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Molecular Docking Study of Flavonoids to Block the Aryl Hydrocarbon Receptor †

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Abstract: Anti-HIF flavonoids have been described for their antitumor activity by interfering with a presumed antioxidant mechanism through direct and indirect ways of overexpression of the hypoxia inducible factor (HIF-1α). The aryl hydrocarbon receptor (AhR) is a protein homologous to HIF-1α and is overexpressed in smoking patients suffering from lung and breast cancer. The interaction of thirteen flavonoids with the AhR has been evaluated by molecular docking. The AhR:ARNT model obtained by SwissModel has been used for docking with the MOE 2019.01 program, as well as several servers for the determination of protein–protein interactions and alanine mutations. Different interaction sites were identified for blocking the AhR: functional ARNT, the interface between the bHLH and PAS-A domains, being important. The blocking capacity to AhR:ARNT proved to be between 50 and 60% for flavonoids 4′,7-dihydroxy-flavone, fisetin, luteolin, 5-hydroxy-2-(4′-hydroxy)-7-methoxy-flavonone, flavone, apigenin, galangin and 7-hydroxy-5-methoxy-flavonone. None of the flavonoids evaluated interact with the PAS-B domain (AhR active site). All the studied flavonoids interact with AhR, except flavone, and with ARNT except the compounds 3,7-dihydroxy-flavone and kaempferol. The best flavonoid for blocking the formation of the AhR:ARNT heterodimer proved to be fisetin, which is found in food sources such as strawberries, apples and grapes, and has shown the ability to reduce pro-cancer inflammatory markers in colorectal cancer patients and lung cancer.

Keywords: flavonoids; molecular docking; aryl hydrocarbon receptor

1. Introduction

The aryl hydrocarbon receptor (AHR), also known as the dioxin receptor, is traditionally defined as a ligand-dependent transcription factor involved in biotransformation and

carcinogenic/teratogenic effects of environmental toxins, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [1]. The AHR is a basic transcription factor that contains helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS). It is present in numerous animal species, including humans, and activates gene expression in a ligand-dependent manner. Ligand binding in the PAS-B domain of AHR leads to nuclear translocation and heterodimerization with the AHR nuclear translocator protein (ARNT). This AHR:ARNT heterodimer binds to DNA sequences, called xenobiotic-responsive elements or dioxin responsive elements (XRE or DRE), that are distributed in the enhancer regions of dioxin-responsive genes and regulate the expression of target genes. The binding of the ligand to AHR occurs on the PAS-B domain. AHR:ARNT dimerization involves interactions between its bHLH and PAS domains, and DNA binding occurs primarily through its basic domains. AHR is overexpressed in patients with lung and colorectal cancer. The search for new antitumor compounds is an important research area to improve effectiveness, increase survival, as well as to decrease multidrug resistance, adverse reactions and mortality in these patients [2–7].

Polyphenols are natural plant compounds and are the most abundant antioxidants in the human diet [8]. In general, antioxidants are defined as substances capable of reducing and preventing the oxidation of a substrate (proteins, lipids, carbohydrates and DNA) at low concentrations, and are present in vegetables, fruits and cereals that are usually consumed as natural diets, because they are safe and provide a large number of health benefits [9,10]. Polyphenols, which are composed of aromatic rings with one or more hydroxyl groups and are grouped in this classification more than 8000 compounds. Polyphenols are subdivided into several groups and the most important polyphenol subclasses are phenolic acids, flavonoids, stilbenes and lignans [10].

Taking this into account and considering that flavonoid compounds are well known and have characteristics that have made them attractive for cancer research, we proceeded to determine by molecular docking the interaction of flavonoids with the receptor AHR to block the formation of the functional heterodimer.

2. Materials and Methods

Molecular docking: A database created of flavonoids was used for modeling, thus a model obtained by SwissModel Web Server of the AHR receptor and its heterodimer complex with ARNT using as the crystal structure of ARNT (PDB: 4zp4) and the AHR sequence. For molecular modeling visualization, presentation of complexes, poses and interactions, the MOE 2019.01 program was used. The server programs Cocomaps (bioCOmplexes Contact MAPS) was used for the determination of atomic contacts between protein interfaces of the AHR:ARNT [11]. Important amino acid residues for protein-protein interaction as well as flexibility of alanine mutations were determined with Robetta and Rosetta Backrub web servers [12,13].

A positive control was used as proof of concept for the antiproliferative activity in the lung cancer cell line (A549) and for the identification of a new binding site: 5,7-diacetoxy-3-phenylcoumarin.

3. Results and Discussion

The activation of AHR is associated with an increase of the oxidative metabolism, and consequently, with the formation of, for instance, reactive oxygen species. Hence, this interaction with DNA in XRE is highly associated with the onset of further toxicity events that include carcinogenicity due to a prolonged AHR activity, development and reproductive toxicity, and immunological deterioration, known as dioxin toxicity effects [1,6,7,14–22].

The architecture of the interaction of AHR with ARNT to form the functional AHR:ARNT heterodimer is presented in Figure 1. Dioxins and dioxin-like substances can cause cancer and the high AHR conservation level shows the binding site of the ligand in the internal cavity at AHR PAS-B domain [1,14,23,24].

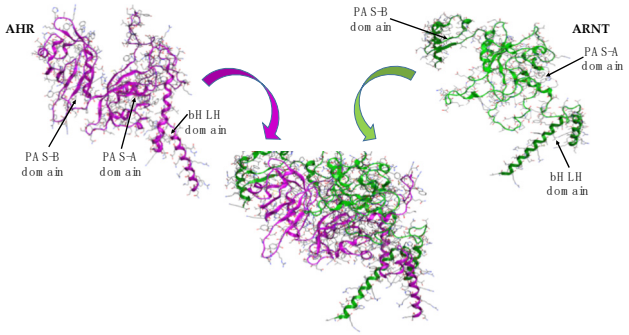


Figure 1. Structural architecture of the interaction of the monomers AHR and ARNT for the formation of the functional heterodimer AHR:ARNT modeled by SwissModel.

The protein-protein interactions (PPIs) are an integral part of most biological functions. Compared to targeting to the active site, inhibition of PPI suffers from the particular problem of more exposed and less defined binding sites, and this poses significant experimental challenges for the development of PPI inhibitors, although the discovery and subsequent refinement of the PPI inhibitors with strong affinity have proven to be a challenging pursuit, although not impossible [25–27].

The flavonoids studied in the molecular modeling study at the surface level of the AHR bind to contact interfaces at the level of the bHLH, PAS-A domains and the bHLH/PAS-A (yellow color circle) (Figures 2 and 3). Therefore, these compounds can present a certain antagonistic behavior due to interactions at the level of the contact surfaces to block PPIs between AHR and ARNT (yellow color circle), is identified as a new binding site where flavonoids can interfere with the formation of the functional AHR:ARNT heterodimer, according to its modeling with Swiss Model [24].

