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Docking, synthesis and evaluation of the antitumoral activity of 3-arylcoumarins in MCF-7 cells

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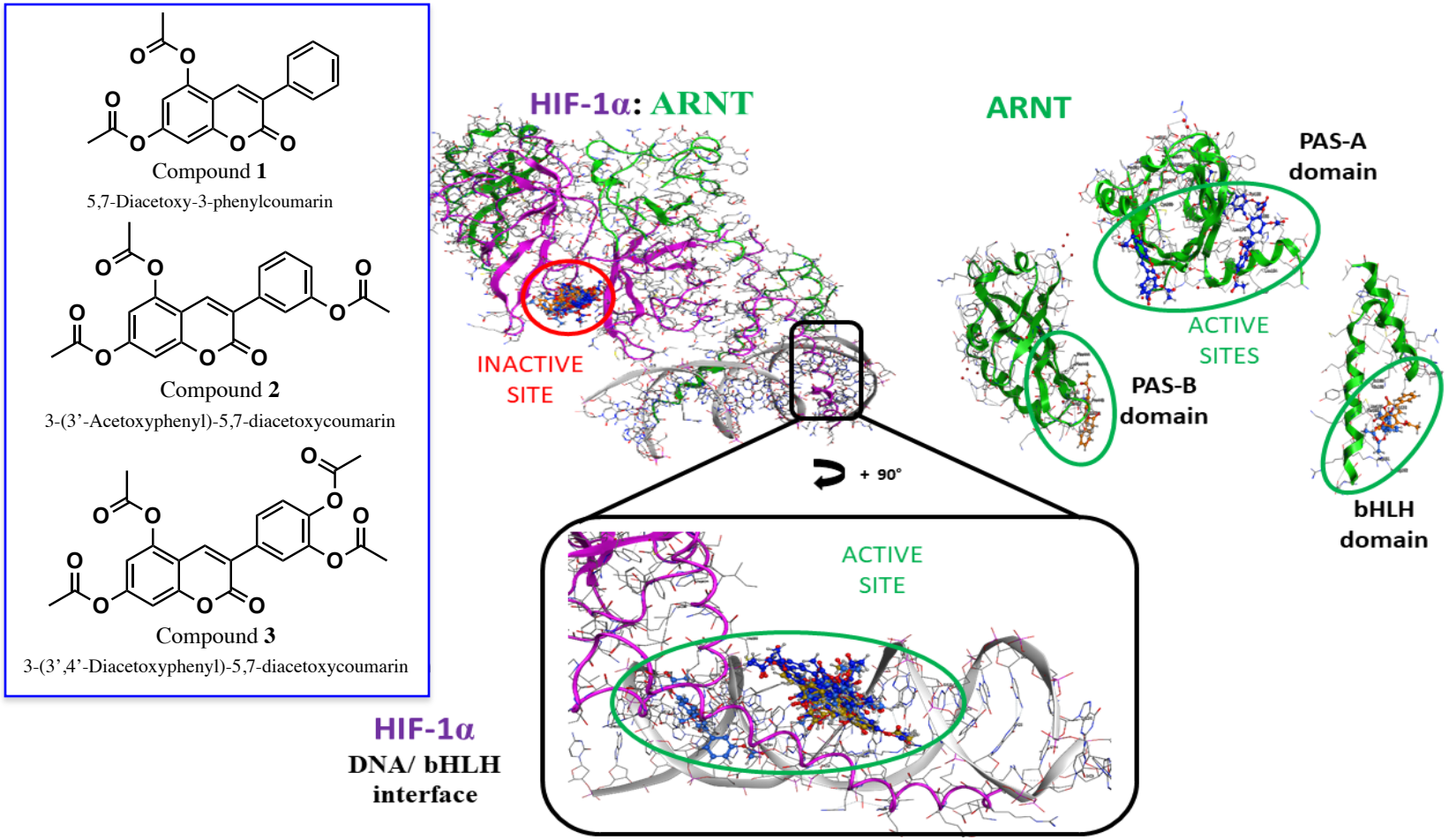
Graphical Abstract



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Abstract: Biological investigations of coumarins have revealed innumerable pathways by which they act as anticancer agents, including the hypoxia-inducible factor (HIF-1α) pathway. Hypoxia is an almost universal hallmark of HIF-1α overexpressing solid tumors in patients with breast cancer. Taking this into account, and considering the central role of HIF-1α, we proceeded to determine by molecular docking the interaction of three compounds bearing the 3-arylcoumarin scaffold with HIF-1α and ARNT, to block the formation of the functional heterodimer, and evaluate the antiproliferative activity in MCF-7 of the synthesized compounds. To perform the molecular docking, crystalline structures of the monomer HIF-1α (PDB: 4zpr), ARNT (PDB: 4zp4) and the heterodimer HIF-1α:ARNT (PDB: 4zpr), the program MOE 2019.01, and several servers, were used. Different interaction sites were identified to block the HIF-1α:ARNT, the interfaces between the DNA/bHLH domains in HIF-1α, and the bHLH and PAS-A domains in ARNT. The probability of blocking HIF-1α:ARNT, resulting from molecular docking, has been 40-60% for the three coumarins –5,7-diacetoxy-3-phenylcoumarin, 3-(3'-acetoxyphenyl)-5,7-diacetoxycoumarin and 3-(3',4'-diacetoxyphenyl)-5,7-diacetoxycoumarin– coinciding with the results of the antiproliferative evaluation in breast cancer cells, as well as with the results of luciferase activity adjusted versus control at the concentration of 256 µg/mL. The three compounds of the 3-arylcoumarin series are identified as interfering with the blockade of the heterodimer HIF-1α:ARNT and exhibiting antiproliferative activity.

Keywords: 3-arylcoumarin, breast cancer, docking, hypoxia-inducible factor, medicinal chemistry.

Introduction

Cancer is characterized as a major public health problem due to its high prevalence worldwide. The United States National Cancer Institute estimates that 12.3% of women will be diagnosed with breast cancer at some point in their life. In Cuba, during 2018, breast cancer represented the second with the highest incidence with 3.748 new cases, and an increase of 215 cases compared to 2017.

