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A Full Computational Evaluation of Two Novel Chalcone Derivatives as Inhibitors for Colon Cancer Related Proteins.

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Abstract: Colorectal cancer is a major threat to the society causing the death through metastasis to several patients with stage IV. Computational tools provide a relatively quick procedure in order to evaluate several molecules for their drug activity. Prenylated flavonoids are well known for their anticancer properties even in colon cancer. Here, we provided altered structures of chalcones, based on theoretical studies that are showing better binding affinities to several colon cancer related proteins. Using molecular docking and dynamics, alongside with density function theory and ADMET studies we are representing two new derivatives of Xanthohumol prenylated flavonoids having promising results against this disease.

Keywords: Xanthohumol; Colon cancer; Molecular docking, DFT, ADMET

1.Introduction

The high mortality rate (90%) amongst patients with stage IV colorectal cancer indicates a necessity in new chemotherapeutic agents with an increased selectivity. In such ways scientific community may be in a position to decrease the risk of metastasis which sometimes it is associate with high resistances in monotherapy [1]. It seems that, nowadays drugs are not yet in position to withstand the resistant mechanisms of tumour cells, which are linked to the tumour microenvironment such as the accumulation of phospholipids [2].

Prenylated flavonoids are secondary metabolites occurring in hops. This group of chemicals is showing a cytotoxic potential even in multi resistant cancer cells [3]. More specifically, 8-prenylnaringenin inhibits breast cancer cells and recently has been evaluated for its antiproliferative and apoptotic activities against human colon cancer cells [4] showing remarkable results. The anticancer action of the flavonoid family is established since several studies are showing that flavonoids can regulate cell death-related cellular signalling via ROS in human colon cancer cells [5,6], oral cancer cells [7], inhibiting enzymes responsible for this disease such as reductases and dehydroxygenases [8] and mediated SIRT1 signalling activation in hepatic disorders [9]. Recently natural flavonoids and chalcones were even tested against several covid-19 related proteins with promising results [10,11].

The molecular docking, is a computational strategy resulted the predicting binding site complementarity between a drug and its therapeutic target and has been massively used to assist drug repositioning for several diseases including cancer [12,13,14]. Additionally, DFT computational approach under the B3LYP/6 311++G(d,p) level of theory by using one-dimensional potential energy surface is another tool that helps the researchers to predict the reactivity of the design molecules [15,16]. On the other hand, molecular dynamics are well used for predicting protein-ligand binding site, including binding pockets and the binding residues in each pocket [17,18].

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Herein, we have performed two major computation experiments, molecular docking and molecular dynamics on several colon cancer related proteins [19], in order to evaluate the anticancer activity of 6-prenylnaringenin, 8-prenylnaringenin, xanthohumol and isoxanthohumol, compared to established anticancer drugs such as Avastin, Oxalilplatin and Xeloda. Based on the best two candidates of that group molecules, we have altered their structure in order to increase their binding affinities with the related proteins. The small molecules also evaluated using density function theory studies. Finally, the two new molecules were evaluated theoretically for their potential drug use, with the help of ADMET studies.

2. Materials and Methods

2.1 Molecular Dynamics

The protein-ligand binding site prediction has been done by using the COACH server (https://zhanglab.ccmb.med.umich.edu/COACH/). The prediction occurred using two comparative methods, TM-SITE and S-SITE, which recognize ligand-binding templates from the BioLiP protein function database by binding-specific substructure and sequence profile comparisons [20]. The molecular dynamic simulation was done in order to evaluate the molecular docking study.

2.2 Molecular Docking

Molecular docking studies were carried out by using iGEMDOCK 2.1 software [21]. The 3F6U, 4P75, 4UYA and 6MFQ proteins coded crystal structures were selected from the Protein Data Bank (www.rcsb.org). Ligand molecules were collected by Drug Bank (www.drugbank.ca). The novel ligands were drawn with the help of ChemDraw Ultra 12.0, Chem3D Pro 12.0 and Avogadro software [22]. The scoring function consisted of a simple empirical scoring function and a pharmacophore-based scoring function to reduce the number of false positives. The energy function can be dissected into the following terms:

$$E_{tot} = E_{bind} + E_{pharma} + E_{ligpre} \quad (1)$$

where E_{bind} is the empirical binding energy used during the molecular docking; E_{pharma} is the energy of binding-site pharmacophores; and E_{ligpre} is a penalty value if the ligand unsatisfied with the ligand preferences. E_{pharma} and E_{ligpre} were used to improve the number of true positives by discriminating active compounds from hundreds of thousands of non-active compounds. The empirical binding energy (E_{bind}) is given as:

$$E_{bind} = E_{inter} + E_{intra} + E_{penal} \quad (2)$$

where E_{inter} and E_{intra} are the intermolecular and intramolecular energy, respectively, and E_{penal} is a large penalty value if the ligand is out of the range of the search box. In this paper, E_{penal} is set to 10000. For screening: The population size was = 200, generations = 70, number of solutions = 3. Fitness is the total energy of a predicted pose in the binding site [23]. Here, the *vdW* term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electrostatic energy, respectively. Screenshots of the ligand-amino acid residue interactions created by CHIMERA software [24].

2.3 Density Function Theory Studies

Geometric optimization calculations were performed in accordance with the DFT method using ORCA software [25,26]. Frequency calculations were performed to obtain

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thermodynamic properties and to verify that each optimization achieved an energy minimum. The quantum chemical descriptors extracted directly from the ORCA output file were total energy, Huckel atomic charges, electronic density, dipole moment, Mayer population analysis, the energy of the highest occupied molecular orbital (HOMO), and the energy of the lowest unoccupied molecular orbital (LUMO) [27,28].

2.4 Structural Modification

The best two candidates according to their binding affinities to the related proteins, Xanthohumol and 8-Prenylnaringenin were altered chemically in structure, adding at first a prenylated group unit to the second ring of their moieties. After that addition we realized that the binding affinities were declined, showing that the increase of the lipophilicity of the second ring of the chalcones altered negatively the chemical relativity with the protein. After that, we decided to introduce diethanolamine structure moiety to the second ring, increasing successfully their binding affinities, even more compared to the original molecules. The structure modification was performed using the Chem3D Pro software and structures were optimised with ORCA softare.

2.5 ADMET Studies

Xanthohumol, 8-Prenylanaringenin and their two novel derivatives were also evaluated for their pharmacological profile using the SwissADME server (http://www.swissadme.ch/). The predicted result consists of physiochemical properties, lipophilicity, water-solubility, pharmacokinetics, drug- likeness, and toxicity studies. The simulation provides in- formation about absorption, distribution, metabolism, excretion, and toxicity of the drug [29]. Moreover, in order to double- check the gained values, we used Biosig Tool online software [30]. The pharmacological profile of the best scoring inhibitors was evaluated by Toxtree software [31,32], using Cramer rules and Cytochrome P450 metabolism prediction, taking information on *pKa* values, *logP* values, solubility, refractivity, and estimated toxicity of the molecules.

3. Results and Discussion

3.1 Molecular Dynamics

The prediction of the binding sites of the related colon cancer proteins, has been done after combination of multiple prediction results of algorithms from TM-SITE, S-SITE, COFACTOR, FINDSITE and ConCavity. The probability of a residue to be a binding residue is calculated from individual methods, which are used as the feature vectors for the residue. The top-scoring predictions from each of the programs are combined using a linear SVM. The detailed prediction it can be found in the supplementary material **S1Table**. On the other hand, in **Figure 1**, we can see the predicted binding sites of the studied protein structures calculated with molecular dynamics simulations as predicted by the COACH server. (A: 6MFQ, B: 4P75, C: 4UYA, D: 3F6U).

6MFQ, 4P75, 4UYA and 3F6U, are 2-chain structure proteins and biomarkers for colon cancer. Defects in these proteins are the cause of hereditary non-polyposis colorectal cancer type 4 (HNPCC4). HNPCC is an autosomal, dominantly inherited disease associated with marked increase in cancer susceptibility. It is characterized by a familial predisposition to early onset colorectal carcinoma (CRC) and extra-colonic cancers of the gastrointestinal [33]. After the prediction of the binding pocket areas of the structures we proceeded with molecular docking studies using for ligands prenylated chalcones with *in vitro* proven anti colon cancer activity, common anticancer drugs that are in use for that disease and two novel derivatives of the best candidates in docking studies.