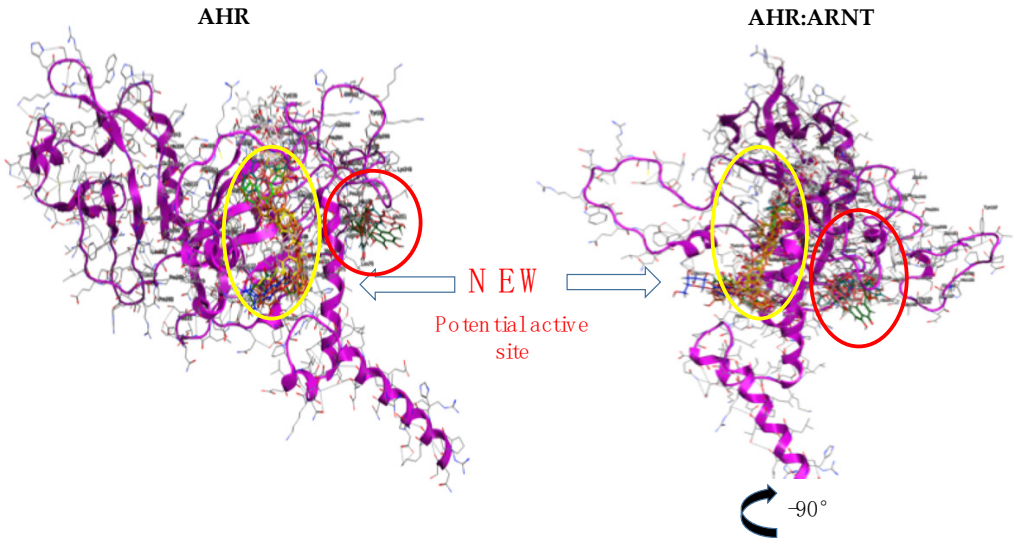


Figure 2. Interaction of flavonoids at the interface of the bHLH and PAS-A domains of AHR modeled by SwissModel. bHLH-PAS-A (yellow color) and PAS-A (Red color).

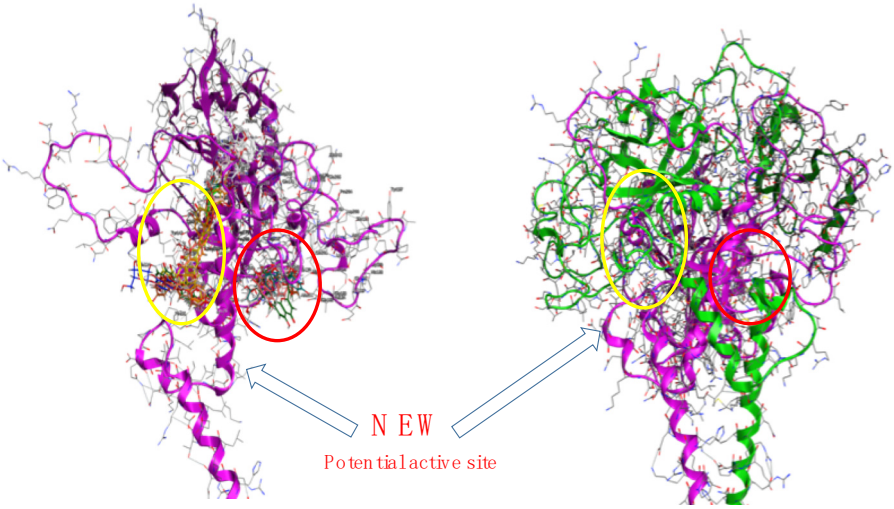


Figure 3. Interaction of flavonoids at the interface of the bHLH and PAS-A domains of AHR modeled by SwissModel. bHLH-PAS-A (yellow) and PAS-A (red).

In the case of ARNT, the flavonoids essentially bind to the surface interfaces of the bHLH and PAS-A domains, in the latter the α-helix is noteworthy, where the Asp161, Leu164, Lys165, Ile168, Leu176 and Tyr188 residues are found, that are visually identified as essential for the interaction between the PAS-A (AHR)/PAS-A (ARNT) domains (Figure 4).

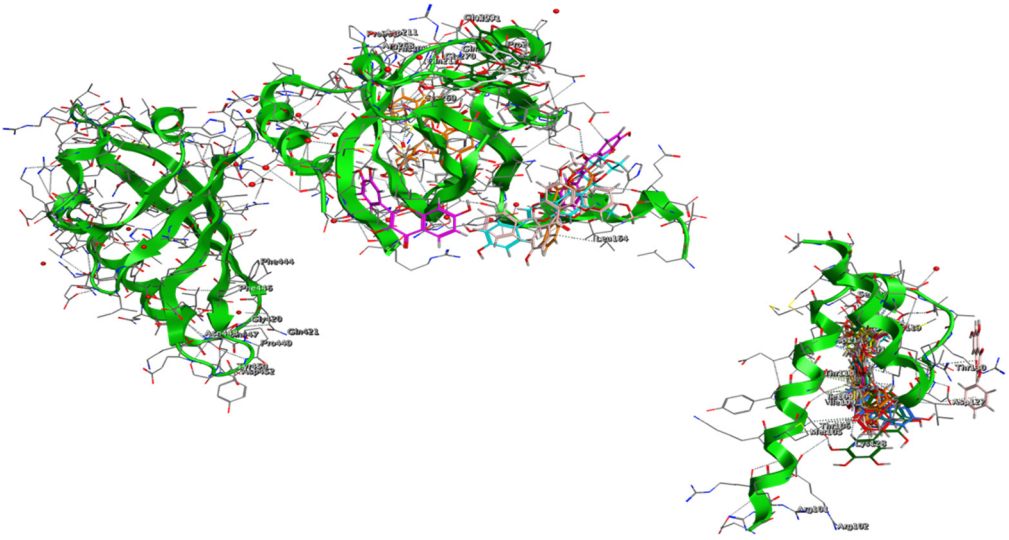
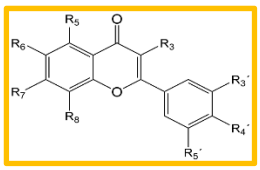


Figure 4. Interaction of flavonoids at the interface of the bHLH and PAS-A domains of ARNT.

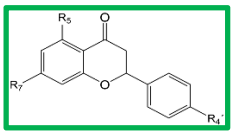
The compounds presented in Tables 1 and 2, as well as compounds **18** and **19** (Figure 5), have different probabilities of blocking the formation of the AHR:ARNT heterodimer, with compounds **1**–**6**, **15**–**16** being those with a blocking percentage ≥ 50%, so they are considered the best, although all the modeled compounds in some way can interfere in the formation of the heterodimer under study by interfering with the PPI. It is important to note that flavonoids are one of the major classes of dietary-derived AHR ligands. The flavonoids are a large class of polyphenolic secondary metabolites that are widely distributed in fruits and vegetables. Quercetin, taxifolin, and robinetin are reported to be able to activate the AHR, while luteolin act as AHR antagonists [28–32]. Other polyphenols are reported to have activity to block PPI at the AHR and ARNT level [24].

Table 1. Structures of flavones and flavonols active in blocking the formation of the functional heterodimer AHR:ARNT. Percentages of probability of the activity estimated by molecular docking.

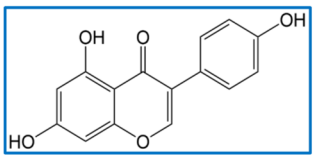


Comp	R_3	R_5	R_6	R_7	R_8	$R_{3'}$	$R_{4'}$	$R_{5'}$	AHR:ARNT Prob (%)
1	H	H	H	OH	H	H	OH	H	60
2	OH	H	H	OH	H	OH	OH	H	60
3	CH_3	OH	H	OH	H	OH	OH	H	60
4	H	OH	H	OH	H	H	OH	H	50
5	H	H	H	H	H	H	H	H	50
6	OH	OH	H	OH	H	H	H	H	50
7	H	OH	H	OCH_3	Glu	OH	OH	H	40
8	OH	OH	H	OH	H	H	OH	H	30
9	H	OH	Glu	OCH_3	H	H	OH	H	30
10	H	H	H	OH	H	H	OCH_3	H	20
11	OH	OH	H	OH	H	OH	OH	OH	20
12	OH	OH	H	OH	H	OH	OH	H	20
13	H	OH	Glu	OH	H	H	OH	H	20
14	OH	H	H	OH	H	H	H	H	10

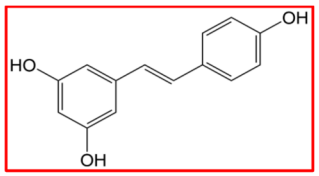
Table 2. Structure of active flavonones to block the formation of the functional heterodimer AHR: ARNT. Percentages of probability of the activity estimated by molecular docking.