The hypoxia-inducible factor (HIF-1α) have become a target for developing new cancer therapies since the early 1990s. Its functions include the regulation of angiogenesis and glucose metabolism, and other genes that play a role in reducing the effectiveness of cancer therapies, such as radiation, chemotherapy and immunotherapy.

Many naturally polyphenols, as well as their derivatives, have been studied for their possible use as anticancer agents. Coumarins have been reported as compounds that present antitumor activities. Considering all the above, we proceeded to determine by molecular docking the interaction of three compounds bearing the 3-arylcoumarin scaffold with HIF-1α and ARNT, to block the formation of the functional heterodimer, and evaluate the antiproliferative activity in MCF-7 of the synthesized compounds.

Docking studies

The results of the molecular docking of the interaction of the three coumarin compounds evaluated indicate that each compound binds to a different interface when the proteins are in the form of monomers (HIF-1α, ARNT) or heterodimer (HIF-1α: ARNT).

At the level of the HIF-1α monomer, the three compounds bind to the interface formed between the DNA chain and the bHLH domain of this protein. Therefore, when this monomer is going to bind to the HREs in target genes to activate transcription, the formation of the functional heterodimer at that interface can be blocked (Figures 1-3). In the case of the ARNT partner (constitutive of the cell nucleus), two of the coumarins (compounds **1** and **2**) also interact with the bHLH domain of ARNT (Figures 4 and 5), thus complementing the blocking of heterodimer formation.

Other possible active sites are identified to block protein-protein interactions in ARNT, resulting consistent with the results of alanine mutations (not shown) for the PAS-A and PAS-B domains (Figures 4 and 6).

When the heterodimer is formed, the three compounds do not find a free interface to bind to the aforementioned sites and can only bind to the interface between the PAS-A and PAS-B domains of HIF-1α.

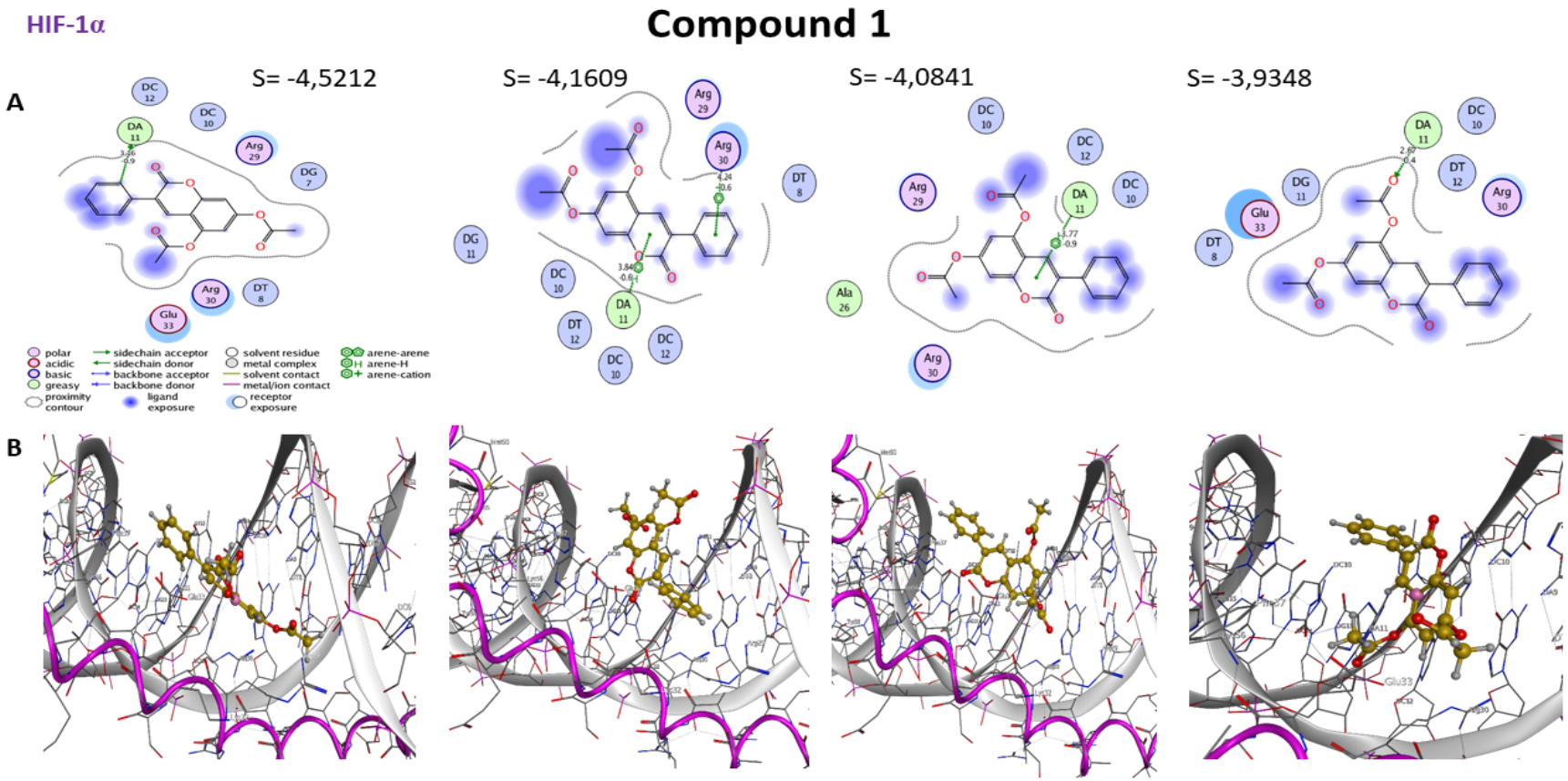


Figure 1. Molecular Docking in the PPI active site. **A.** Interaction network between monomeric protein HIF-1α and the studied compound **1**. **B.** Active poses of compound **1** in DNA/bHLH interface HIF-1α. S: Scoring.

