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Original Article

MOLECULAR DOCKING STUDIES ON FLAVONOID COMPOUNDS: AN INSIGHT INTO AROMATASE INHIBITORS

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ABSTRACT

Objective: Aromatase is a key enzyme that plays a crucial role in the synthesis of estrogen and has a major effect in pathogenesis of estrogen-dependent disease, including breast cancer, endometrial cancer, and endometriosis**.** The abnormal over expression of aromatase can be inhibited by aromatase inhibitors. The objective of the present study is to evaluate the binding interaction of flavonoid compounds with cytochrome P450 enzyme aromatase, which is involved in the metabolism of estrogens and considered as a powerful target for treatment of estrogen-dependent breast cancers.

Methods: To understand the mechanisms involved in the binding of flavonoid compounds and their interactions with the binding site of aromatase, molecular docking studies were carried using *Autodock* 4.2.

Results: The docking results revealed that, benzoflavanones showed higher binding affinity compared to other class of compounds. The presence of hydrogen bond interaction and cation–π interaction contributed to their higher binding affinity. The flavonoid compounds with unsubstituted or less substituted rings showed higher binding affinity than those with substituted rings. The hydrogen bonding interactions were predominant in all the classes of compounds considered for the study and were found to be important for inhibition. The docking studies showed that the binding energies mainly depend on aromatic properties like cation–π and π–π interactions. These properties play a key role in determining the biological activity of flavonoid compounds.

Conclusion: The present findings provided valuable information on the binding process of flavonoid compounds to the binding site of aromatase and revealed the structural requirement needed for binding.

Keywords: Breast cancer, Estrogen, Aromatase, Aromatase inhibitors, Flavonoid compounds, Docking studies.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and leading cause of death in females, worldwide. Many breast cancers are sensitive to the hormone estrogen, which causes breast cancer tumor to grow. The cancer of this type is called as HER2 positive breast cancer. HER2 refers to a gene that helps cells to grow, divide, and repair themselves. Mutation or damage in this gene or in the cell signalling pathway, leads to cancer [1]. Since 2008, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%. It is also estimated that breast cancer is the most prevailing cancer worldwide [2]. Other than radiotherapy, chemotherapy many other surgical approaches are used for the treatment [3]. Estrogen plays a crucial role by binding to estrogen receptors, in promoting the proliferation of both the normal and neoplastic breast epithelium by inducing intracellular signalling cascades [4,5].

Aromatase is the enzyme involved in estrogen biosynthesis, which can be inhibited for limiting the growth and development of estrogen dependent tumors. Human aromatase is the product of the CYP19A1 gene. This enzyme is located in the endoplasmic reticulum of the cell and its activity is regulated by tissue specific promoters that are in turn controlled by hormones, cytokines, and other factors. Inhibition of aromatase is an important approach for reducing growth-stimulatory effects of estrogens in estrogen-dependent breast cancer. Two primary approaches have been developed to reduce the growth stimulatory effects of estrogens in breast cancer like interfering with the ability of estrogen to bind to its receptor, and decreasing circulating levels of estrogen. Anti-estrogens (Tamoxifen) compete for binding to the estrogen receptors and reduce the number of receptors available for binding to endogenous estrogen [6]. Both steroidal and non-steroidal aromatase inhibitors have also shown clinical efficacy in the treatment of breast cancer. The potent and selective third- generation aromatase inhibitors, like anastrozole, letrozole, and exemestane were used in postmenopausal patients failing anti-estrogen therapy alone or multiple hormonal therapies. Use of an aromatase inhibitor as initial therapy or after treatment with tamoxifen is now recommended as adjuvant hormonal therapy for a postmenopausal woman with hormone-dependent breast cancer [7].

As like anti-estrogen, steroidal and non-steroidal aromatase inhibitors, flavonoids also play a major role in aromatase inhibition. Flavonoids are a group of phytochemicals produced by various plants in high qualities. Based on their skeleton, flavonoids classified to flavans, flavones, flavanones, isoflavones, isoflavanones, anthocyanidines, chalcones, flavanoligans and flavonols, each possessing the benzopyranone ring system as the common chemical scaffold. Flavonoids exert anti-inflammatory, analgesic, anti-allergic effects, apoptotic, estrogenic and anti-estrogenic properties. Flavonoids interacts with CYP450 enzymes in atleast three way: by inducing biosynthesis of several CYPs, by modulating the enzymatic biosynthesis of CYPs (inhibited or stimulated) and the last is the metabolism of flavonoids by several CYPs in turn. They poses both induction of CYP mediated carcinogenesis as well as inhibition of the same by scavenging free radicals formed by CYP catalysed reactions. As aromatase inhibitors, flavonoids has structure resembling the estrogen skeletons, they bind to the estrogen receptor in turn modulates its activity [8]. Flavones have been demonstrated to be competitive inhibitors of aromatase with respect to the androgen substrate, with K_i values at low micromolar concentrations. The binding characteristics and the structural requirement necessary for the inhibition of human aromatase by flavone and isoflavone phytoestrogens were obtained using computer modelling and confirmed by site–directed mutagenesis. It was found that these compounds bind to the active site of aromatase in an orientation in which their A-ring and C-ring mimic rings D and C of the androgen substrate, respectively [9]. Initial attempts to study the aromatase and its inhibitors interaction have been relied on homology derived

models. At present there are crystal structures available for human placental aromatase. Thus the availability of the structural details of aromatase active sites help to understand the binding nature of aromatase inhibitors and also to evaluate key residues needed for binding. Molecular docking is one of the foremost approaches to understand the binding interactions that govern the enzyme inhibition [3]. In this study, docking calculations were performed to elucidate the binding modes of all flavonoid compounds with aromatase. The crystal structure of the human placental aromatase (**PDB ID: 3EQM**) solved by Gosh *et al*, 2009 [10] was considered for docking studies. The tertiary structure consists of 12 major helices and 10 strands distributed into one major and 3 minor sheet, and follow the characteristic CYP450 fold.

