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# Design, molecular docking and biological evaluation of (*E*)-3-[4-(Difluoromethoxy)-3-hydroxyphenyl]-1-phenylprop-2-en-1-ones

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**ABSTRACT**

*A series of eleven(E)-3-[4-(Difluoromethoxy)-3-hydroxyphenyl]-1-phenylprop-2-en-1-oneswere designed and the molecular docking was carried out for this fluorochalcones with 1SA0 protein. The in silico docking studies reveal that all the compounds are bounded well with receptor via. hydrogen bond, halogen, polar, pi-pi, metal-ligand and other interactions. The chalcones were tested against bacterial strains viz. Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.*

**Keywords:** Fluorochalcones, molecular docking, 1SA0 protein, hydrogen bond and halogen interaction.

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## INTRODUCTION

Molecular docking is a vital tool used to understand and explore the steric and electrostatic complementarity between the protein and the ligand. The interactions between the receptor and ligand are quantum mechanical in nature. Molecular docking has a large number of applications in studying the biological phenomena. For instance, binding interactions between a ligand and an enzyme protein may result in activation or inhibition of an enzyme, or if the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design, lead optimization and bioremediation.

The 1,3-diarylprop-2-en-1-ones, an α,β-unsaturated ketones known as chalcones. The chalcones are synthesized by Claisen-Schmidt condensation of an arylaldehyde with aryl methyl ketone. The organic chemists are attracted towards the synthesis ofchalcone. Because, chalcone and its derivatives, among the large families of plant constituents, have various therapeutic benefits including antimicrobial[1], anti-inflammatory[2], antitumor [3], antiulcerative[4], antifungal [5], antibacterial[6], anticancer[7] and anti-HIV[8] properties.Chalcone are important intermediate for the syntheses of heterocyclic compounds such as flavonoids[9], isoflavonoids[10], pyrazole[11],pyrimidine[12,13] etc.[14,15]The fluorinated drugs play major role in the present pharmaceutical market. Fluorinated drugs are used for treatment of diseases of the central nervous system (CNS), various cardiovascular diseases and obesity, anti-cancer, antibacterial agents, and antifungal therapy [16,17]. Fluorine atomsare useful in providing connectivity information in carbon NMR spectrum due to its long-range spin-spin coupling constant [18,19].

## MATERIALS AND METHODS

**Synthesis:**

*Procedure for the preparation of (E)-3-(4-(difluoromethoxy)-3-hydroxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (11)*

A mixture of 4-difluoromethoxy-3-hydroxybenzaldedye (5 mmol), 4-hydroxy acetophenones (5 mmol) and sodium hydroxide was ground together in mortar with pestle for 5 min and left to harden at room temperature for 30 min. The solid was dissolved in cold distilled water and acidified with dilute HCl and kept aside for overnight. The solid that separated was filtered, dried and recrystallized from ethanol. Yield: 85%; M.P.: 184-186°C; FT-IR (KBr, $cm^{-1}$): 3293 (OH), 3052-3011 (Aromatic C-H), 2923-2849 (Aliphatic C-H), 1657 (C=O); $^{1}$H NMR(DMSO-$d_6$, 400 MHz, ppm): δ = 10.15 (broad singlet, 2H, OH), 7.12 (t, 1H, H-7), 7.56 (d, 1H, H-9), 7.74 (d, 1H, H-8), 8.31-7.17 (aromatic); $^{13}$C NMR (DMSO-$d_6$, 100 MHz, ppm): δ = 187.07 (Carbonyl C-10), 122.02 (C-9), 141.93 (C-8), 116.40 (C-7), 6.88-8.04 (aromatic); Mass (m/z): 307 (M+1)The synthesis and characterization ofcompounds **1-10** have been reported in literature [20].

**Molecular docking studies:**

*Ligand Preparation:* Docking calculations were carried out using DockingServer [21]. The MMFF94 force field [22] was used for energy minimization of ligand molecule using molecular docking server Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined.

*1SA0 Protein Preparation:*Docking calculations were carried out on 1SA0 protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [23]. Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

*Computational Methods:* Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [24]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

**Antibacterial activity:**

The antibacterial activity of compounds was determined by disc-diffusion method [25,26]. The test organisms were subculture using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37ºC ±1ºC for 18 hours, they were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121ºC for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160 ºC, for an hour. Into each sterilized petriplate (20 cm diameter), was poured about 125 mL of molten nutrient agar medium which was already inoculated with the respective strain of bacteria (5 mL of inoculum to 250 mL of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification. After solidification, the paper discs containing the derivatives were placed at different areas on the surface of each plate and labeled accordingly. Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 mL Analar grade) to give a concentration of 1000 µg/mL. Ciprofloxacin solution was also prepared to give a concentration of 1000 µg/mL in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using con. HCl. All the compounds were tested at dose levels of 1000 µg and DMSO used as a control. The solutions of each test compound, control and reference standard were added separately in the cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37±1 °C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