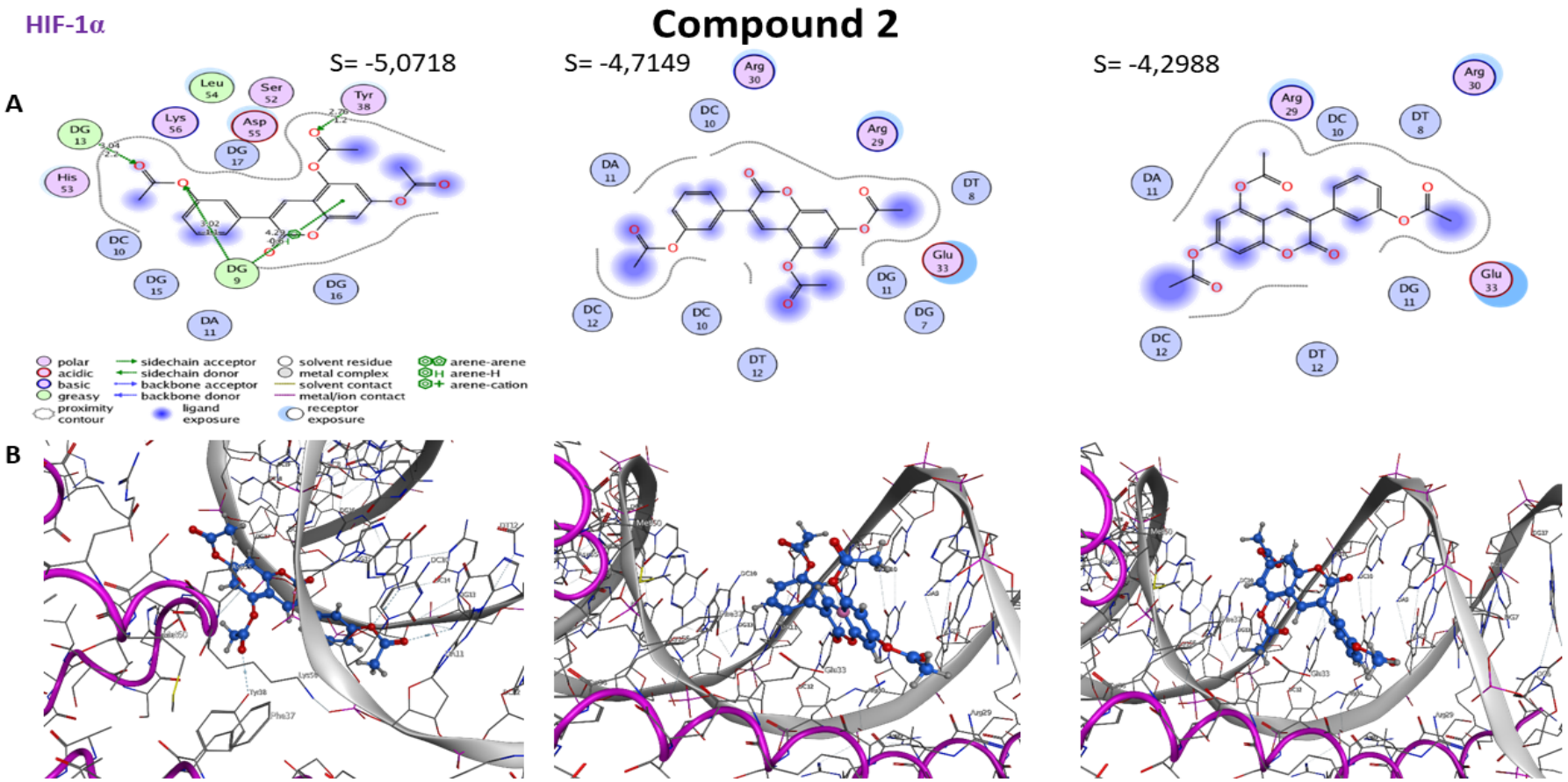


Figure 2. Molecular Docking in the PPI active site. **A.** Interaction network between monomeric protein HIF-1α and the studied compound **2**. **B.** Active poses of compound **2** in DNA/bHLH interface HIF-1α. S: Scoring.