A striking feature of the tertiary aromatase structure is that long loops interconnect well–defined secondary structure elements, was observed with other P450 structures. The active site of aromatase is the distal cavity of the heme–binding pocket and the Heme Fe is the reaction centre of the enzyme. The amino acid Asp309 is proposed to be a catalytically important residue. The residue Asp309 appears to be involved not only in catalysis but also in substrate binding. Several flavonoid compounds were synthesized and tested for aromatase inhibitor [11-16]. These structural features play an important role in determining the binding mode of several drugs including aromatase inhibitors [17].

MATERIALS AND METHODS

Preparation of the protein and Ligand Structures

The three dimensional X–ray structure of aromatase complexed with androstenedione (ASD), was obtained from Brookhaven Protein Data Bank (**PDB ID: 3EQM**). The crystal structure was refined by removing water molecules and the cofactor, phosphate ion. The heme was retained in the binding site. Hydrogen atoms were added and electronic charges were assigned to the protein atoms using kollman united atoms force field by using AutoDockTools–1.5.6 [18].

To understand the binding nature of the ASD, the ASD was removed and redocked into the binding site of aromatase. The docking calculations were carried out using both the Genetic Algorithm and Lamarckian Genetic Algorithm.

The docked conformations obtained using LGA could able to reproduce the binding modes reported in the crystal structure solved by Ghosh *et al*. In this paper, the Lamarckian Genetic Algorithm (LGA) was used to explore the binding conformational landscape of flavonoid compounds. The flavonoid compounds considered for the present study are given in the **Scheme 1** [11-16]. The compounds were geometrically optimized using AM1 Hamiltonian in MOPAC software package [19]. The gasteiger partial atomic charges were added and all possible flexible torsion angles of the ligand was defined by using *AUTOTORS*. The structures were saved in a PDBQT format for *AutoDock* calculations.

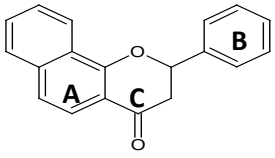
Docking Studies

Docking calculations were performed to predict the binding affinity between the aromatase and flavonoid compounds using *AutoDock* 4.2 [20]. *AutoDock* requires pre–calculated *grid maps*, one for each atom type present in the ligand being docked. These maps were calculated by using the auxiliary program *AutoGrid*. The compounds treated as flexible molecules were allowed to move in the six spatial degrees of freedom for orientation and torsional degrees of freedom within the grid box. Lennard–Jones parameters 12–10 and 12–6, implemented with the program, were used for modeling H–bonds and van der Waals interactions, respectively.

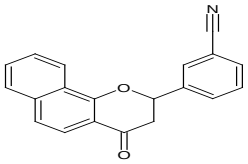
AutoDock uses a semi empirical free energy force field to evaluate conformations during docking simulations. The energy functions define what contributes to the energy of the molecule, like bond stretching, bending etc., so that energy of the molecule can be calculated for any particular conformation. The force field evaluates binding in two steps. In the first step, the intramolecular energetics is estimated for the transition from unbound states to the conformation of the ligand and protein in the bound state. The second step then evaluates the intermolecular energetics of combining the ligand and protein in their bound conformation.

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$$

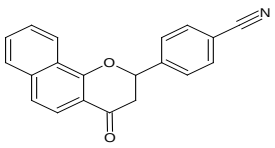
First three terms are for van der Waal's, hydrogen bonding, electrostatics respectively. The term ΔG_{tor} is for rotation and translation and ΔG_{desolv} is for desolvation upon binding and the hydrophobic effect [20].



