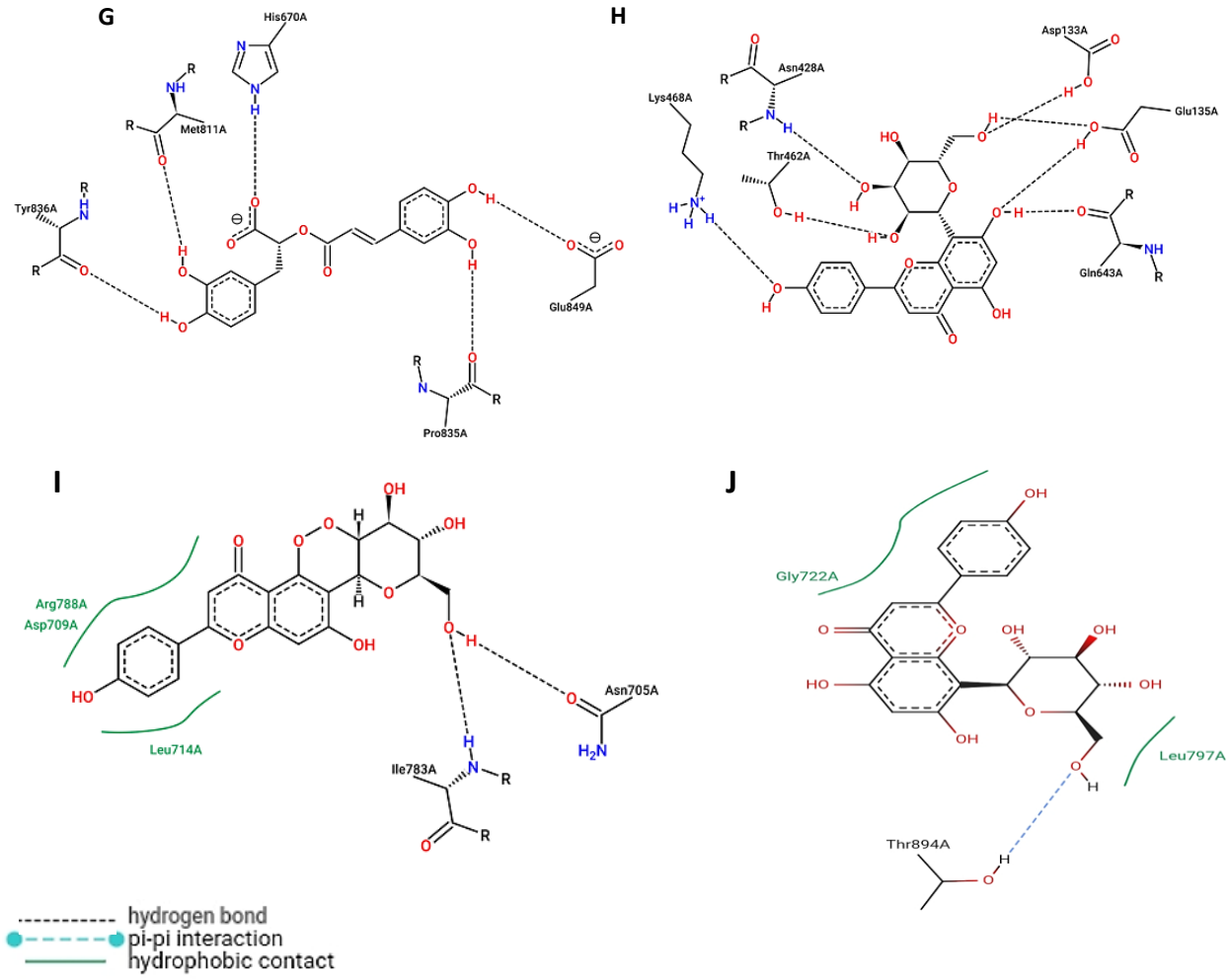

Figure S2. Binding pose of interaction of: (A) Isovitexin and EFGR (3POZ). (B) Vitexin and EFGR (3POZ). (C) Luteolin and ER (3ERT). (D) Nepetoidin A and ER (3ERT). (E) Isovitexin and HER2 (3PP0). (F) Luteolin and HER2 (3PP0). (G) Rosmarinic acid and PI3K (4JPS). (H) Vitexin and PI3K (4JPS). (I) Isovitexin and PR (4OAR). (J) Vitexin and PR (4OAR).