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3.2 Molecular Docking

Using the predicted binding pockets of the studied structures, we performed molecular docking studies with the ligands: 6-Prenylnaringenin, 8-Prenylnaringenin, Isoxanthohumol, Xanthohumol, 8-Prenylnaringenin derivative, Xanthohumol derivative, Avastin, Oxylplatin and Xeloda. Avastin, Oxylplatin and Xeloda are common anticancer drugs that are in use for colon cancer treatment. 8-Prenylnaringenin and Xanthohumol exhibited relatively high binding affinities with the selected proteins, so we decided to alter their structures chemically, by substituting the second ring of the prenylated chalcone molecules with diethanolamine. As we can see on **Table 1**, the binding affinities of the molecules increased after the substitution. In general, all the prenylated chalcone molecules that can be found in hops plant, showed similar or even better in some cases binding affinities compared to the anticancer commercial drugs. The two substituted novel chalcone molecules exhibited even higher binding affinities with the selected proteins. In **Figure 2** we can observe depicted structures of the binding protein-drug complexes. (A: 3F6U-Xanthohumol, B: 6MFQ-8Prenylnaringenin, C: 3F6U-Xanthohumol derivative, D: 6MFQ-8Prenylnaringenin derivative).

3.3 Density Function Theory Studies

Using density function theory studies, we were able to determine the optimized 3d structures of the two novel chalcone derivatives. Selected bond lengths and angles alongside with atomic charges, can be found in supplementary tables, **S2Table, S3Table and S4Table** respectively. The optimized structures and van der Waals sphere structures of the two novel chalcone molecules can be found in **Figure 3**.

The distribution and energy of HOMO is an important parameter to explain the antioxidant potential of phenolic antioxidants. The electron donating capacity of the molecule can be predicted by looking at the energy values of HOMO [34]. So, in **Figure 4**, we can see the HOMO and LUMO orbitals of the chalcone derivatives indicating Δ_{GAP} for Xanthohumol derivative equal to 11.265 eV and Δ_{GAP} for 8-Prenylnaringenin derivative equal to 11.403 eV. The results are in a good agreement with the docking studies revealing that 8-Prenylnaringenin derivative has higher activity than the Xanthohumol derivative.

3.4 Structural Modification

At first, we substituted the two molecules with a prenylated group leading to an increase in lipophilicity of the molecules. This resulted a decrease to the binding affinities of the molecules on the selected proteins. After this step we substitute the second ring of the chalcone derivatives with a diethanolamine, a strategic synthesis reported previously in the literature [35]. By doing this we have increased the hydrophilicity of the molecules and further more their binding affinities to the studied proteins. This will probably increase their biological activity as well, a fact that should be studied further *in vitro* in another work. The substitution of the two chalcone molecules can be found in **Figure 5**. This structural modification had an impact on the amino acid residue that the molecules interacted as well. A detailed information of the binding amino acid residues with the molecules Xanthohumol, Xanthohumol derivative, 8-Prenylnaringenin and 8-Prenylnaringenin, can be found in **Table 2**.

2.5 ADMET Studies

Our final approach was to evaluate theoretically the pharmacological properties of the two novel molecules. The predicted pharmacological properties of these two candidate molecules are depicted in **Table 3.** The pharmacological properties of the drugs, including the absorption, distribution, metabolism, excretion, and toxicity, are also presented. It is worthy to say that these two derivatives have very similar pharmacological values and

better than the *in vitro* evaluated Xanthohumol and 8-Prenylnaringenin, a fact that has to do probably with their increase in hydrophilicity. We believe though that further studies should be done in order these molecules to be fully evaluated as drug candidates. Regarding their toxicity their increase in polarity decreases their toxicity values as well, increasing more their potential use.

4. Conclusions

In this work we have investigated the binding pockets of several colon cancer proteins using molecular dynamics and we were evaluated theoretically the binding affinities of a family of prenylated chalcones with the help of molecular docking studies. The prenylated chalcones were used in the literature against colon cancer previously *in vitro* with promising results. So, Xanthohumol and 8-Prenylnaringenin were computationally revealed as promising candidates evenly compared with traditionally anticancer drugs. The molecules were then studied with density function theory and finally, we managed to increase the binding affinity of that molecules on cancer proteins by substitution with diethanolamine molecule, attached on the second phenolic ring. We do believe that these two novel chalcone derivatives will show promising results when tested *in vitro* and should be used in further studies against colon cancer.