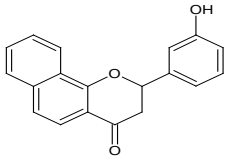
BF1



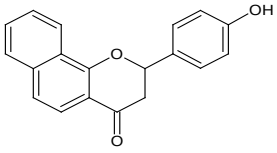
BF2



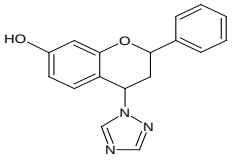
BF3



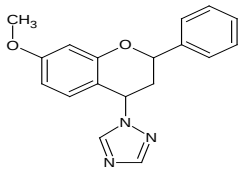
BF4



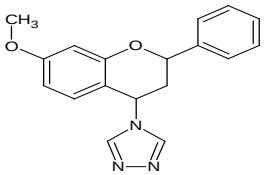
BF5



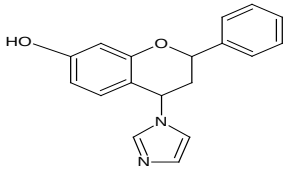
TF1



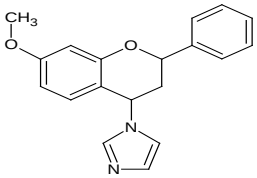
TF2



TF3



IF1



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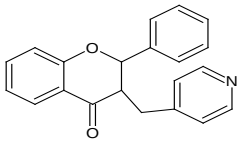
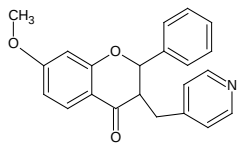
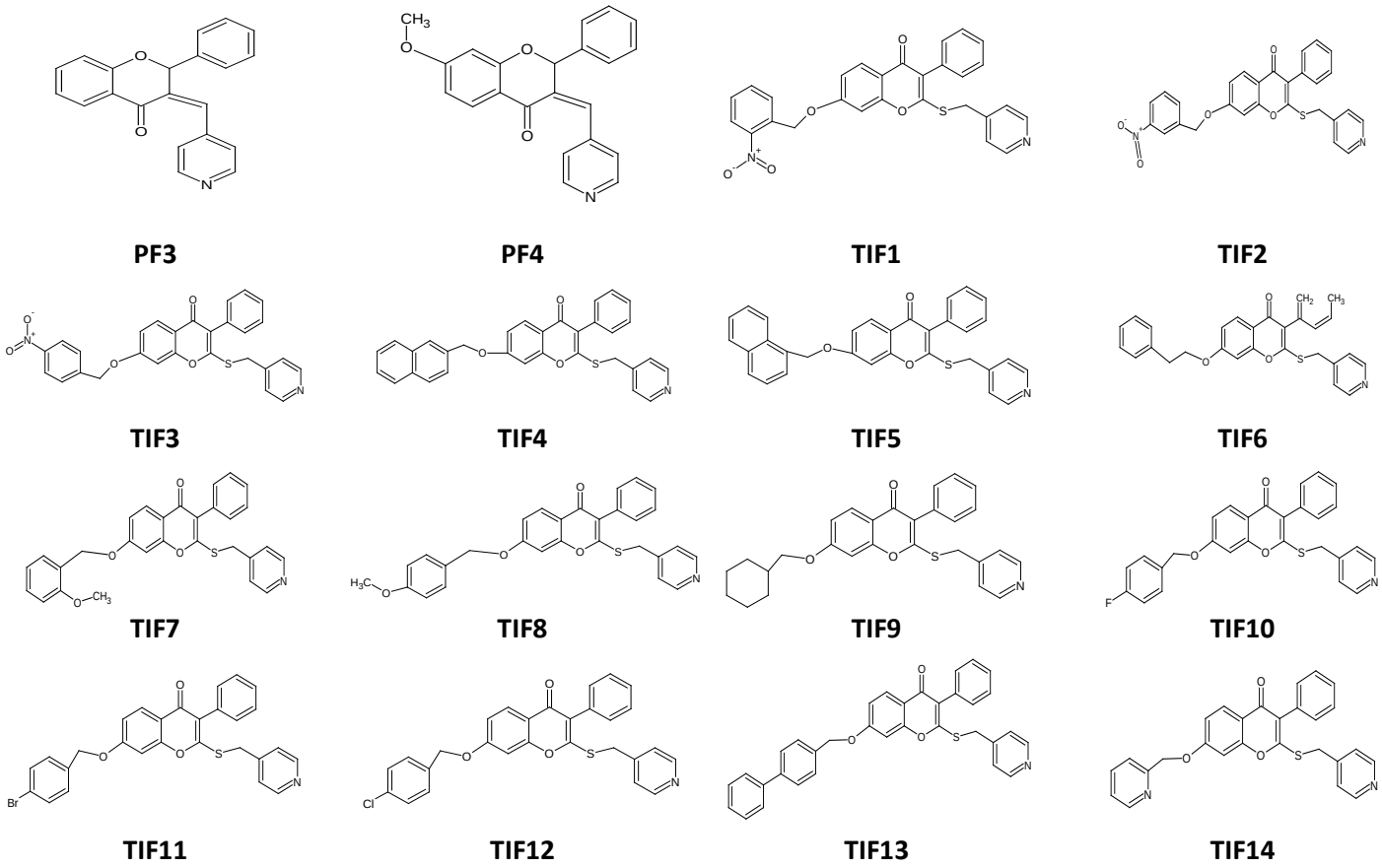