## RESULTS AND DISCUSSION

The*Insilico* molecular docking studies were carried out for a series of eleven fluorochalones (Figure1) with 1SA0 protein. To investigate the potency of the ligands **1-11**, we proceeded to examine the interaction of ligands with tubulin (1SA0). The molecular docking was performed by simulation of ligands into the binding site of tubulin. The binding modes of ligands and tubulin were depicted in Figure 2a & 2b. The 2D plot and HP plot were exhibited in Figure 3(a&b) and Figure 4(a&b) respectively. The docking results reveal that all the ligands inside 1SA0 protein are bounded well.The Free Energy of Binding, Inhibition Constant, Vander Waal + Hydrogen bond + dissolve Energy, Electrostatic Energy and Total Intermolecular Energy are given in Table 1.The ligand molecules are bound to protein 1SA0 by four binding modes such as Hydrogen bonds, Van der Waals, electrostatic and hydrophobic

interactions (Table 2). The ligands **1,3,5,7** and **10** only have hydrogen bond interaction with receptor. The calculated free energy of binding for substituted compounds is larger than the parent chalconeespecially the halo and napthyl substituted ligands. The free energy of binding for compounds chloro-(**3**), napthyl-(**10**), fluoro-(**2**) and bromo-(**6**) substituted ligands in the inhibitor binding site (IBS) were -7.39, -7.11, -7.01 and -7.00 kcal/mol respectively in the best pose.