Supplementary Materials: S1Table, S2Table, S3Table, S4Table, S5Table.

Author Contributions: "Conceptualization, Manos C. Vlasiou.; methodology, Manos C. Vlasiou.; software, Christos C. Petrou.; validation, Yiannis Sarigiannis., resources, Christos C. Petrou.;; writing—original draft preparation, Kyriaki S. Pafiti; writing—review and editing, Manos C. Vlasiou; visualization, Kyriaki S. Pafiti.; supervision, Manos C. Vlasiou. All authors have read and agreed to the published version of the manuscript."

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Data Availability Statement: In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics. You might choose to exclude this statement if the study did not report any data.

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Conflicts of Interest: "The authors declare no conflict of interest."

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Figures

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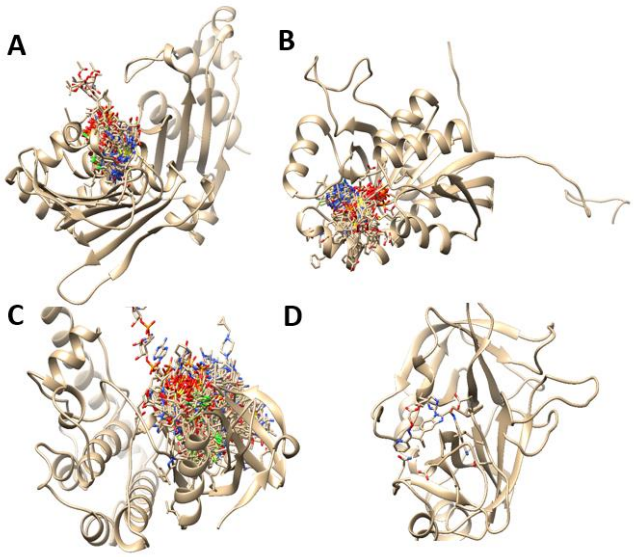

Figure 1: Predicted binding sites of the studied protein structures calculated with molecular dynamics simulations. (A: 6MFQ, B: 4P75, C: 4UYA, D: 3F6U).

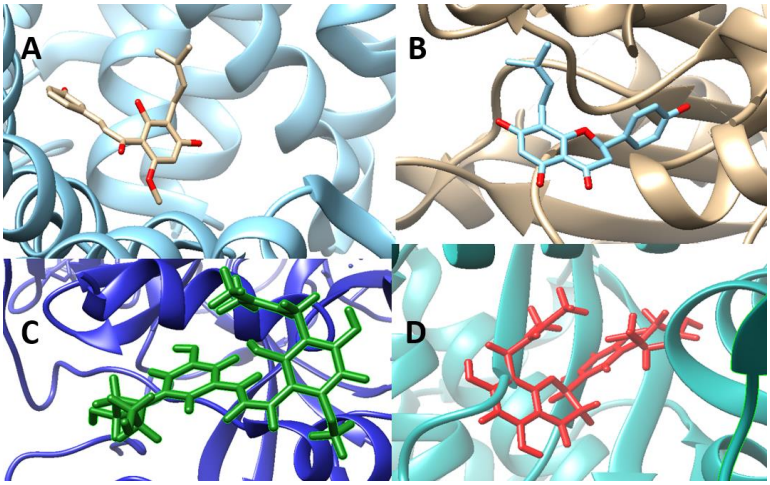

Figure 2: Screen shots of the binding protein-drug complexes. (A: 3F6U-Xanthohumol, B: 6MFQ-8Prenylnaringenin, C: 3F6U-Xanthohumol derivative, D: 6MFQ-8Prenylnaringenin derivative).

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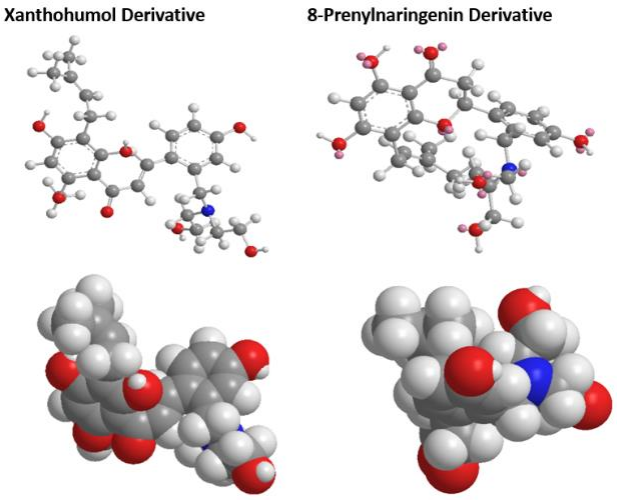

Figure 3: Optimized structures and van der Waals sphere structures of the two novel chalcone molecules.