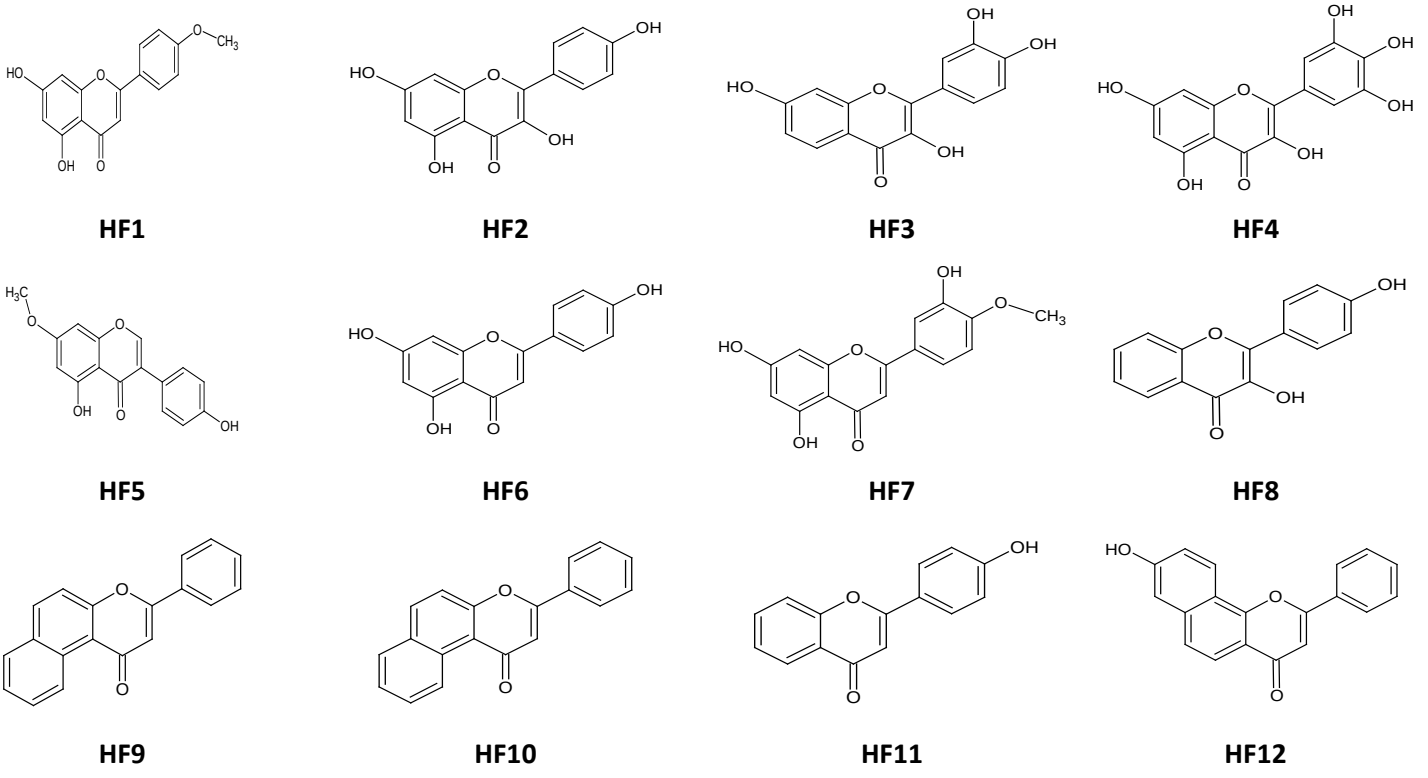
PF1

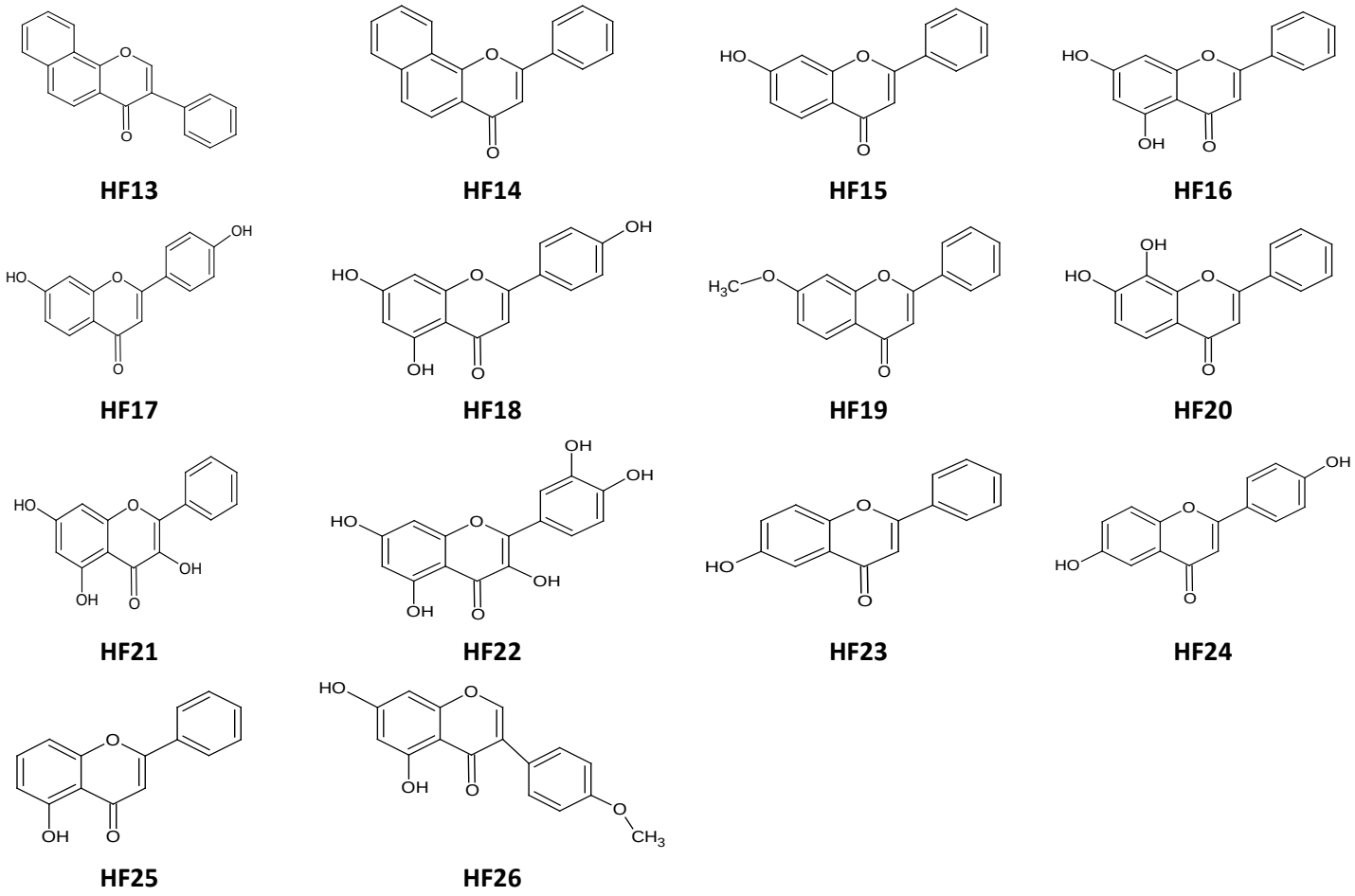


PF2



Scheme 1: The analogs of flavonoid compounds are shown.





Scheme 1 (continued): The analogs of flavonoid compounds are shown.

RESULTS AND DISCUSSIONS

The binding energy and their corresponding intermolecular energy, electrostatic energy, internal energy and torsional energy for each class of flavonoids with aromatase are given in **Table 1.** All the energies are reported in kcal/mol. **Figure 1** shows the lowest binding energy conformation of each flavonoid class. Their interacting residues in the binding site of aromatase are represented in elemental coloured ball and stick model. The maroon colour dashed lines between the atoms represent the hydrogen bonds.

Table 1: Molecular docking energies of flavonoids with aromatase are shown.

Compounds	ΔG_{BE}	$\Delta G_{intermol}$	ΔG_{elect}	$\Delta G_{internal}$	ΔG_{tor}
BF1	−7.24	−7.54	−0.05	−0.32	0.30
BF2	−7.56	−7.86	−0.19	−0.34	0.30
BF3	−7.16	−7.46	−0.02	−0.33	0.30
BF4	−7.40	−7.99	−0.16	−0.26	0.60
BF5	−6.93	−7.52	−0.24	−0.27	0.60
TF1	−5.99	−6.89	−0.16	−0.63	0.89
TF2	−6.53	−7.43	−0.31	−0.42	0.89
TF3	−6.64	−7.54	−0.17	−0.60	0.89
IF1	−5.97	−6.86	−0.23	−0.22	0.89
IF2	−6.13	−7.02	−0.21	−0.46	0.89