Comp	R_5	R_7	$R_{4'}$	AHR:ARNT Prob (%)
15	OH	OCH_3	OH	60
16	OCH_3	OH	H	50
17	OH	OCH_3	H	30



Compound 18: Genistein. **AHR:ARNT Prob (%):** 40



Compound 19: Resveratrol. **AHR:ARNT Prob (%):** 30

Figure 5. Genistein and resveratrol, with the probability of blockage.

3.1. Active Flavonoids with a Probability of Blockage Higher Than 50%

Compound **1** (Figure 6) can interact with the AHR and the ARNT to block the interactions between both proteins. As can be seen in the three poses represented in the environment of the interactions with the amino acid residues, this compound interacts through hydrogen bonds with the Lys79 residue and through π-π interactions with the Phe260 residue, also through hydrophobic interactions this compound is probably capable of interfering by causing steric impediments and affecting the atomic contacts established

between the following residues at the level of the bHLH/PAS-A interface and the PAS-A domain of AHR. Phenylalanine Phe260 is reported by Corrada *et al.* (2017) as an important residue at the PAS-A domain level for interaction with ARNT [6]. In the case of interactions with ARNT, compound **1** interacts through hydrogen bonds with residues Thr110, Ser120, where only serine has atomic contacts (≤6Å) with AHR residues, so it can affect these interactions. In the case of the threonine residue (Thr110), it is not an important residue nor does it present atomic contacts with its partner (ARNT) at a distance ≤ 6Å. The amino acid residues between 88 and 128 of ARNT are important for DNA binding to develop its activity in combination with its partner (UniProt P53762) [33].

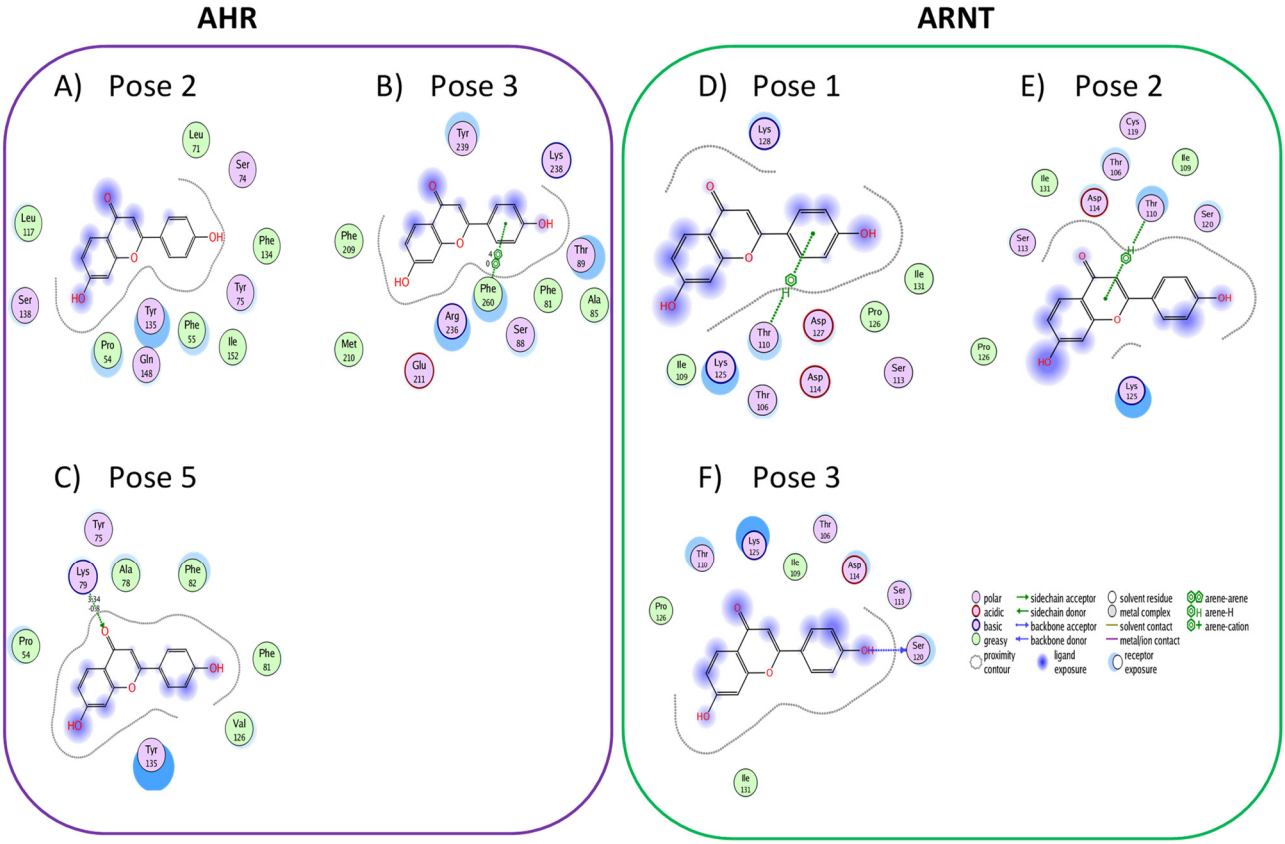


Figure 6. Interaction network between monomeric proteins AHR, ARNT and the studied compound **1**.

Similarly to compound **1**, compound **2** (fisetin) also interacts with the two proteins under study (putative target), with a greater probability of direct interaction with ARNT compared to AHR (Figure 7). Interactions at the AHR level are established at the bHLH/PAS-A interface of the AHR. The binding by hydrogen bonds to the Arg236 and Gly250 residues enable the interaction and interference with amino acid residues in that region and that present some atomic contacts (≤6Å) with the amino acids of the ARNT partner. In this specific case, the identified amino acid residues are listed below: Val73, Leu76, Arg77, Phe81, Phe82, Ser88, Arg236, Lys238 and Phe260. It is important to note that in this region the amino acid Lys238 is reported to be important for the binding of the AHR:ARNT heterodimer to the DRE box to develop target gene activation activity (UniProt P30561) [1,24,33]. The interaction of compound **2** with ARNT is also important, and like compound **C**1, it interacts at the level of the bHLH domain with bonds to the two threonine and serine residues highlighted above, but also establishing a bond of this type with the Ser228 residue that is not a residue with atomic contacts with the AHR, but in a region where there are amino acid residues capable of interacting with the AHR (e.g., pose 5, Arg261, Ile312, Lys313, Ala314 and Phe335). Compound **2** can cause interference through hydrophobic interactions

at the level of these bHLH and PAS-A domains at ARNT, in the latter by binding to the α-helix where residues Leu164, Lys165, Ile168, Leu176 and Tyr188 are found, all with atomic contacts ($\leq$6Å) with AHR according to the analyzed model. This is in correspondence with those reported in UniProt, which reports that the surface region between residues 167 and 171 of AHR is essential for the dimerization process of ARNT with AHR (UniProt P53762) [33].