**Figure 1.Fluoro chalcones (1-11)**

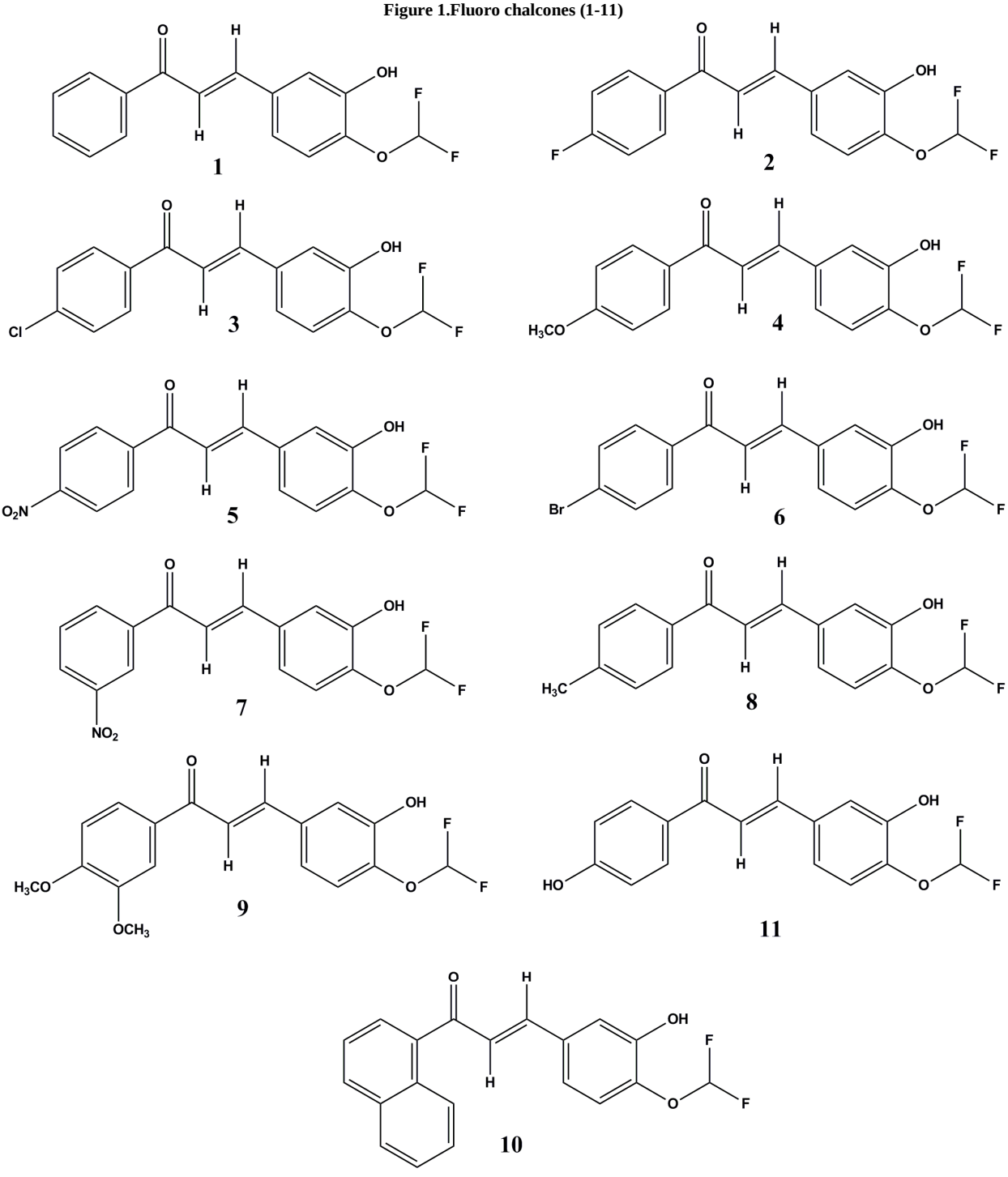


**Figure 2a.Docking pose of fluorochalcones (1-6) in binding site of 1SA0 protein**

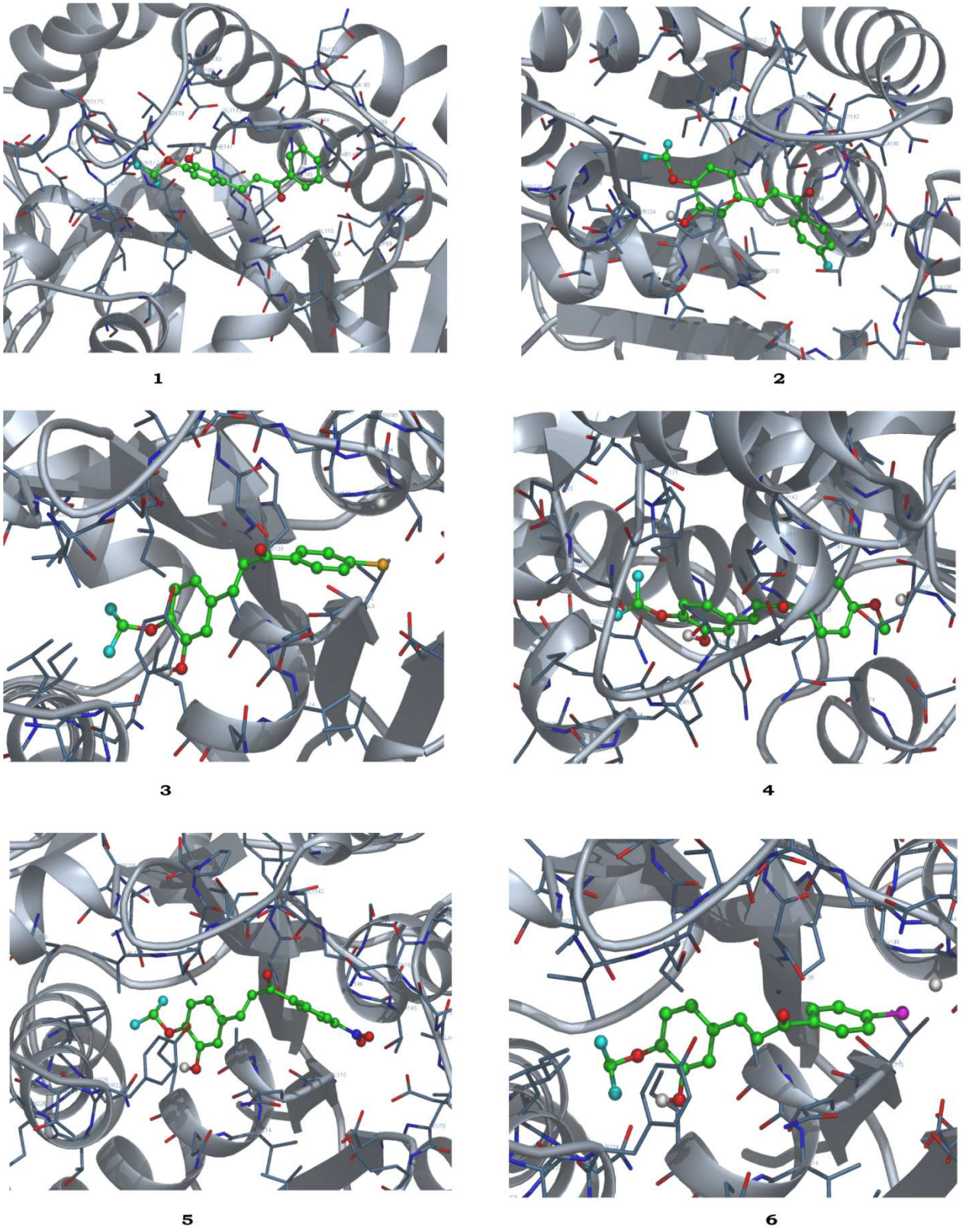
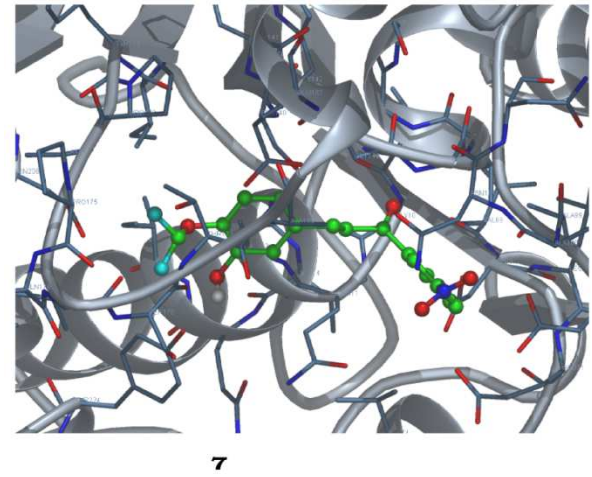
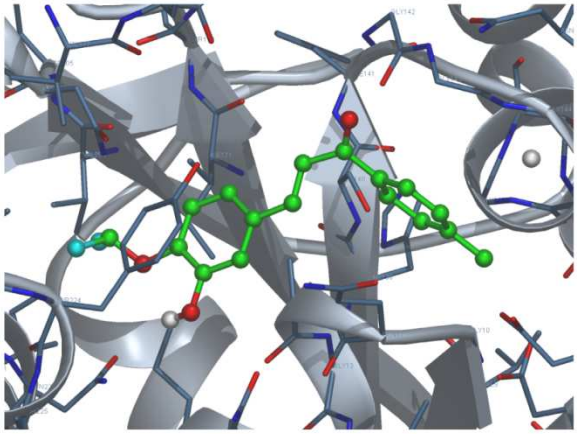