PF1	−7.02	−7.62	−0.14	−0.72	0.60
PF2	−5.45	−6.34	0.02	−0.82	0.89
PF3	−6.97	−7.57	−0.07	−0.61	0.60
PF4	−7.04	−7.94	−0.12	−0.72	0.89
HF1	−5.63	−6.82	−0.21	−0.25	1.19
HF2	−5.88	−7.37	−0.24	−1.20	1.49
HF3	−6.13	−7.62	−0.28	−1.21	1.49
HF4	−5.70	−7.78	−0.78	−1.42	2.09
HF5	−5.30	−6.50	−0.50	−0.31	1.19
HF6	−6.75	−7.94	−0.51	−0.80	1.19
HF7	−6.48	−7.98	−0.78	−0.94	1.49
HF8	−5.96	−6.86	−0.24	−0.66	0.89

Docking analysis of Benzoflavanones

The benzoflavanones (**BF**) [11] binding energy were in the range ~ –7.0 kcal/mol. They possessed lowest binding energy compared to all other class of compounds considered for study. The common binding site residues interacting with **BF** were Met374, Ser478, Ala306, Asp309, Thr310 and Arg115. Interestingly **BF1**, **BF3** and **BF5** showed cation–π interactions between Arg115 and the benzo ring of the flavanone.

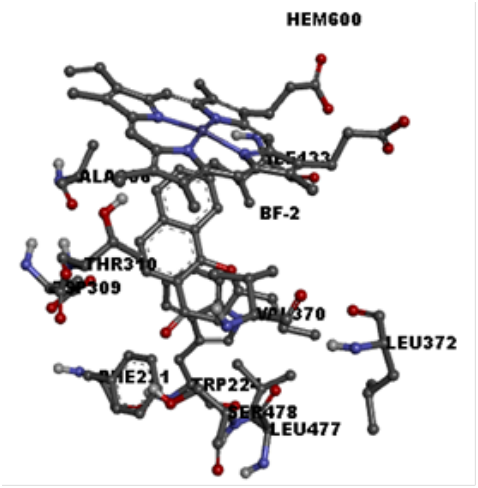
Even though the above compounds exhibited cation–π interactions, comparing to **BF3** and **BF5** the binding energy was low for **BF1** compound, which may be due to the unsubstituted benzo group in **BF1**. In **BF2** and **BF4** there are hydroxy (–OH) and cyano (–CN) groups present at the *meta* (C_3') positions of the B-ring.