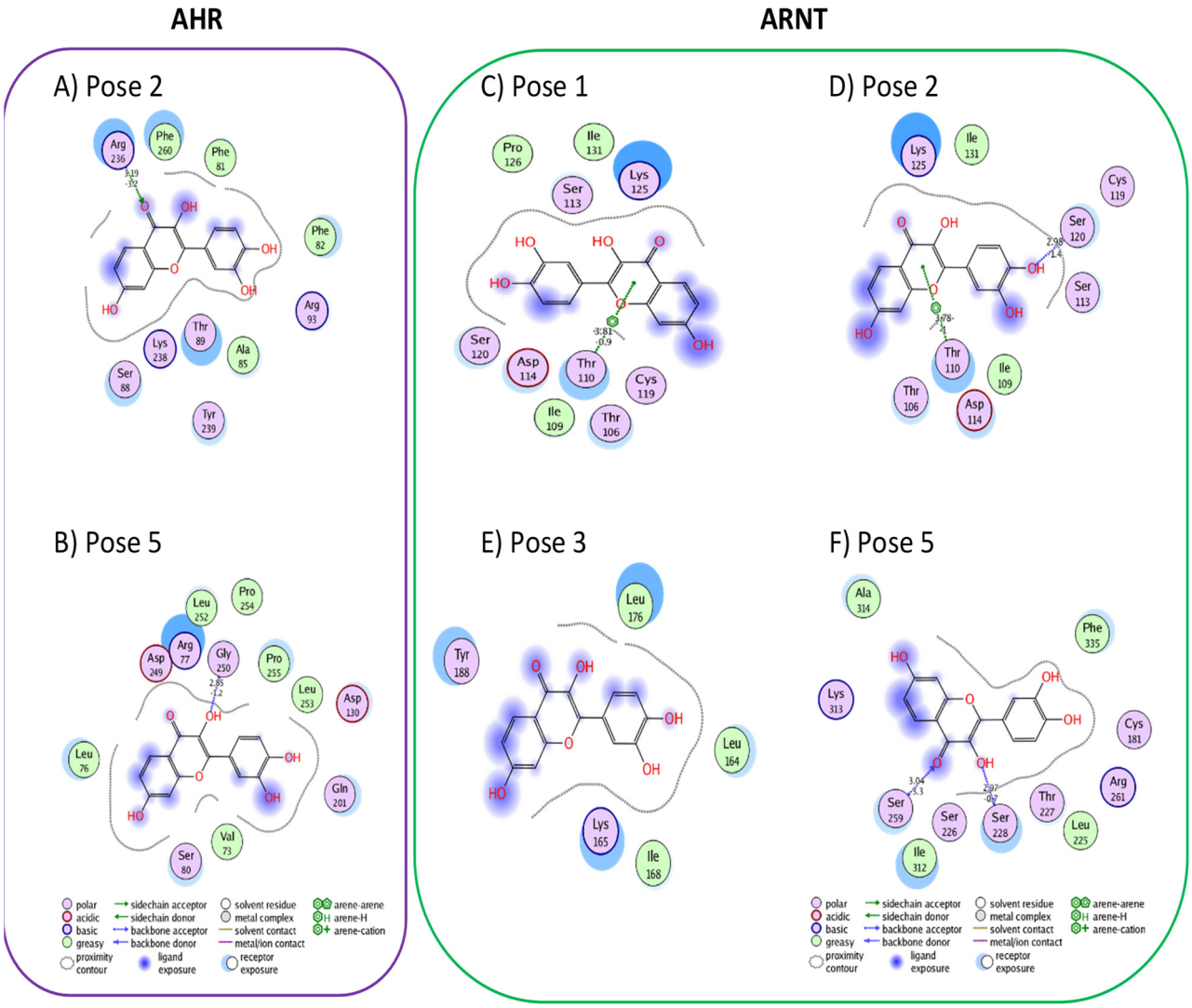


Figure 7. Interaction network between monomeric proteins AHR, ARNT and the studied compound **2**.

Compound **3** (3-methylleuteoline) preferentially interacts with AHR than with ARNT (Figure 8). At the AHR level, this compound interacts with the surface interface between the bHLH and PAS-A domains (bHLH/PAS-A). This compound binds via hydrogen bonds to amino acids Phe55 and Lys79 that establish atomic contacts ($\leq$6Å) with the ARNT partner, and also binds to Asp249, but this is not an important residue for the formation of the heterodimer. In the surface region of interaction of compound **3**, the following amino acids present some type of atomic contact ($\leq$6Å) with ARNT, and are listed below: Pro54, Phe55, Leu71, Ser74, Try75, Arg77, Ala78, Lys79, Phe81, Phe82, Val124, Leu125, Val126, Phe134, Tyr135, Ser138, Gln148, Ile152, Phe260, Ala261 and Ile262. It should be noted that in this surface region are the amino acids Pro54 and Phe55 that are described in UniProt as important, the amino acid sequence 37 to 65 of the peptide chain of AHR as important for DNA binding, Val124 and Phe260 is required for the integrity of the AHR:ARNT,

Phe134 and Ile152 are important for the activity of the AHR (UniProt P30561) [33]. By also binding the compound **3** to the ARNT, it can therefore block the formation of the functional heterodimer, in this case at the interface that encompasses the PAS-A domains and the surface between the PAS-A and PAS-B domains (PAS-A/PAS-B), so through a hydrogen bond with the amino acid Lys334 and hydrophobic interactions it can cause steric impediments for the formation of the heterodimer and thus interfere with the atomic contacts (≤6Å) that can be established at the level of some of the ARNT amino acids listed below: Asp173, Ala185, Val 187, Phe205, Val305, Trp315, Lys334, Phe335, Leu343, Gln344 and Tre346. It is important to highlight that Val 305 of this group is reported as important to guarantee the stability of AHR:ARNT (UniProt P53762) [33].