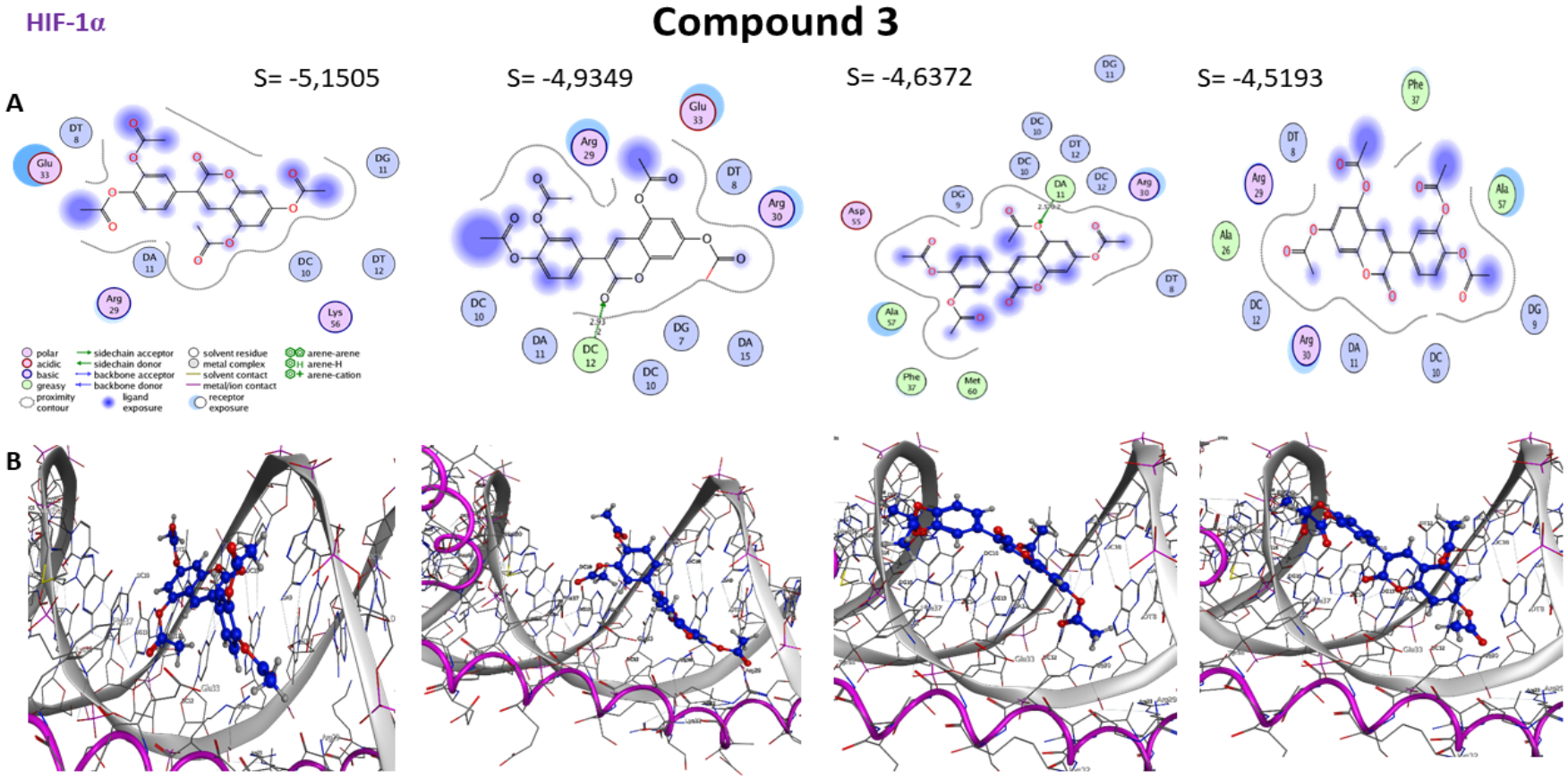


Figure 3. Molecular Docking in the PPI active site. **A.** Interaction network between monomeric protein HIF-1α and the studied compound **3**. **B.** Active poses of compound **3** in DNA/bHLH interface HIF-1α. S: Scoring.

Compared to targeting to the active site, inhibition of protein-protein interactions (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, it is not impossible.

The binding of the three compounds to the DNA/bHLH interface is established through hydrogen bonding and hydrophobic interactions. The expected result is that these compounds produce steric impediment by blocking the interactions that are established between the amino acid residues of the bHLH domains of HIF-1α (Arg29 and Glu33) that have atomic contacts (≤ 6Å). The Glu33 residue establishes interactions with the Leu132 residue (ARNT) which is important for the formation of the heterodimer as it is determined by the analysis of alanine mutations ($\Delta\Delta G^{\circ}$ = 1.29 kcal / mol).