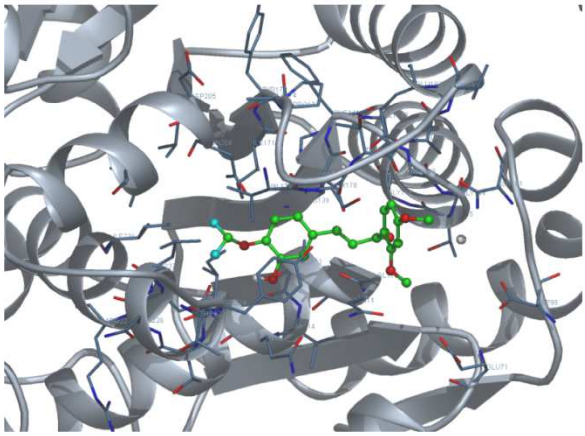
**Figure 2b.Docking pose of fluorochalcones (7-11) in binding site of 1SA0 protein**



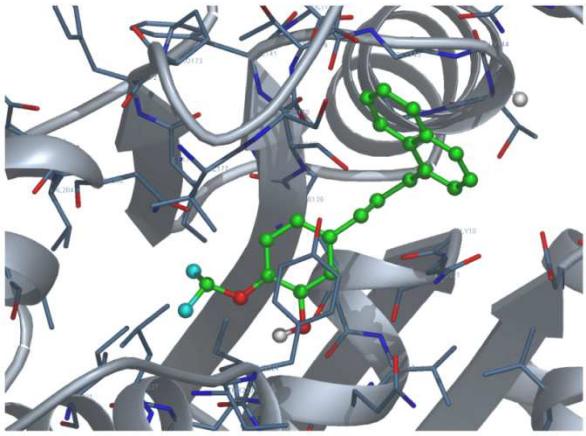
7