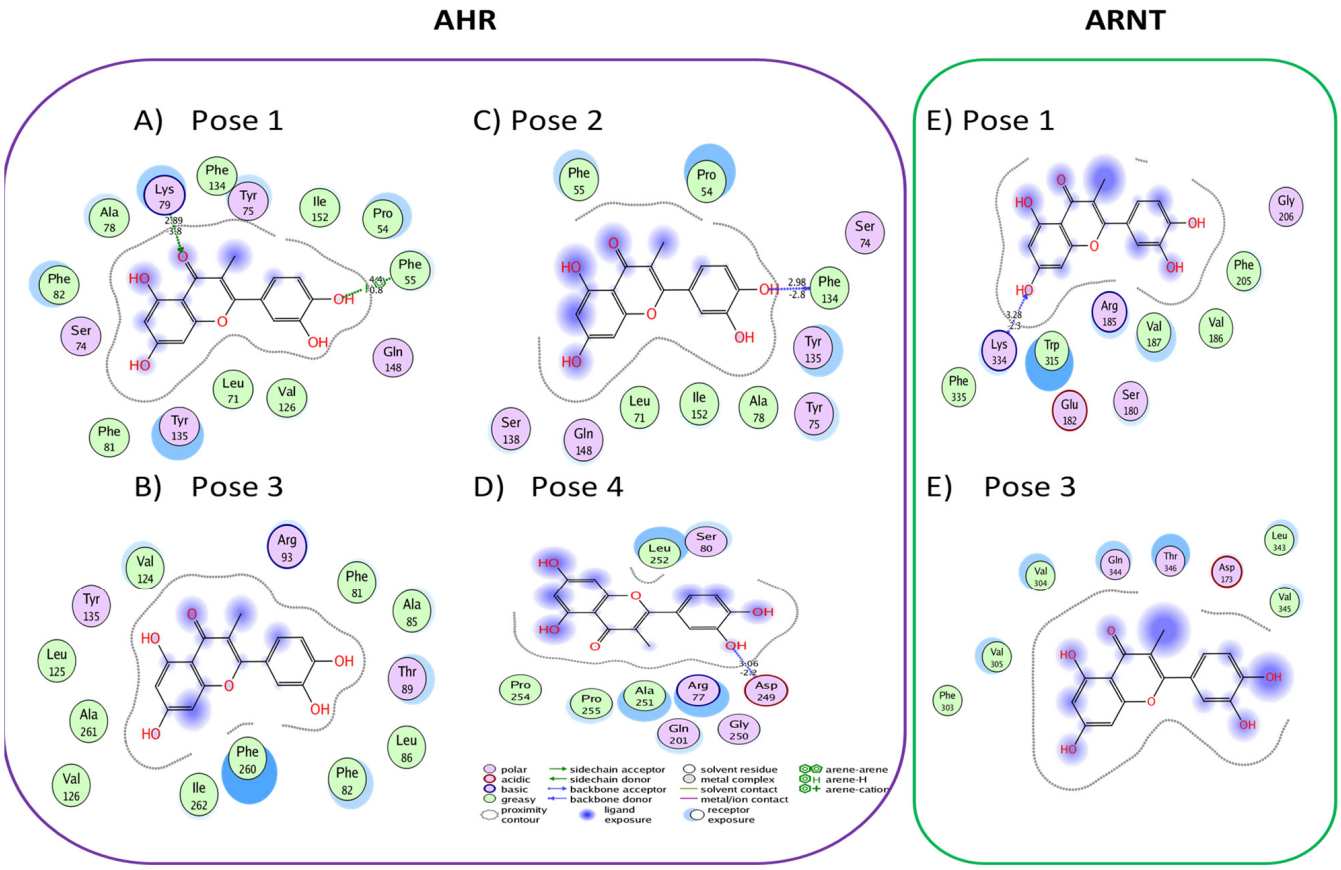


Figure 8. Interaction network between monomeric proteins AHR, ARNT and the studied compound **3**.

Compound **4** (Apigenin, Figure 9) and presents hydrogen bond bonds with the AHR and with ARNT. At the AHR level, this compound interacts at the level of the surface that forms between the bHLH and PAS-A (bHLH/PAS-A) domains, in the environment of the same amino acids listed for the case of compound **C3**, except for the following: Phe55, Arg77, Ser138, Gln148, Ala261 and Ile262 and including Ser88 with which it establishes a hydrogen bond and which is a residue that establishes atomic contacts (≤6Å) and Lys238 whose importance has already been mentioned. In the case of interactions with ARNT, compound **C4** interacts in a similar way to compound **1**.

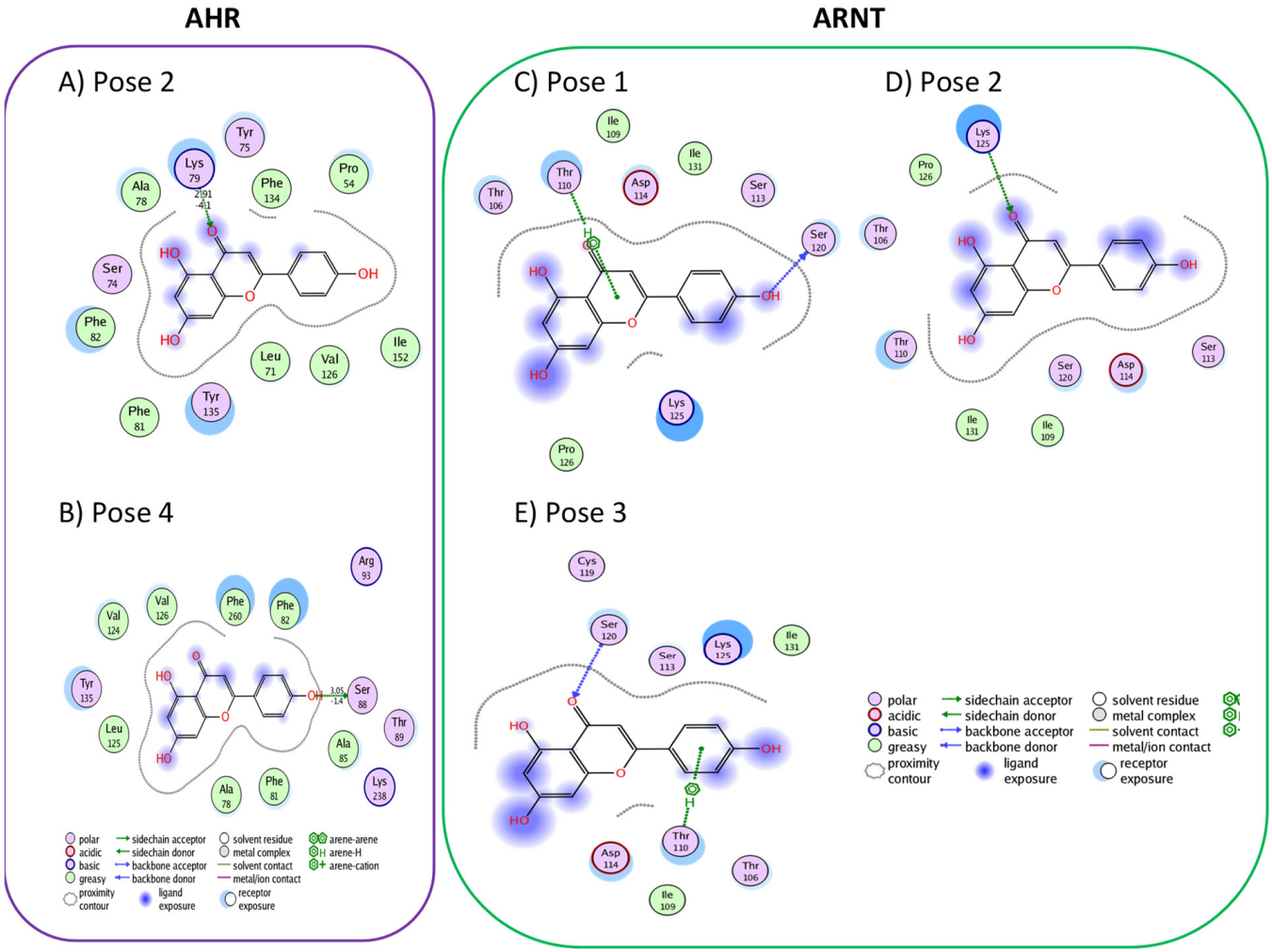


Figure 9. Interaction network between monomeric protein AHR, ARNT and the studied compound **4**.

3.2. Active Flavonones with a Probability of Blockage Higher Than 50%

Compound **5** (flavone) exhibits a preferential interaction with ARNT over AHR, but with a 50% probability of blocking the formation of the AHR:ARNT heterodimer (Figure 10). The binding of compound **5** to AHR is established by interaction through hydrogen bonding with Ser88 in a region where other amino acids also establish atomic contacts (≤6Å, Thr89, Arg236, Lys238 and Phe260), standing out the same considerations as those mentioned above for Lys238 and Phe260. The level of ARNT, compound **5** binds in the bHLH domain to Thr106, Thr110, Ser113 and Lys125, which do not have atomic contacts with the amino acids of the AHR, but it does bind to the Leu164, which does have contacts with AHR. However, they can interfere with some of the other amino acids that do interact with the AHR (e.g., Ile109, Asp114, Asp127 and Lys128). At the level of the α-helix in the PAS-A domain of ARNT where the Leu164 found (Lys165, Ile168, Leu176 and Tyr188), compound **5** can interfere with this surface by binding to this residue, which is important for establishing interactions with the AHR.