Presence of electron-withdrawing groups like cyano and electron-donating groups such as the hydroxyl on B-ring, increased the inhibitory effect can be seen in **BF2** and **BF4**. Among all the five compounds only the **BF4** hydroxyl group which is in the *meta* position in B-ring showed hydrogen bond with Met374 (Met374 NH···OH). In benzoflavanones, ΔG_{elect}, $\Delta G_{internal}$ interactions contributed very less to the binding energy and the significant contribution to the binding energy was due to $\Delta G_{intermol}$ energy, which is the sum of vdW, hydrogen bonding and desolvation, and torsional energies.

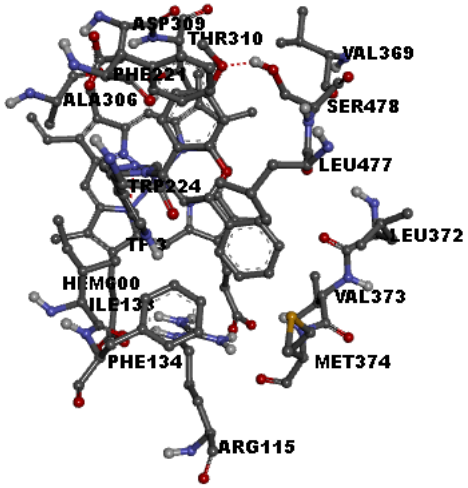
The torsional energy was almost the same for **BF1**–**3** which was ~0.3 kcal/mol and **BF4**–**5** showed torsional energy of ~0.6 kcal/mol. Torsion angles within a molecule are defined by four atoms connected by three bonds. **BF1**–**3** had one rotatable bond and **BF4**–**5** had two rotatable bonds, therefore, higher torsional energy than **BF1**–**3.**

Docking analysis of Triazolylflavans

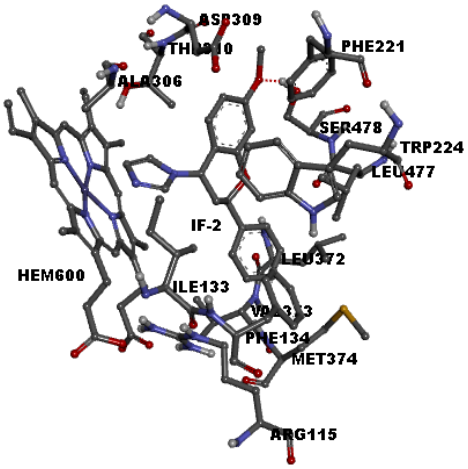
Binding energy of Triazolylflavan (**TF**) [12] was in the range ~–6.0 kcal/mol and among them the lowest binding energy was shown by **TF3.** The common residues in the binding site interacting with **TF** were Ser478, Thr310, Asp309, Trp224, Leu477, Met374 and Ile133. The oxygen of oxymethyl groups in **TF2** and **TF3** showed hydrogen bond with the hydrogen ion of hydroxyl group in Ser478 (Ser478-OH···OCH_3) residue at a distance of 1.745 Å and 1.778 Å respectively. Though **TF2** showed a stronger hydrogen bond than **TF3**, its binding affinity was less than **TF3**. **TF1** exhibited a π–π interaction between the C-ring of flavan and imidazo group of tryptophan (Trp224) residue but its binding energy was very high compared to **TF2** and **TF3**.



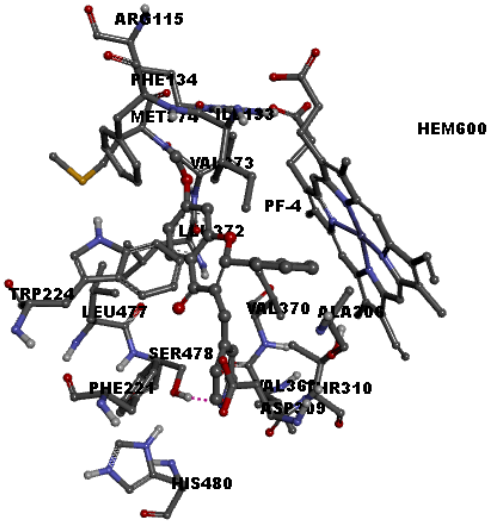
BF2



TF3



IF2



PF4

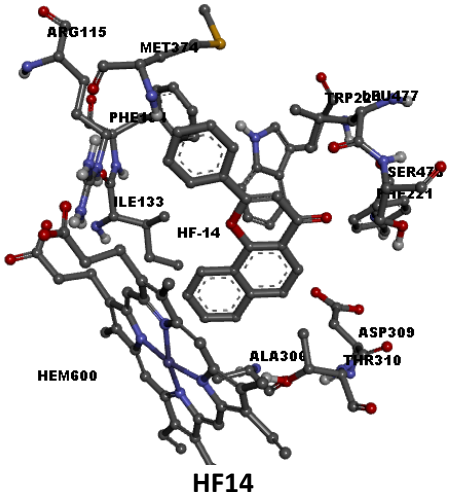


Fig. 1: Flavonoid compounds having lowest binding energy with aromatase are shown.

Table 1 (Continued): Molecular docking energies of flavonoids with aromatase are shown.