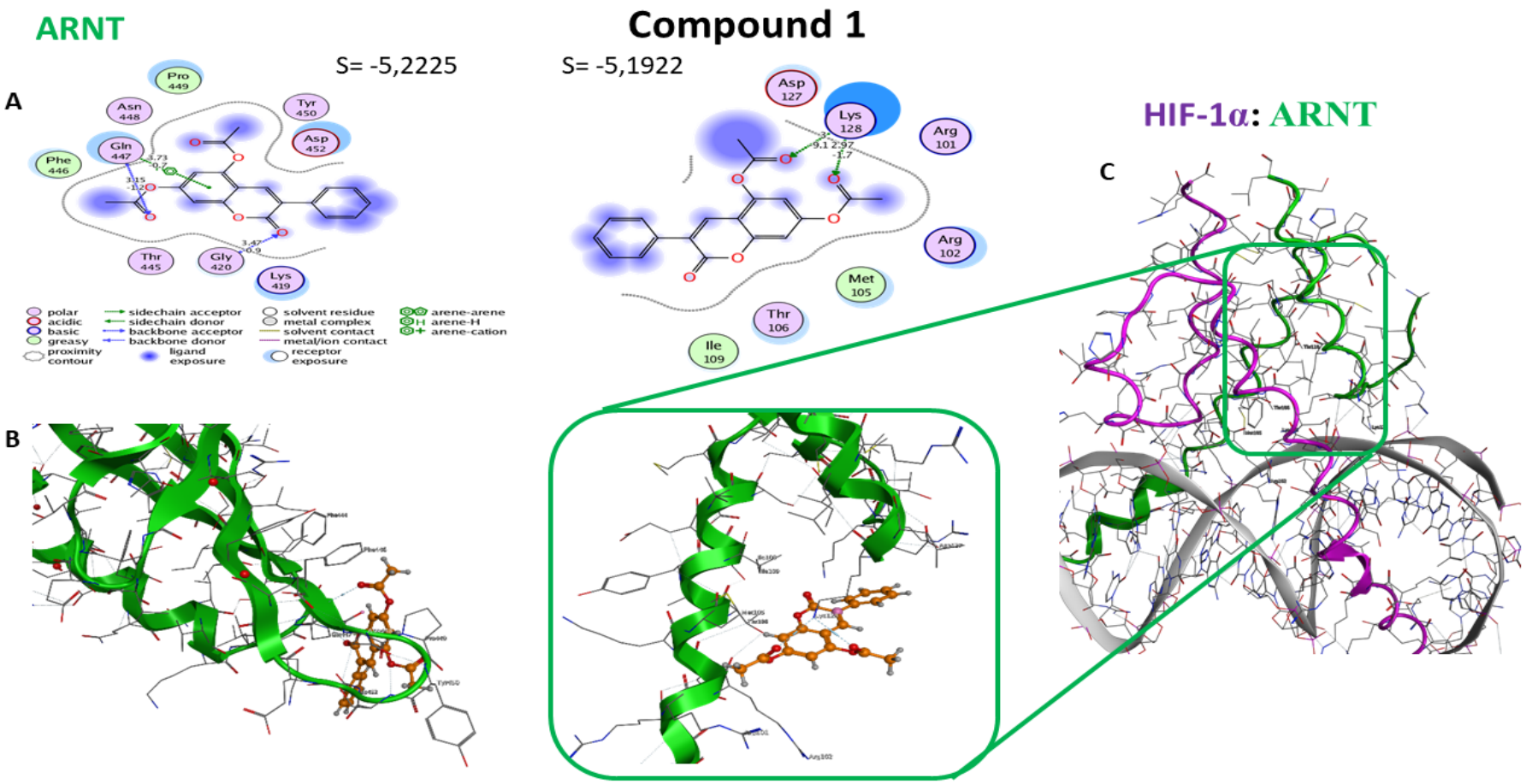


Figure 4. Molecular Docking in the PPI active site. **A.** Interaction network between monomeric protein ARNT and the studied compound **1**. **B.** Active poses of compound **1** in interface ARNT. S: Scoring.

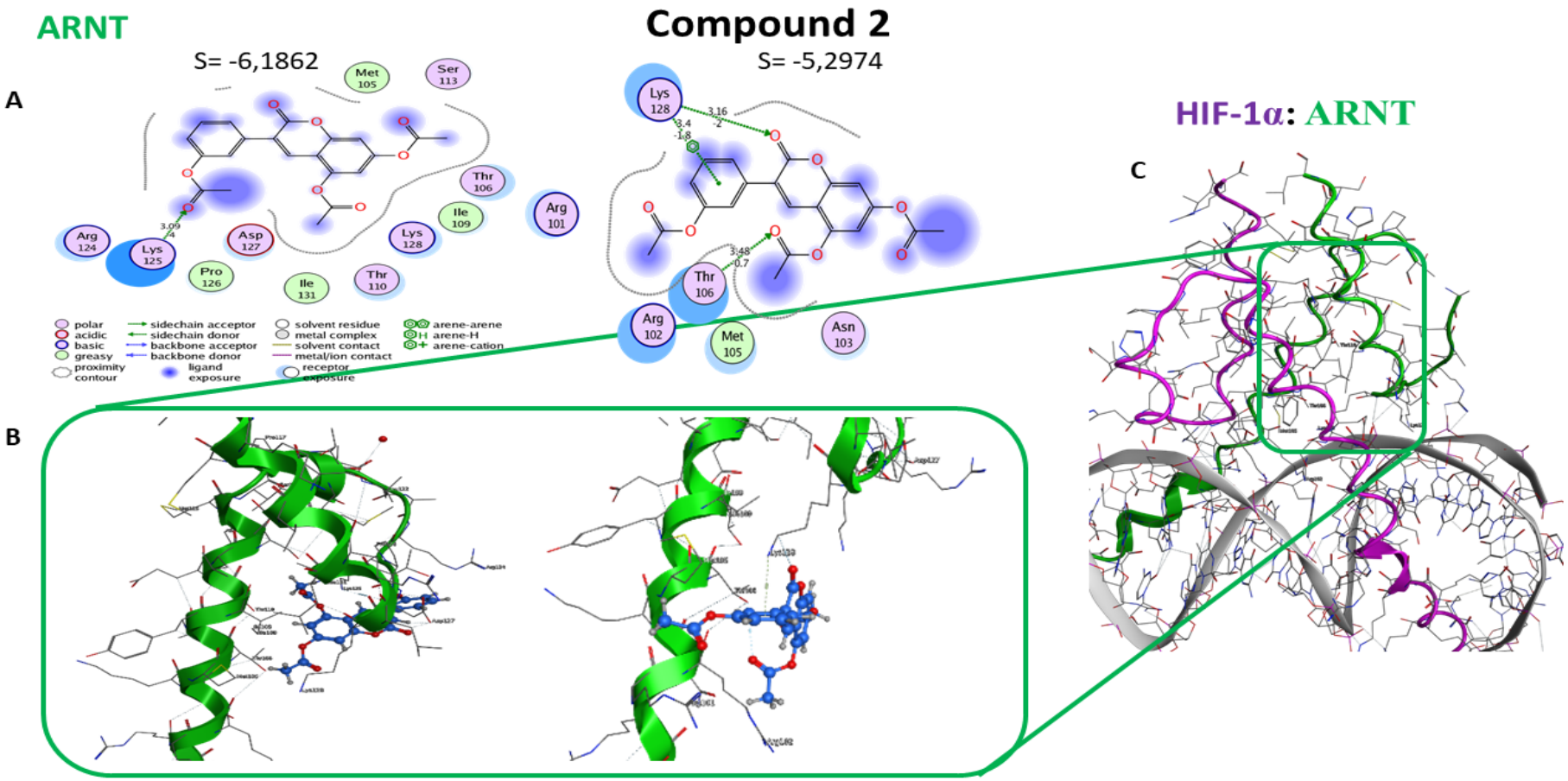


Figure 5. Molecular Docking in the PPI active site. **A.** Interaction network between monomeric protein ARNT and the studied compound **2**. **B.** Active poses of compound **2** in bHLH/bHLH domain ARNT. S: Scoring.