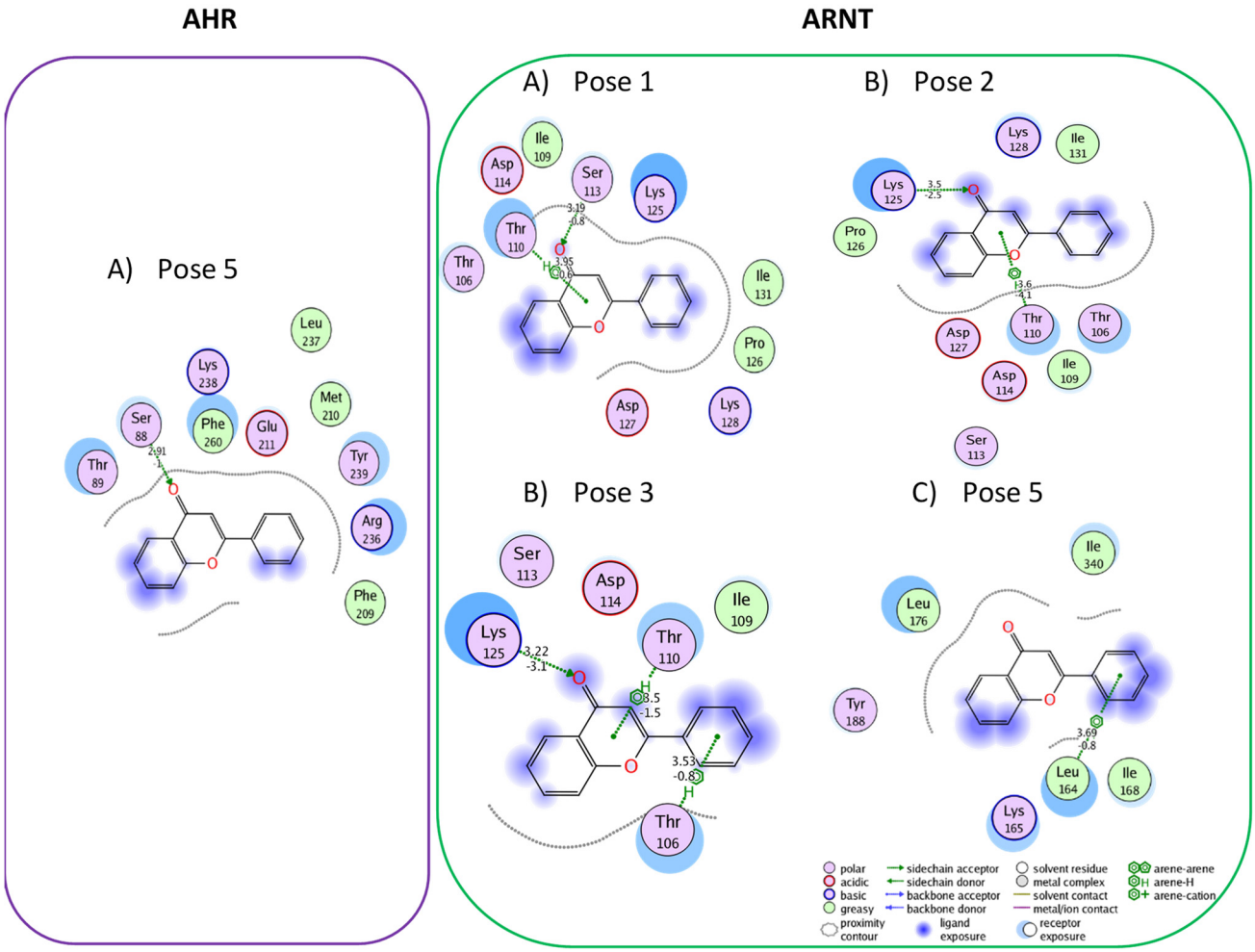


Figure 10. Interaction network between monomeric proteins AHR, ARNT and the studied compound **5**.

Compound **6** (galagin) exhibits a preferential binding behavior for ARNT comparing to AHR; However, based on the structural analysis criteria, it is possible that it interferes by 50% in the formation of the AHR:ARNT heterodimer (Figure 11). Like the previous compounds, this compound binds to the bHLH/PAS-A surface interface through interactions that are established with the following amino acid residues that have atomic contacts (≤6Å) with ARNT and include Lys79, Ser88 and Phe260. The importance of its interaction and bind with Phe260 is also highlighted, which is in correspondence with that reported by Corrada et al. 2017 and UniProt P30561 [6,33], as well as the presence on that surface of Pro54, that is present in the region that is important for the binding of AHR to DNA between residues 37 and 65, the Val124 that is reported as necessary to guarantee the integrity of the AHR, the Phe134 whose mutation by aspartic acid causes the decrease in the activity of the AHR, and Lys238 which mutation by aspartic acid would reduce the binding affinity of the heterodimer to the DRE (UniProt P30561) [33]. Compound **6** binds in ARNT to amino acids Thr106, Thr110, Ser113, Pro126, Asp114 and Asp127, where the first four do not have atomic contacts (≤6Å) with AHR, but Asp114 and Asp127 do. It should also be noted that somehow, compound **6** can interfere with the interactions established by the following ARNT amino acids that have already been previously mentioned (Ile109 and Ser120).