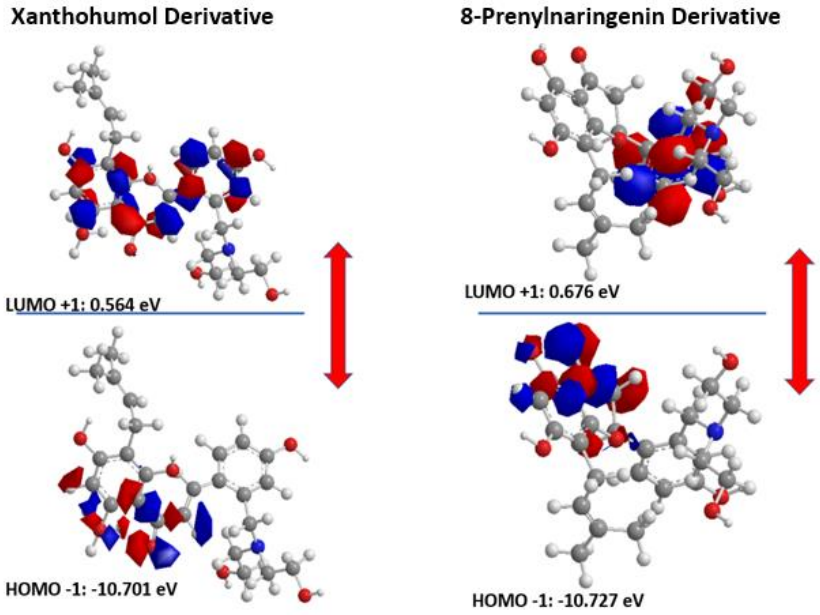

Figure 4: HOMO-LUMO orbitals of the two novel chalcone molecules.

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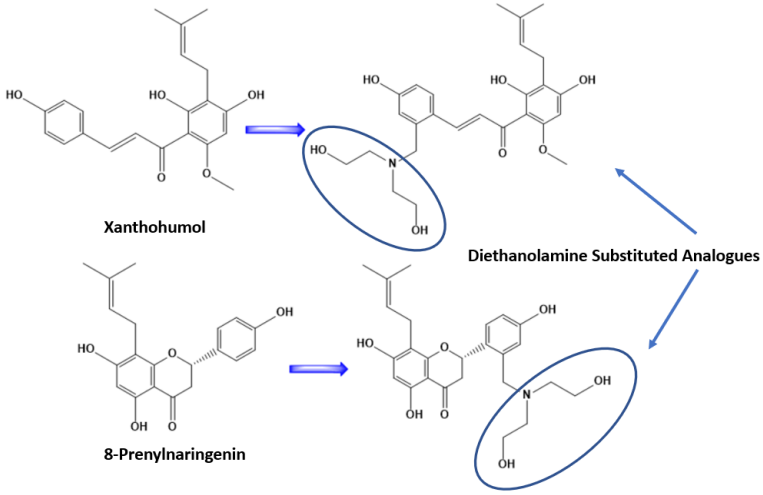

Figure 5: Structural modification of Xanthohumol and 8-Prenylnaringenin, after substitution with diethanolamine on the second ring.

Tables

Table 1: Binding affinities and energy distribution of the studied protein-ligand complexes.