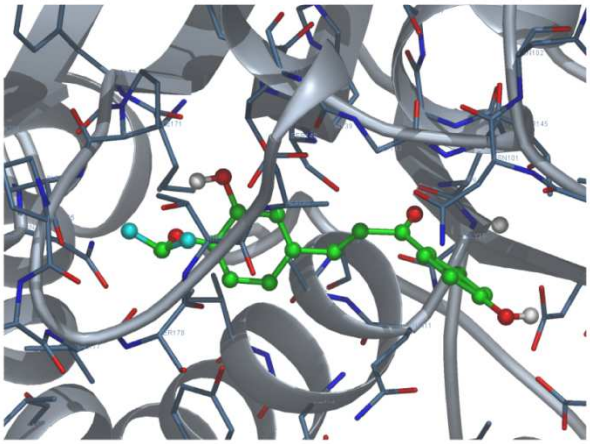
8



9



10



11

**Figure 3a. 2D plot of fluorochalcones (1-6)**

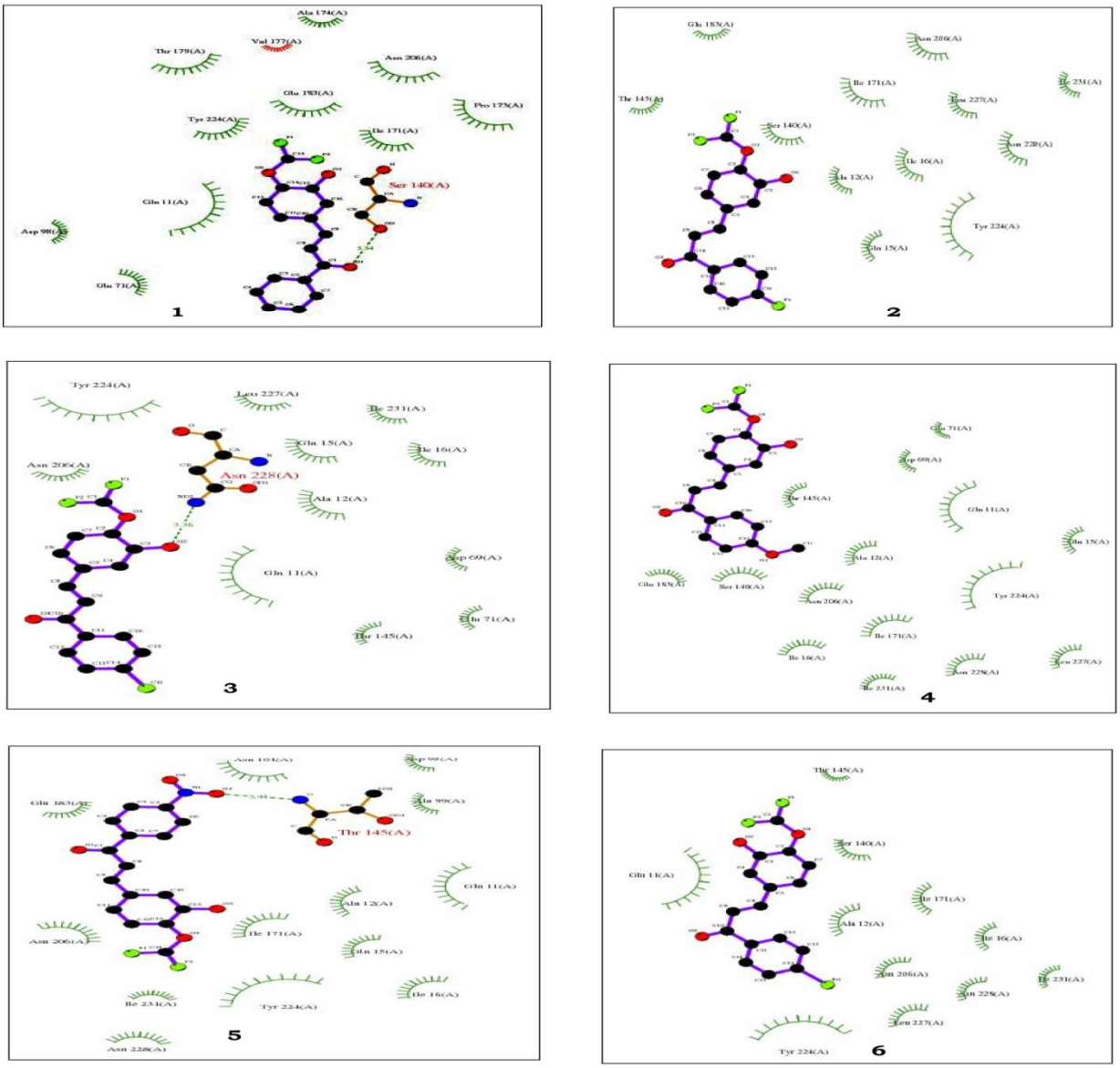


**Figure 3b. 2D plot of fluorochalcones (7-11)**

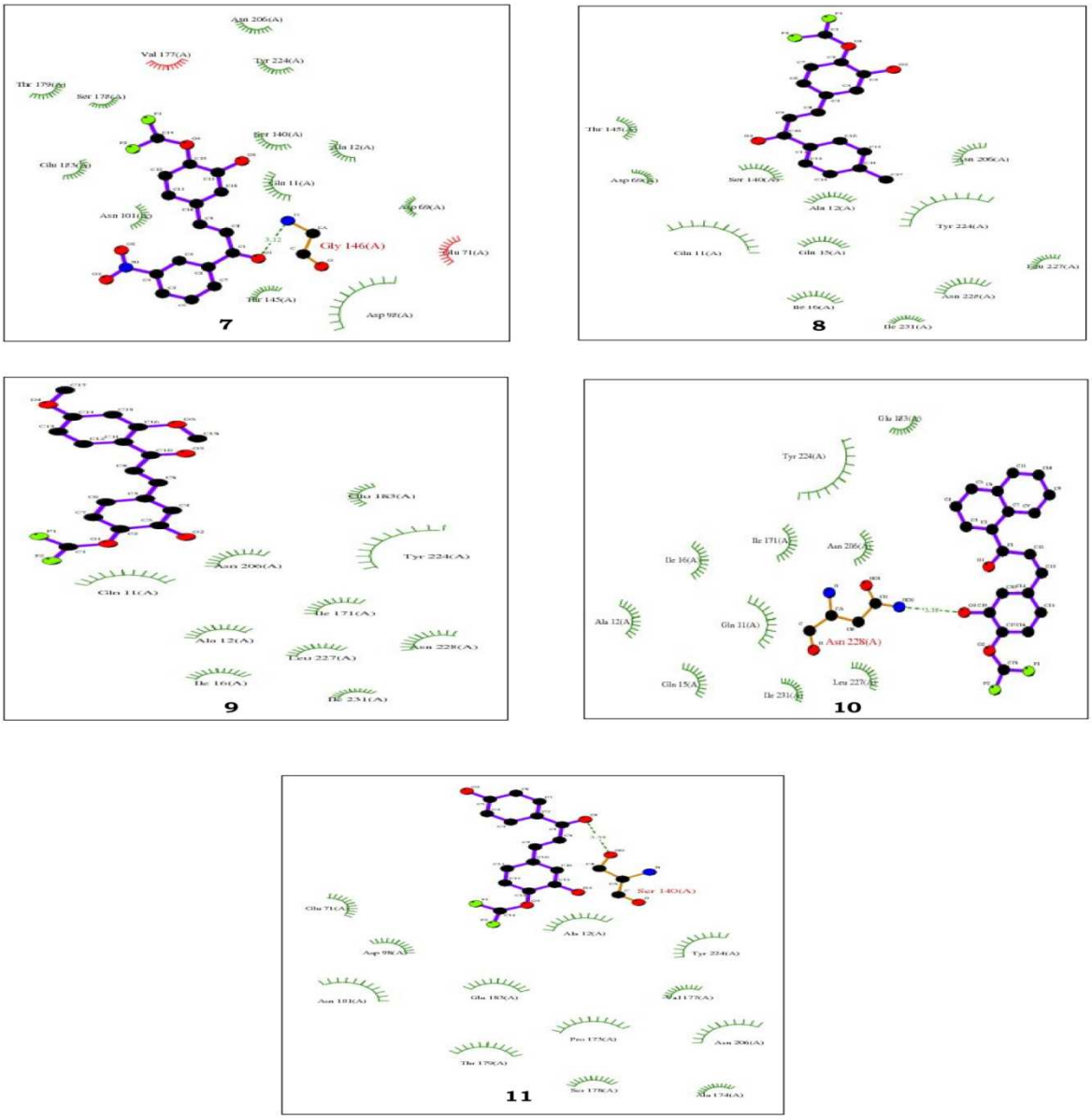


**Figure 4a. HP plot of fluorochalcones (1-6)**

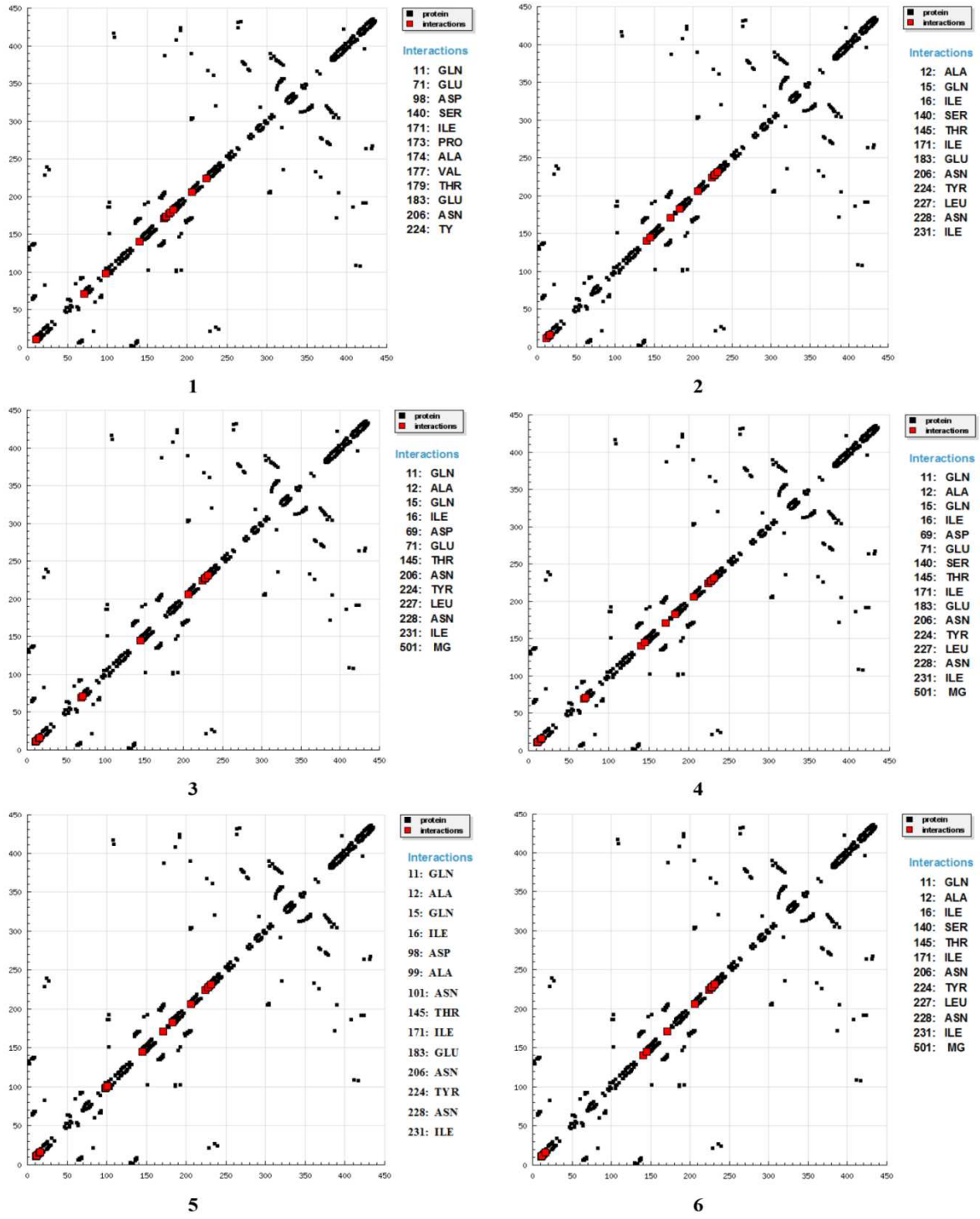
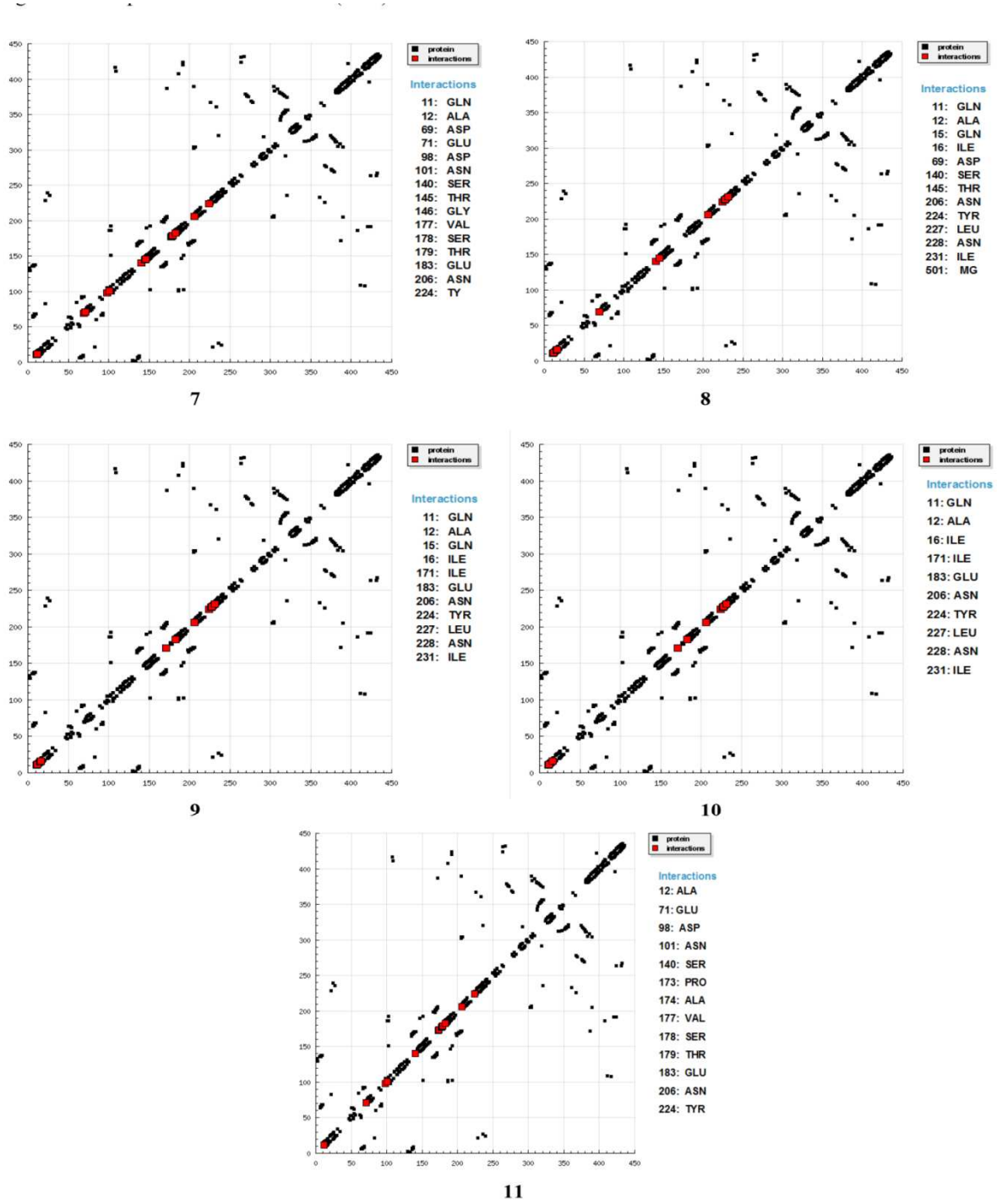