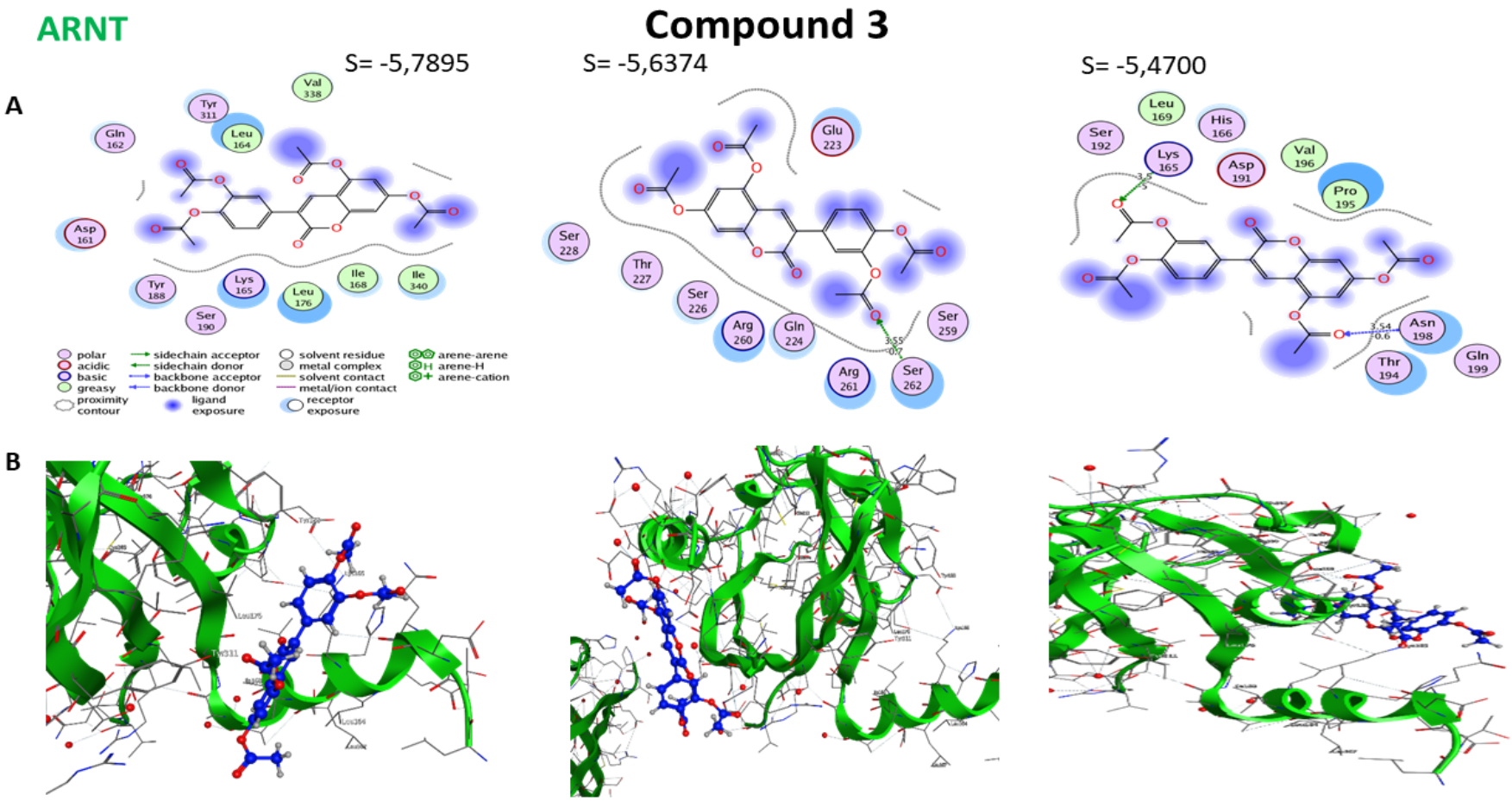
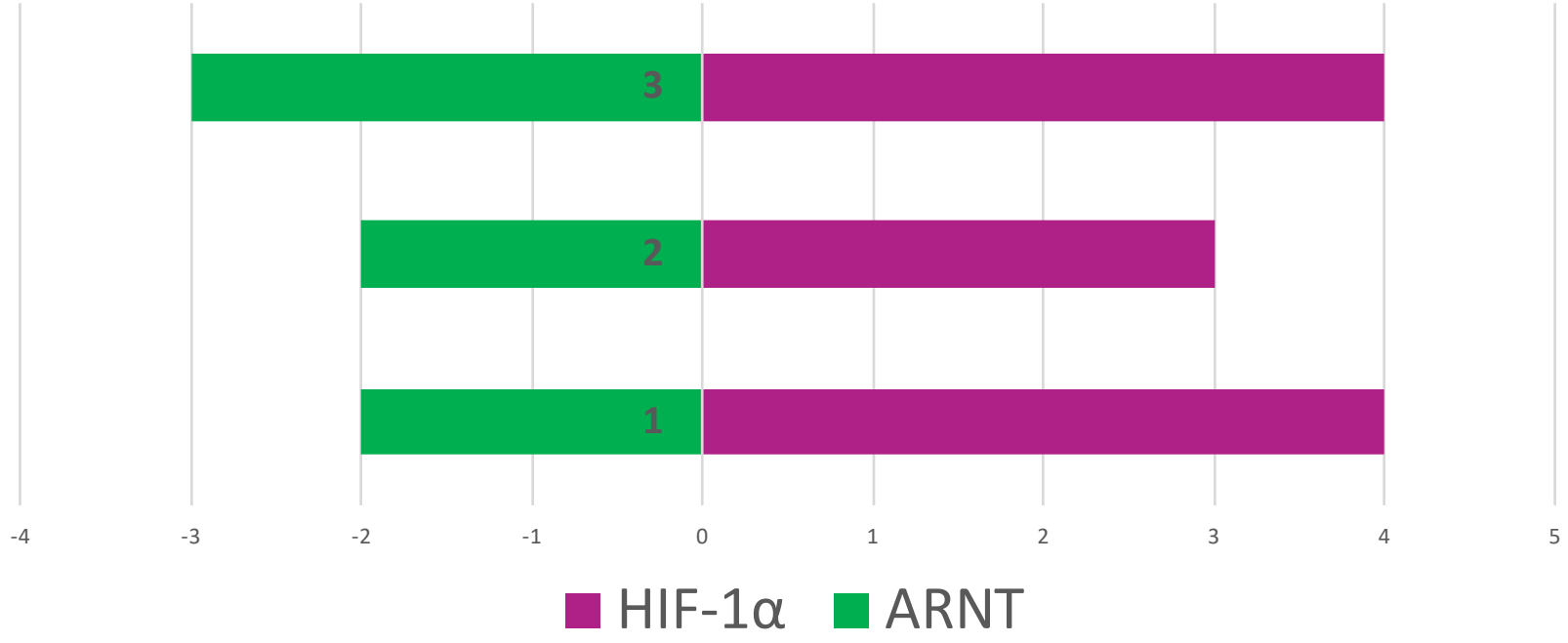


Figure 6. Molecular Docking in the PPI active site. **A.** Interaction network between monomeric protein ARNT and the studied compound **3**. **B.** Active poses of compound **3** in PAS-A domain ARNT. S: Scoring.