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Complex (Protein-Ligand)	Binding Energy (Kcal/mol)	Van der Waals Contribution (Kcal/mol)	Hydrogen Bond Contribution (Kcal/mol)
3F6U-6Prenylnaringenin	-8.35	-7.87	-0.48
3F6U-8Prenylnaringenin	-8.16	-6.83	-1.33
3F6U-Isoxanthohumol	-8.17	-7.01	-1.16
3F6U-Xanthohumol	**-8.42**	-0.98	-7.44
3F6U-Xanthohumol Derivative	**-9.91**	-1.16	-8.75
3F6U-AVASTIN	-7.55	-6.70	-0.85
3F6U-OXALILPLATIN	-7.95	-4.82	-3.13
3F6U-XELODA	-8.55	-6.99	-1.56
4P75-6Prenylnaringenin	**-9.95**	-7.84	-2.11
4P75-8Prenylnaringenin	-8.95	-7.90	-1.05
4P75-Isoxanthohumol	**-9.05**	-7.99	-1.06
4P75-Xanthohumol	-8.63	-6.25	-2.38
4P75-AVASTIN	-7.78	-6.83	-0.95
4P75-OXALILPLATIN	-6.96	-4.75	-2.21
4P75-XELODA	-10.4	-2.75	-7.65
4UYA-6Prenylnaringenin	-7.53	-7.53	0
4UYA-8Prenylnaringenin	-7.36	-6.54	-0.82
4UYA-Isoxanthohumol	-7.37	-6.86	-0.50
4UYA-Xanthohumol	-6.83	-6.43	-0.40
4UYA-AVASTIN	-7.08	-6.38	-0.70
4UYA-OXALILPLATIN	-5.96	-4.7	-1.26
4UYA-XELODA	-7.83	-6.81	-1.02
6MFQ-6Prenylnaringenin	-8.92	-7.97	-0.95
6MFQ-8Prenylnaringenin	**-9.11**	-0.25	-8.86
6MFQ-8Prenylnaringenin Derivative	**-9.22**	-1.79	-7.43
6MFQ-Isoxanthohumol	**-9.25**	-8.98	-0.27
6MFQ-Xanthohumol	**-9.95**	-7.94	-2.01
6MFQ-AVASTIN	**-9.07**	-6.87	-2.20
6MFQ-OXALILPLATIN	-8.05	-5.05	-3.00
6MFQ-XELODA	**-9.16**	-6.60	-2.56

Table 2: Amino acid residue of the binding complexes with the two novel chalcone molecules.

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Complex (Protein-Ligand)	Hydrogen Bonded Amino Acid Residue	Hydrophobic Interacted Amino Acid Residue
3F6U-Xanthohumol	ARG-554, TRP-553, ASN-641	LYS-560, TRP-593, ASN-594 (M), ASN-594 (S), ALA-597, HIS-598 (M), HIS-598 (S)
3F6U-Xanthohumol Derivative-3	ARG-554 (M), ARG-554 (S)	TRP-553, ARG-554 (M), ARG-554 (S), ALA-553, TRP-593, ASN-594 (M), ASN-594 (S), ALA-597
6MFQ-8-Prenylnaringenin	ASP-55	ASN-53, ASP-70, ASN-71 (M), ASN-71 (S), PRO-152 (M), PRO-152 (S), ARG-153 (M), ARG-153 (S), THR-52, ARG-153
6MFQ-8-Prenylnaringenin Derivative	ASN-45, ASP-48, CYS-73 (M), CYS-73 (S), GLY-74	ASN-45, ALA-49, GLY-74, VAL-75, LEU-83, GLU-109

Table 3: Pharmacokinetic characteristics of the design molecules compared with their parent ones.

Molecule	Xanthohumol	Xanthohumol Derivative	8-Prenylnaringenin	8-Prenylnaringenin Derivative
Log Po/w	2.75	3.05	2.59	2.53
Consensus Log Po/w	3.72	2.89	3.26	2.32
Log S	-5.18	-4.46	-4.91	-4.10
Class	Poorly Soluble	Moderately Soluble	Moderately Soluble	Moderately Soluble
Solubility	$9.26*10^{-3}$ mg/ml	$5.06*10^{-4}$ mg/ml	$7.55*10^{-3}$ mg/ml	$7.84*10^{-3}$ mg/ml
Inhibitor	CYP1A2, CYP2C9, CYP3A4	CYP3A4	CYP1A2, CYP2C9, CYP2D6, CYP3A4	P-gp Substrate, CYP2D6
Log Kp	-4.86 cm/s	-6.66 cm/s	-5.22 cm/s	-7.12 cm/s
Lipinski	Yes	Yes	Yes	Yes
Bioavailability Score	0.55	0.55	0.55	0.55