**Figure 4b. HP plot of fluorochalcones (7-11)**




In the binding mode, ligand **1** was nicely bound to binding site of receptor by hydrogen bond. The oxygen of the α,β-unsaturated carbonyl system formed one hydrogen bond with amino hydrogen of SER140 (O…H—N, bond length=3.34Å). The 2D plot of ligand**1** showed in Figure 3a. Apart from hydrogen bonding the ligand has halogen, polar and other interactions with receptor 1SA0. The ligand**3** contacts with ASN228, ASN206, GLU71, ASP69, GLN15, TYR224, ALA12, ILE16, GLN11, LEU227, ILE231 and THR145 of 1SA0*via*hydrogen bond(O…H—N ASN228, bond length=3.36Å),halogen bond, polar, hydrophobic, pi-pi, metal-ligand interactions. The ligand **5** bound well with 1SA0 receptor *via* hydrophobic, pi-pi interactions and binding is stabilized by one hydrogen bond. The oxygen of nitro group formed hydrogen bond with amino hydrogen of THR145 (O…H—N bond length=3.44Å).The ligand **7**also exhibit one hydrogen bond, the carbonyl oxygen formed hydrogen bond with amino hydrogen of GLY146(O…H—N, bond length=3.12Å). The 2D PLOT of ligand **10** (Figure 3b)clearly shows that hydrogen bond formed between oxygen of hydroxyl group situated at aromatic ring and amino hydrogen of ASN228 with the bond length value of 3.38Å. The ligand**11** inside protein is outlined by different amino acids *viz*. SER140,

ASN206, GLU183, SER178, VAL177, ALA174, ASP98, ALA12, ASN101, PRO173, TYR224 and GLU71 through halogen bond, polar, hydrophobic, pi-pi, metal-ligand and hydrogen bond interactions. The oxygen of the α,β-unsaturated carbonyl system formed one hydrogen bond with hydrogen of hydroxyl group of SER140 (bond length=3.34Å). The ligands **2,4,6,8** and **9** have exhibits halogen bond, polar, hydrophobic, pi-pi, cation-pi and metal-ligand interactions except hydrogen bond interactions with receptor 1SA0.

**Table 1.Molecular docking energyscore of ligands 1-11**

| Ligands | Estimated Free Energy of Binding kcal/mol | Estimated Inhibition Constant (Ki) µM | vdW + Hbond + desolv Energy kcal/mol | Total Intermolecular Energy kcal/mol |
|---|---|---|---|---|
| 1 | -5.94 | 44.07 | -7.62 | -7.71 |
| 2 | -7.01 | 7.26 | -8.34 | -8.21 |
| 3 | -7.39 | 3.81 | -8.55 | -8.53 |
| 4 | -6.40 | 20.29 | -7.85 | -7.86 |
| 5 | -6.50 | 17.30 | -8.16 | -7.96 |
| 6 | -7.00 | 7.43 | -8.28 | -8.32 |
| 7 | -6.86 | 9.40 | -8.57 | -8.86 |
| 8 | -6.74 | 11.53 | -8.03 | -8.06 |
| 9 | -5.13 | 175.02 | -6.83 | -6.83 |
| 10 | -7.11 | 6.10 | -8.08 | -8.12 |
| 11 | -6.72 | 11.94 | -7.86 | -8.18 |