The bonds that establish the three compounds with the constitutive partner ARNT are generally established in three active sites or hot spots that were determined by alanine mutations (not shown). Each compound has its peculiarity for at least one different binding site.

Compound **1** first binds the PAS-B domain of ARNT in an important region that includes the amino acid residues Phe446, Asn448 and Tyr450, that are important according to the results of alanine mutations. Compound **1**, when binding to Gln447 residue, can cause steric impediment to binding to partner HIF-1α. The other binding site is in the bHLH domain which, due to steric impediment, and can also interfere with the formation of the heterodimer (Figure 4). In the case of the interactions of compound **2**, they are presented in a similar way to the latter type (Figure 5).

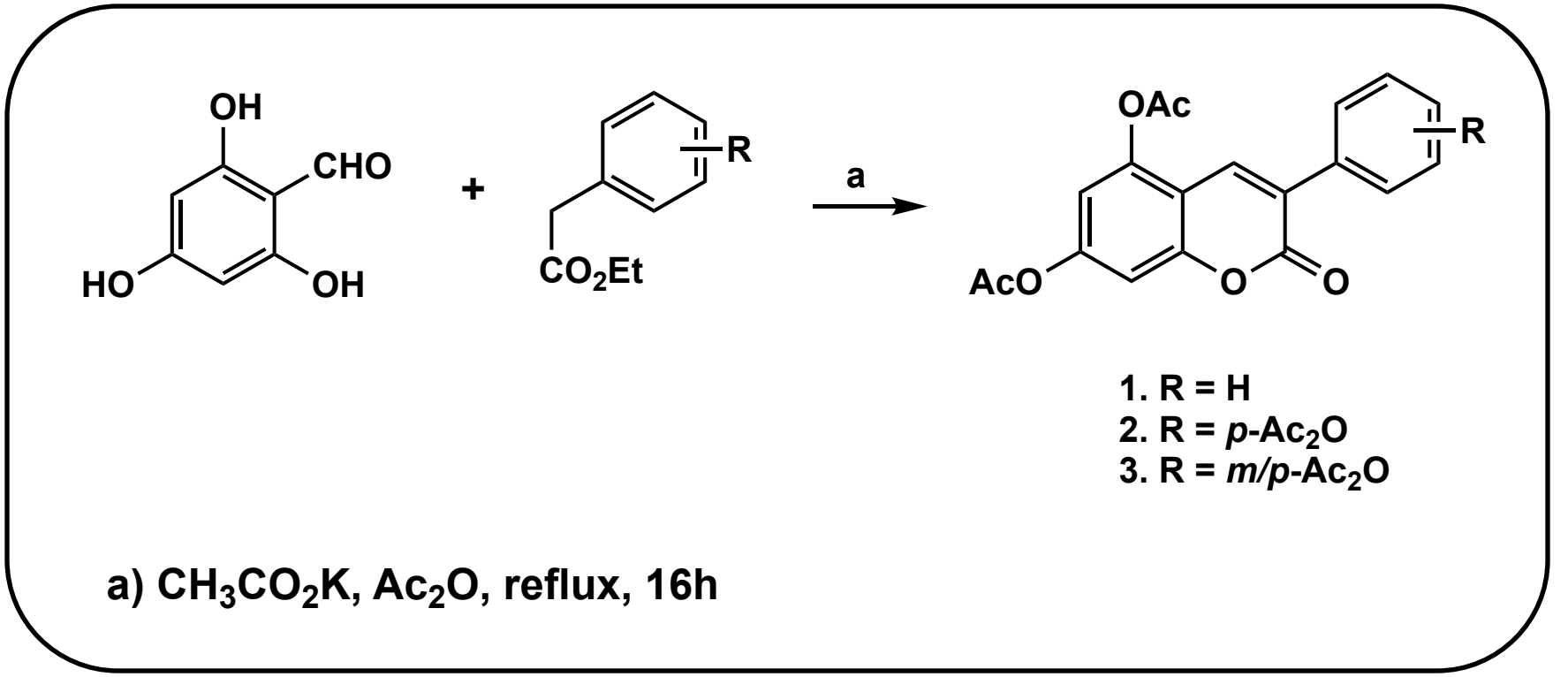
Compound **3**, whose structural difference with the two previous ones is that it has two acetoxyl substituents in the 3-aryl ring, binds preferentially to the PAS-A domain in three interfaces, thus increasing the possibilities of interfering in the formation of the functional heterodimer.



Graphic 1. Poses of binding to active sites in monomers HIF-1α and ARNT for compounds **1**, **2** and **3**. Maximum number of poses evaluated: 5.

None of the three studied compounds binds in their entirety to active sites to block PPIs.