Compounds	ΔG_{BE}	$\Delta G_{intermol}$	ΔG_{elect}	$\Delta G_{internal}$	ΔG_{tor}
HF9	−5.85	−7.35	−0.46	−0.85	1.49
HF10	−6.77	−7.07	−0.18	−0.25	0.30
HF11	−7.03	−7.63	−0.10	−0.26	0.60
HF12	−7.02	−7.62	−0.25	−0.27	0.60
HF13	−6.80	−7.10	−0.07	−0.27	0.30
HF14	−7.15	−7.45	−0.05	−0.30	0.30
HF15	−6.55	−7.15	−0.18	−0.21	0.60
HF16	−6.53	−7.43	−0.12	−0.81	0.89
HF17	−6.43	−7.33	−0.31	−0.18	0.89
HF18	−6.47	−7.66	−0.48	−0.78	1.19
HF19	−6.32	−6.92	−0.09	−0.30	0.60
HF20	−6.21	−7.10	−0.31	−0.81	0.89
HF21	−6.06	−7.26	−0.16	−1.29	1.19
HF22	−6.16	−7.95	−0.90	−1.64	1.79
HF23	−6.30	−6.90	−0.13	−0.21	0.60
HF24	−6.17	−7.06	−0.36	−0.17	0.89
HF25	−6.47	−7.07	−0.22	−0.82	0.60
HF26	−5.27	−6.47	−0.37	−0.88	1.19

As in benzoflavonones, the contribution of ΔG_{elect}, $\Delta G_{internal}$ interactions were very less to binding energy in the triazolylflavans, and energy contribution was due to the $\Delta G_{intermol}$ energy. The torsional energy for **TF1–3** was same of ~0.9 kcal/mol as they had three rotatable bonds.

Docking analysis of imidazolylflavans

The imidazolylflavans (**IF**) [13] showed binding energy in the range ~−6.0 kcal/mol. The lowest energy was exhibited by **IF2** which has a 7-oxymethyl substitution. The most common residues in the binding site that interact with **IF** were Ser478, Thr310, Ala306, Asp309, Phe221, Leu477, Met374 and Ile133. In IF2, the oxygen atom of the 7–oxymethyl group showed hydrogen bond with hydroxyl hydrogen of Ser478 (Ser478 OH···OCH_3) at a distance of 1.725Å whereas the hydroxyl group of **IF1** showed no hydrogen bond with the nearby residues.

This could be one of the reasons why **IF1** had higher binding energy or lower binding affinity than **IF2**. In imidazolyl flavans, ΔG_{elect}, $\Delta G_{internal}$ interactions contributed less to the binding energy and the significant contribution to the binding energy was due to $\Delta G_{intermol}$ energy. The compounds **IF1** and **IF2** had three rotatable bonds and a same torsional energy of ~0.9 kcal/mol.

Docking analysis of pyridinyl-substituted flavanones

The Pyridinyl-substituted flavanones (**PF**) [14] showed binding energies between −7.0 and −6.0 kcal/mol and of which the lowest energy was shown by **PF4**. The binding site residues that commonly interacted with **PF** were Ser478, Thr310, Ala306, Trp224, Leu477, Met374 and Ile133. The hydrogen bond with the hydroxyl group of Ser478 (Ser478 OH···N) were with the nitrogen of the pyridinyl ring in **PF3** and **PF4** at a distance of 2.097Å and 2.024Å respectively.

The oxygen of 7-oxymethyl group of **PF4** also made hydrogen bond with the amino hydrogen of Met374 (Met374 HN···OCH_3) at a distance of 1.974Å. This could be one of the reasons for the lowest binding energy or highest binding affinity of **PF4** compared to **PF1–3** as the 7-methoxy group of **PF4** acts as hydrogen bond acceptor and interacts well with amino acid residues of aromatase.

The benzo group of all the compounds from **PF1–4** showed cation–π interactions with the Fe of heme, and pyridinyl ring of **PF2** showed cation–π interaction with Arg115. From the docking results obtained for **PF1–4**, Z-isomer of oxymethyl pyridinyl substituted flavanones and E-isomer of unsubstituted pyridinyl flavanones showed lower binding energy or higher binding affinity as the restrained configuration of E–isomer could bring the pyridine nitrogen closer to heme Fe for a possible interaction.

In pyridinyl-substituted flavanones, the contribution of ΔG_{elect}, $\Delta G_{internal}$ interactions was less to the binding energy and the significant contribution to the binding energy was due to $\Delta G_{intermol}$ energy. **PF1** and **PF3** had two rotatable bonds and the same torsional energy of ~0.5 kcal/mol. **PF2** and **PF4** had three rotatable bonds with a torsional energy of ~0.9kcal/mol.