**Table 2. Amino acid residues of 1SA0 receptor and its interactions with ligands 1-11**

| Ligands | Amino acids | Hydrogen bond | | | Other interactions |
|---|---|---|---|---|---|
| | | Atom of ligand[a] | Amino acid | Distance Å | |
| 1 | SER140, GLU183, GLN11, SN206, PRO173, ALA174, THR179, VAL177, TYR224, ILE171, GLU71, ASP98. | C=**O** | SER140 | 3.34 | halogen, polar |
| 2 | GLN15, TYR224 , ILE171,ASN206, ASN228, GLU183, ILE16, SER140, ALA12, LEU227, ILE231, THR145 | - | - | - | halogen bond, polar, pi-pi, hydrophobic, cation-pi |
| 3 | ASN228, GLN15, TYR224, ALA12, GLN11, ASN206, ILE16, LEU227, GLU71, ILE231, ASP69, THR145 | **O**—H | ASN228 | 3.36 | halogen bond, polar, pi-pi,hydrophobic, metal-ligand |
| 4 | GLN15, TYR224, ALA12, GLN11, ASN228, GLU183, ILE171, SN206, SER140, ILE16, LEU227, THR145, ILE231, ASP69, GLU71 | - | - | - | halogen bond, polar, pi-pi, cation-pi, hydrophobic, metal-ligand |
| 5 | THR145, GLN15, TYR224, GLN11, ASN228, GLU183, ILE16, ASN101, ASP98, ALA12, ILE171, ASN206, ILE231, ALA99 | **O**—N | THR145 | 3.44 | halogen bond, polar, pi-pi, hydrophobic, |
| 6 | ALA12, GLN11, TYR224, LEU227, SER140, ASN206, ILE171, THR145, ASN228, ILE16, ILE231 | - | - | - | halogen bond, polar, pi-pi, hydrophobic, metal-ligand |
| 7 | GLY146, ASP98, ALA12, GLN11, ASN206, ASN101, SER140, TYR224, THR145, THR179, SER178, GLU183, VAL177, ASP69, GLU71 | C=**O** | GLY146 | 3.12 | halogen bond, polar, cation-pi, hydrophobic |
| 8 | GLN15, TYR224, GLN11, ASN228, ALA12, ILE16, ASN206, SER140, THR145, LEU227, ILE231, ASP69 | - | - | - | halogen bond, polar, cation-pi, hydrophobic |
| 9 | GLN11, ALA12, ILE16, TYR224, ILE171, LEU227, ASN206, GLU183, ASN228, ILE231 | - | - | - | halogen bond, polar, pi-pi, cation-pi, hydrophobic |
| 10 | ASN228, GLN15, TYR224, ALA12, GLN11, ASN206, ILE16, ILE171, ILE231, LEU227, GLU183 | **O**—H | ASN228 | 3.38 | halogen bond, polar, pi-pi, cation-pi, hydrophobic |
| 11 | SER140, ASP98, ALA12, ASN101, ASN206, PRO173, GLU183, THR179, SER178, TYR224, VAL177, GLU71, ALA174 | C=**O** | SER140 | 3.34 | halogen bond, polar, hydrophobic, metal-ligand |

[a]*The ligand's atom participating in H-bond interaction is demonstrated as boldstyle.*

Overall, the results of the docking study reveal that the freeenergy of binding of fluorochalcones is dependent on the substituted moiety on the aromatic ring of this scaffold. Regarding this substitution, the calculated free energy of binding for substituted ligands is larger than the parent chalcone except ligand **9**.

The compounds (**1-11**) were screened for their antibacterial activities against gram-positive *viz. Bacillus subtilis*and *Staphylococcus aureus*and *gram-negative bacteria viz. Escherichia coli* and*Pseudomonas aeruginosa* by disc diffusion method. The inhibitory activities were compared with Ciprofloxacin as reference standard.

Each compound was tested against the above mentioned four bacterial pathogenic micro-organisms. All the compounds (**1-11**) exhibited a moderate to poor activity against all tested bacteria (Table 3). The halogen and nitro substituted compounds are showing higher activity compared to other compounds. The *meta* and *para* nitro substituted compounds and napthyl substitution compound exhibits moderate activity.

**Table 3. Antibacterial activity of (*E*)-3-[4-(Difluoromethoxy)-3-hydroxyphenyl]-1-phenylprop-2-en-1-ones**

| Bacteria | Zone of inhibition in diameter in mm | | | |
|---|---|---|---|---|
| | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* |
| Standard* | 32 | 38 | 36 | 32 |
| 1 | 15 | 14 | 13 | 10 |
| 2 | 23 | 22 | 21 | 22 |
| 3 | 19 | 19 | 18 | 18 |
| 4 | 10 | 13 | 11 | 8 |
| 5 | 23 | 18 | 22 | 17 |
| 6 | 18 | 19 | 16 | 16 |
| 7 | 19 | 18 | 24 | 18 |
| 8 | 16 | 10 | 15 | 12 |
| 9 | 17 | 14 | 18 | 14 |
| 10 | 19 | 22 | 22 | 17 |
| 11 | 18 | 16 | 20 | 14 |

**ciprofloxacin*

## CONCLUSION

A series eleven of fluorochalcones are examined for the *in silico* molecular docking studies with 1SA0 protein and results reveal that all the compounds inside 1SA0 protein are bounded well.The ligands have shown inhibition to 1SA0, especially which contains substituents at aromatic ring. It is observed that the compounds have hydrogen, halogen, polar and other interactions which are important for the folding of proteins. It could be concluded that the binding mode of our target compounds in the binding site of 1SA0 receptor suggested that they might act through inhibition of tubulin.All the compounds exhibited a moderate to poor activity against all tested bacteria.

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