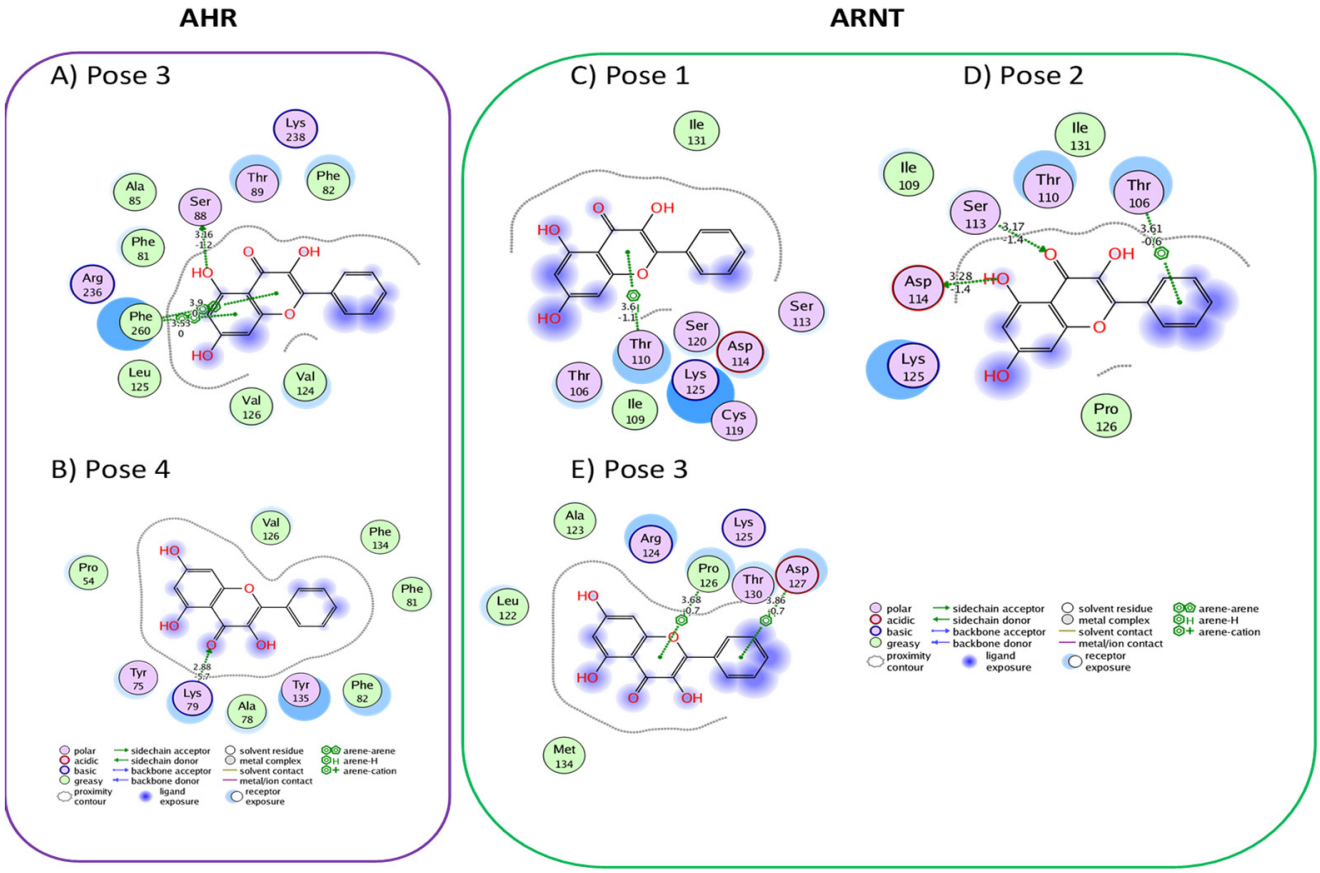


Figure 11. Interaction network between monomeric protein AHR, ARNT and the studied compound **6**.

The compound **15** [5-hydroxy-2-(4'-hydroxy)-7-methoxy-flavonone, Figure 12] also interacts with AHR and ARNT, with AHR it binds through the amino acids Arg78 and Lys79 that present atomic contacts (≤6Å). In this surface environment, there are amino acids that in various ways contribute to the formation and stability of the AHR:ARNT heterodimer, such as Phe260 and Leu240, which are reported to be also important for the affinity of the heterodimer to the DRE (UniProt P3056) [33]. At the level of the interfaces that are formed in bHLH, PAS-A, and PAS-A/PAS-B at ARNT, compound **15** interacts through hydrophobic interactions, highlighting the interaction that occurs in the α-helix where the residues are found Asp161, Leu164, Lys165, Ile168, Leu176 and Ile340, all with atomic contacts (≤6Å) with AHR.

Compound **16** (7-hydroxy-5-methoxy-flavonone, Figure 13) has a preferential binding percentage for AHR to ARNT. Due to hydrophobic interactions, it binds to the bHLH domain where Ile109, Asp114, Asp127 and Lys128 have atomic contacts (≤6Å) with AHR. With the AHR, compound **16** only binds to two arginine residues (Arg217 and Arg236), Arg217 does not have atomic contacts with ARNT and Arg236 does, as already mentioned for compound **5**. As previously described, the binding of this compound on the surface of AHR developed in the bHLH/PAS-A interface, and in the PAS-A/PAS-B of AHR (Lys238 and Phe260), are important residues for the formation of the heterodimer.

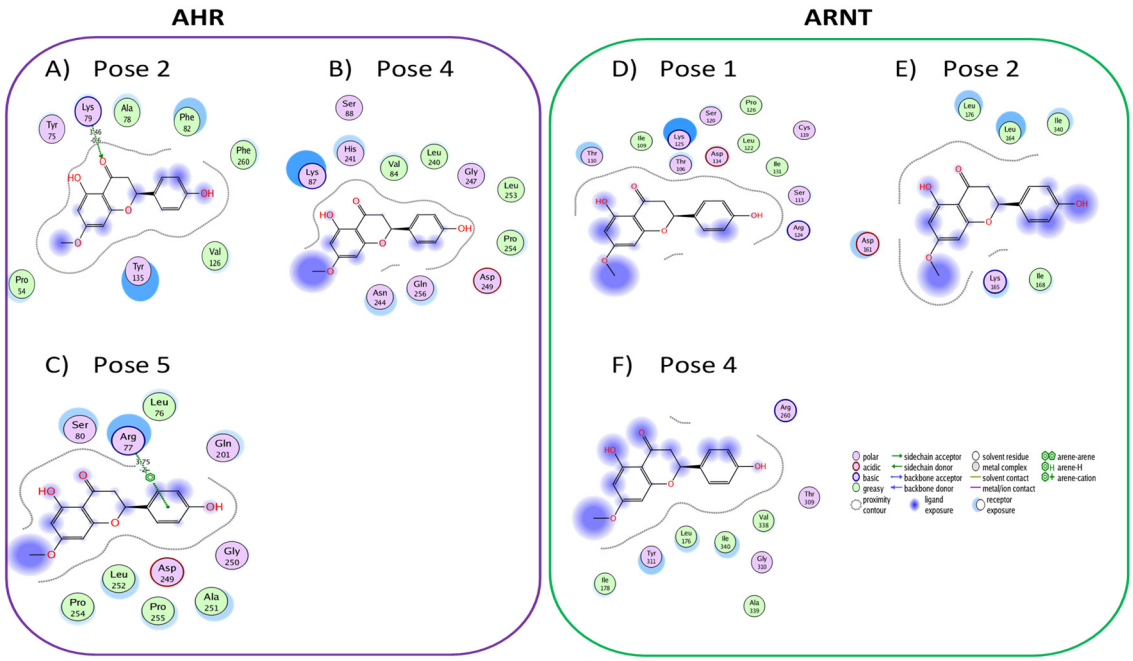


Figure 12. Interaction network between monomeric protein AHR, ARNT and the studied compound **15**.