Docking analysis of hydroxy flavones

Hydroxyflavones (**HF**) [15] showed binding energies between –7.0 and –5.0 kcal/mol and the lowest binding energy was shown by **HF14**. The residues of binding site interacting with the HF were Ser478, Thr310, Ala306, Phe221, Leu372, Arg115, Trp224, Met374 and Ile133. **HF14**, which has the lowest binding energy among all the hydroxyflavones, showed a cation–π interaction between the B-ring and Arg115. **HF14** was the only unsubstituted flavone in that class and studies have shown that unsubstituted flavones showed higher aromatase inhibiting activity as they can correctly fit in the binding site. Out of the 26 hydroxyflavones taken for the study, 20 of them showed cation–π interactions with Fe of heme. **HF11** and **HF12** followed **HF14** in having low binding energies, where, **HF11** showed a cation–π interaction between B-ring and Fe of heme and Arg115, and hydroxyl oxygen of its B–ring showed a hydrogen bond with amine hydrogen of Met374 (Met374 NH···OH) at a distance of 1.797Å. **HF12** also showed cation–π interaction with Arg115 and a hydrogen bond between hydroxyl oxygen of 7,8-benzo ring and amine hydrogen of Met374 (Met374 NH··OH) at a distance of 1.634Å. In addition, studies have shown that presence of hydroxyl group at the C_7 position increases inhibitory potency as they serve as hydrogen bond acceptor to interact with the nearby residues, as seen in **HF11** and **HF12**.

As mentioned in the literature isoflavones showed less binding affinity and less inhibition of aromatase than flavanones and flavones. **HF12** formed a stronger hydrogen bond than the rest of the hydroxyflavones and therefore, is lower binding energy. **HF26** is an isoflavone and showed the highest binding energy –5.27 kcal/mol. It also showed a hydrogen bond between C_7 hydroxyl hydrogen and hydroxyl oxygen of Thr310 (Thr310 HO··HO) at a distance of 2.102Å, which is a weak interaction, compared to that seen in **HF12** and **HF14**. Hydroxyl substituted derivatives showed lower binding energies than methoxy derivatives as the hydroxyl groups showed the ability to form hydrogen bonds. In hydroxyflavones, ΔG_{elect}, $\Delta G_{internal}$ interactions contributed less to the binding energy and the significant contribution to the binding energy was due to $\Delta G_{intermol}$ energy. The torsional energy ranges from 0.30–2.09 kcal/mol. **HF4** had eight rotatable bonds, therefore, showed the highest torsional energy 2.09 kcal/mol.

Docking analysis of pyridylmethyl thio isoflavones

The binding energies obtained for **TIF** [16] ranged between –6.0 and 2.0 kcal/mol. The lowest energy was exhibited by **TIF1**. These compounds were found to bind near a binding site other than the experimentally identified binding site. Therefore, a search was conducted in the Q-site finder [21] to identify the possible binding sites in the aromatase. Q-site finder showed ten binding sites in aromatase and the thio compounds were found to bind near site 4, which is located away from the experimentally identified binding site. The common residues that interact with thio compounds were Tyr424, Phe427, Phe430, Lys 448, Met444 and Val 445. Though these compounds were bound at a different site, they displayed significant hydrogen bond and cation–π interactions and high torsional energies. The torsional energies obtained were 2.09 and 2.39 kcal/mol. The compound with the lowest binding energy (**TIF1**) showed there hydrogen bond interactions, two π–π interaction between and one cation–π interactions. A hydrogen bond was formed between amine hydrogen of Gln428 and nitrogen of the pyridine ring of **TIF1** (Gln428 NH···N) at a distance of 1.962Å. The other hydrogen bonds were formed between hydroxyl hydrogen of Tyr361 and carbonyl oxygen of C-ring of flavone (Tyr361 OH···OC) at a distance of 2.233Å and amine hydrogen of Lys448 and oxygen of the nitrobenzene ring of **TIF1** (Lys448 NH_2···O_2N) at a distance of 1.809Å. This could be one of the reasons why **TIF1** showed higher activity than the other compounds of the same class.

CONCLUSION

In the present study, the binding and interactions of flavonoid compounds (flavans, flavanones, isoflavones and flavones) with aromatase have been studied using molecular docking calculations. Most of the compounds have shown significant binding interactions with the aromatase. It was observed that the benzo ring is an important but not the only structural requirement to evoke aromatase inhibition. The hydrogen bond interactions and cation–π interactions also contributed to the strong binding of these compounds to the binding site of aromatase. An increase in binding energy was observed when there is substitution at C_7 and C_8 position of A-ring of the flavanone. Compounds with unsubstituted or less substituted rings showed higher activity than substituted rings.

This is possibly because the binding cavity was not large enough to accommodate bulky substituted compounds without steric clashes. Hydrogen bonding interactions were predominant in all the classes of compounds taken for study and were found to be important for inhibition. Among the compounds considered for the study, **BF4, TF2, TF3, IF2, PF3, PF4, HF1–9, HF11–12** and **HF15–26** showed hydrogen bonding with some of the residues in the binding site of aromatase. **BF1, BF3, BF5, PF1–4, HF1–5, HF7, HF8, HF10–17, HF19** and **HF21–26** showed cation–π interaction. Amino acids **Thr310, Met374** and **Ser478** are the common residues that interact with the flavonoid compounds **BF, TF, PF, IF** and **HF**. The amino acid **Asp309** which is involved in ASD substrate binding also plays a key role in binding of flavonoid compounds **BF, TF** and **IF**. Interestingly the thio isoflavones, which had an extended hydrocarbon chain showed decreased activity and those compounds, were found to bind at a different binding site in the enzyme rather than the experimentally identified binding site. The decrease in activity compared to the other class of compounds may be due to unfavourable interactions with the binding site residues. This study provides a detailed insight into binding of the flavonoid compounds, to the aromatase.

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