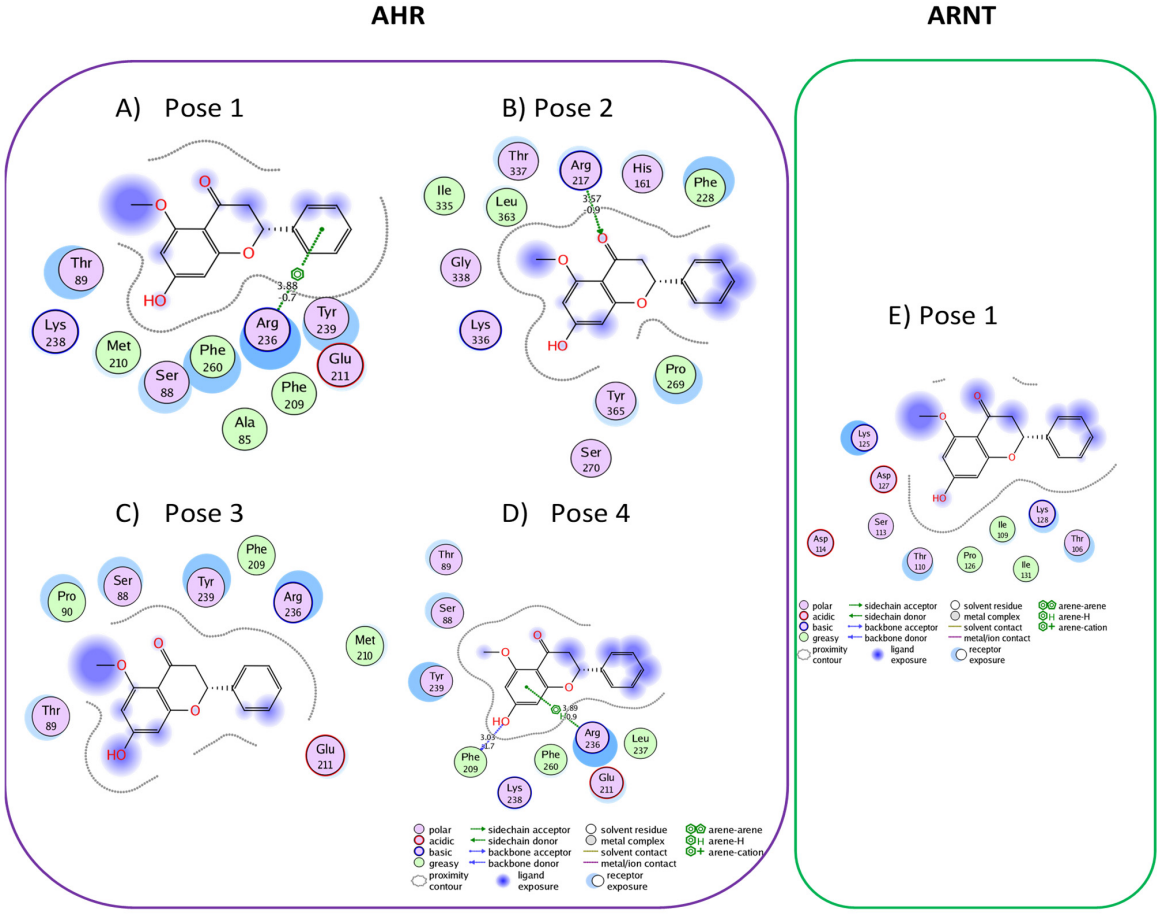


Figure 13. Interaction network between monomeric protein AHR, ARNT and the studied compound **16**.

In summary, the compounds that present the highest percentages of probability to modulate the blockade of the formation of the functional AHR:ARNT heterodimer due to their binding to important interfaces according to the results of the AHR molecular docking are compounds **3**, **1**, **15** and **16**, while for ARNT are compounds **2**, **5**, **1**, **4**, **6** and **15**. From all the studied flavonoids, compounds **1** and **15** have equivalent potential to modulate the blockade of this heterodimer. The best compound to bind to AHR is compound **3**, and best compounds to bind ARNT it is compounds **2** and **5**.

3.3. Proof of Concept

In the A549 lung cancer cell line in which the AHR is overexpressed [34–37], compound **20** presented a percentage of activity of 40% in the selected modeling conditions. This compound (5,7-diacetoxy-3-phenylcoumarin) proved to bind to the bHLH/PAS-A interface (Figure 14), interfering through possible steric impediments, with the formation of the AHR:ARNT heterodimer by binding to AHR through hydrophobic interactions. The coumarin ring has the ability to establish different types of non-covalent interactions including hydrophobic, π-π and electrostatic forces, as well as hydrogen bonds, van der Waals forces, among others, with other active molecules and the active site on different targets of living organisms [38].The structure of the coumarin nucleus allows the formation of π-π type interactions in the rings with residues of a hydrophobic nature in the environment of the protein surface (hydrophobic patches), in particular with Phe260, Tyr75 and Tyr135.

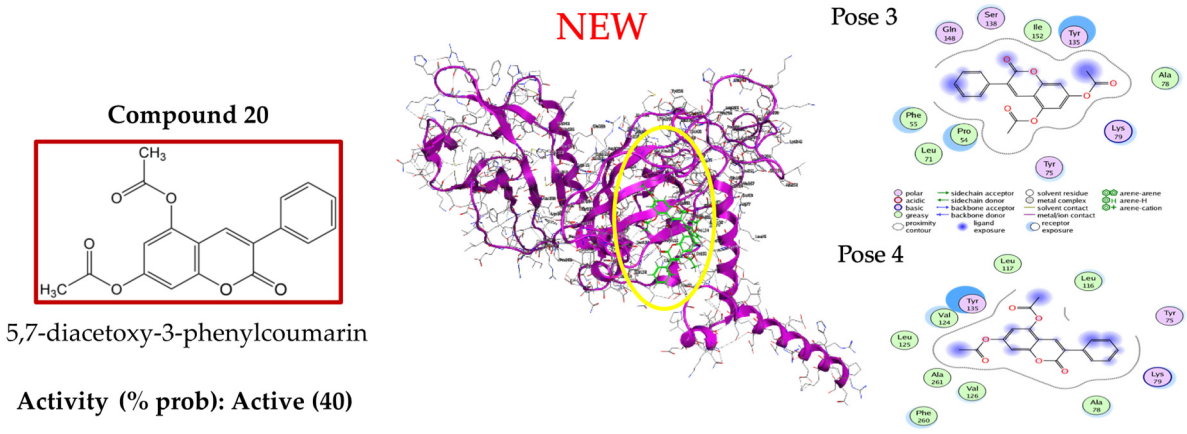


Figure 14. Interaction network between monomeric protein AHR, ARNT and the studied compound **20**.

The proof of concept carried out with coumarin **20** comparing to the studied flavonoids obeys more than a structure-activity relationship, and is directed towards a structural identification at the level of the AHR receptor as a possible new surface binding site (site allosteric) for the modulation of the activity of this receptor in the formation of the functional AHR:ARNT heterodimer, since the antiproliferative effect of flavonoids is fundamentally associated with their activity as an antioxidant [9,10,39]. Compound **20** decreased the proliferation of A549 lung cancer cells below 50% (Table 3). Therefore, it is expected that flavonoids that also bind to this interface bHLH/PAS-A of AHR, may modulate the antiproliferative activity via another mechanism of action. The properties of the surfaces of the domains and protein-protein interfaces can be predicted by appropriate docking methods or by template-based models. Therefore, these models only represent the starting point to analyze the processes by which ligands can affect AHR activation, dimerization, and transformation in their functional form and DNA binding [40]. Therefore, in a preliminary way, all this structural information can be used for the identification of new molecular entities capable of interfering in the activity of the AHR.

Table 3. Antiproliferative activity of compound **20** on A549 lung cell line.

Concentration (μg/mL)	Determination 1	Determination 2	Mean	% CV
8	14,455	19,804	17,129	2208
16	12,551	9956	11,254	1630
32	14,018	15,413	14,716	670
64	8487	9921	9204	1102
128	2486	2740	2613	686
256	−1702	−1501	−1602	−885

4. Conclusions

Eight flavonoids with potential activity blocking the AHR:ARNT heterodimer have been identified by molecular docking, with probability percentages between 50 and 60%. The flavonoid interactions at the interface surfaces of the bHLH and PAS-A domains of AHR and ARNT have proved to occur mainly by hydrogen bonding and hydrophobic interactions, without binding to the PAS-B domain of AHR. A new surface binding site is proposed at the level of the bHLH/PAS-A interface as the most likely site to which 74% of the studied flavonoids bind to the interfere with the formation of the complex. Glycosylated flavonoids showed AHR:ARNT blocking percentages of lower than 50%. In the case of flavonones, the incorporation of a hydroxyl group in position R4′ proved to increase the activity by 30%. It is estimated that the flavonoids identified as active display antiproliferative activity in lung cancer cells (A549) due to the structural similarity with the nucleus of the evaluated coumarin, which presented a blocking percentage of the AHR:ARNT of 40%, binding to the same site of the AHR surface interface. As proof of concept, the synthetic 5,7-diacetoxy-3-phenylcoumarin have been studied. This compound proved to decrease the proliferation of A549 lung cancer cells below 50%.

Supplementary Materials: The poster presentation can be downloaded at: https://www.mdpi.com/article/10.3390/ecsoc-25-11766/s1.

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