Synthesis



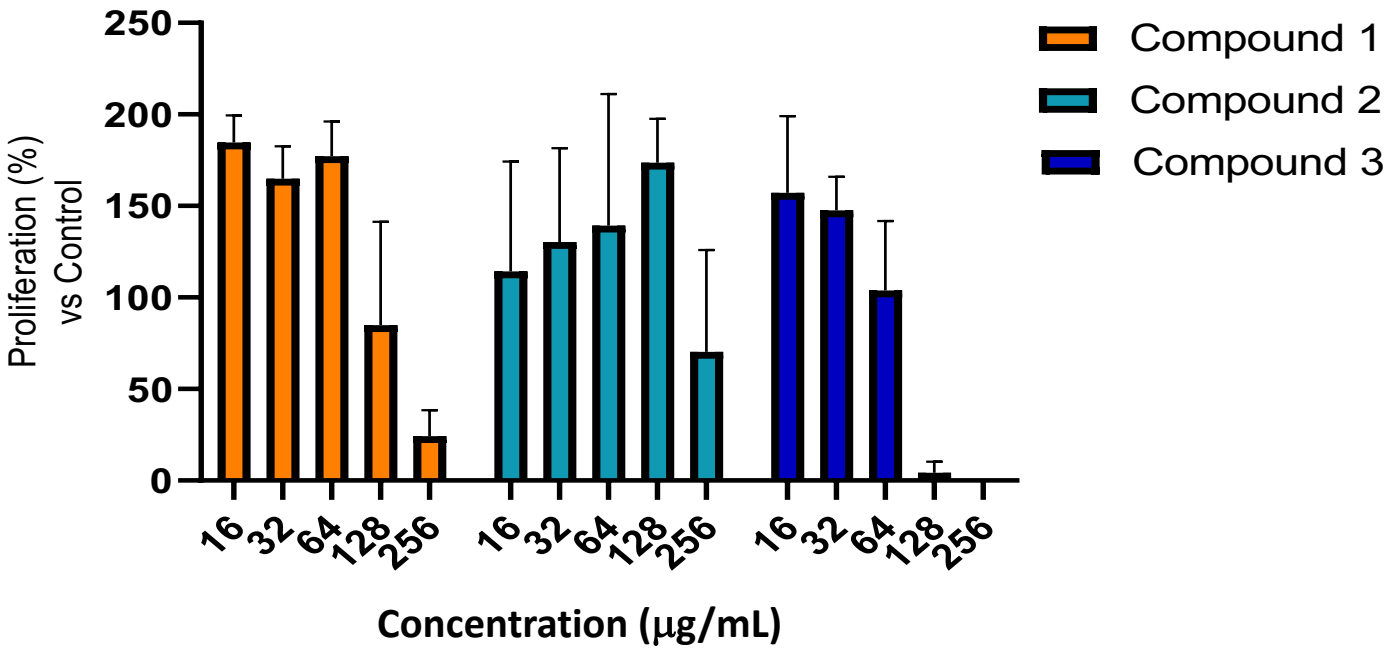
Scheme. General synthetic methodology for compounds **1**-**3**.

3-arylcoumarins were synthesized in good yields via Perkin−Oglialoro condensation reaction.

General procedure: A solution containing the 2,4,6-trihydroxybenzaldehyde (1.67 mmol), anhydrous CH_3CO_2K (2.94 mmol) and the corresponding arylacetic acid (1.67 mmol), in Ac_2O (1.2 mL), was refluxed for 16 h. The reaction mixture was cooled, neutralized with 10% aqueous $NaHCO_3$, and extracted with EtOAc (3 × 30 mL). The organic layers were combined, washed with distilled water, dried (anhydrous Na_2SO_4) and evaporated under reduced pressure. The product was purified by recrystallization in EtOH, and dried to afford the desired compound **1**, **2** or **3**.

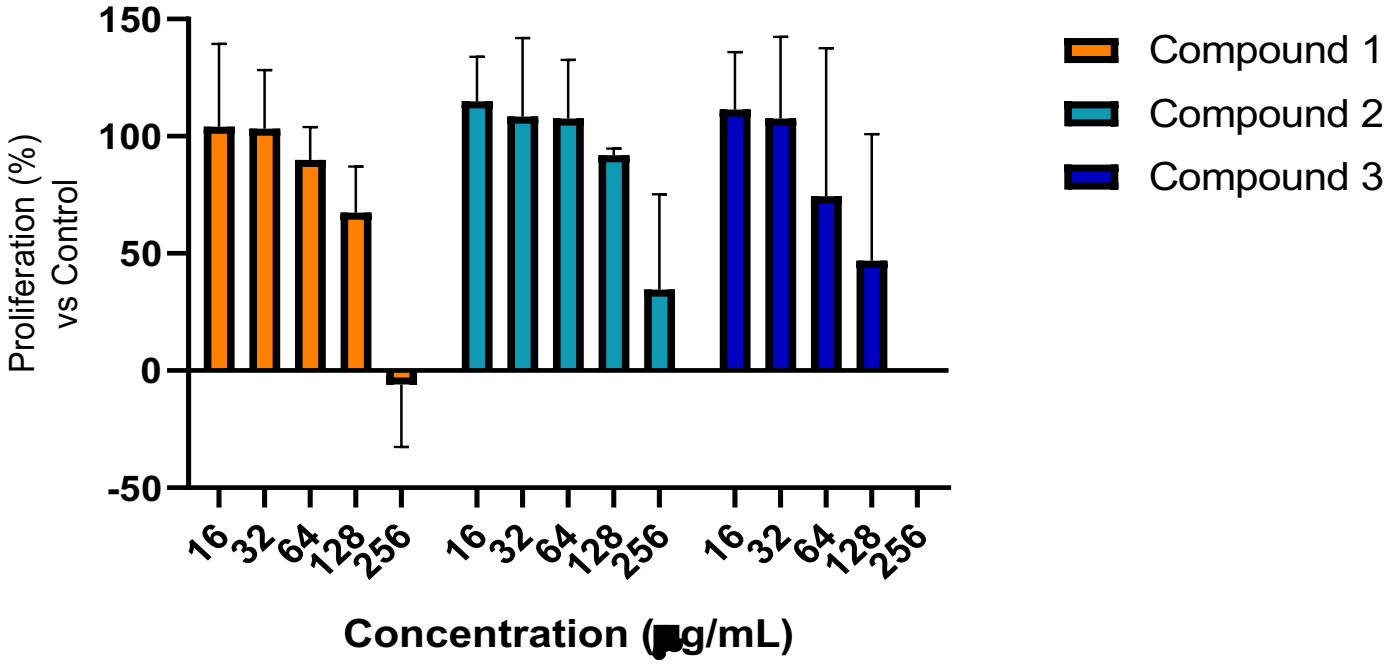
Biological assays

Comp **1** IC_{50} = 177.74 µg/mL
Comp **2** IC_{50} = 132.63 µg/mL
Comp **3** IC_{50} = 128.19 µg/mL



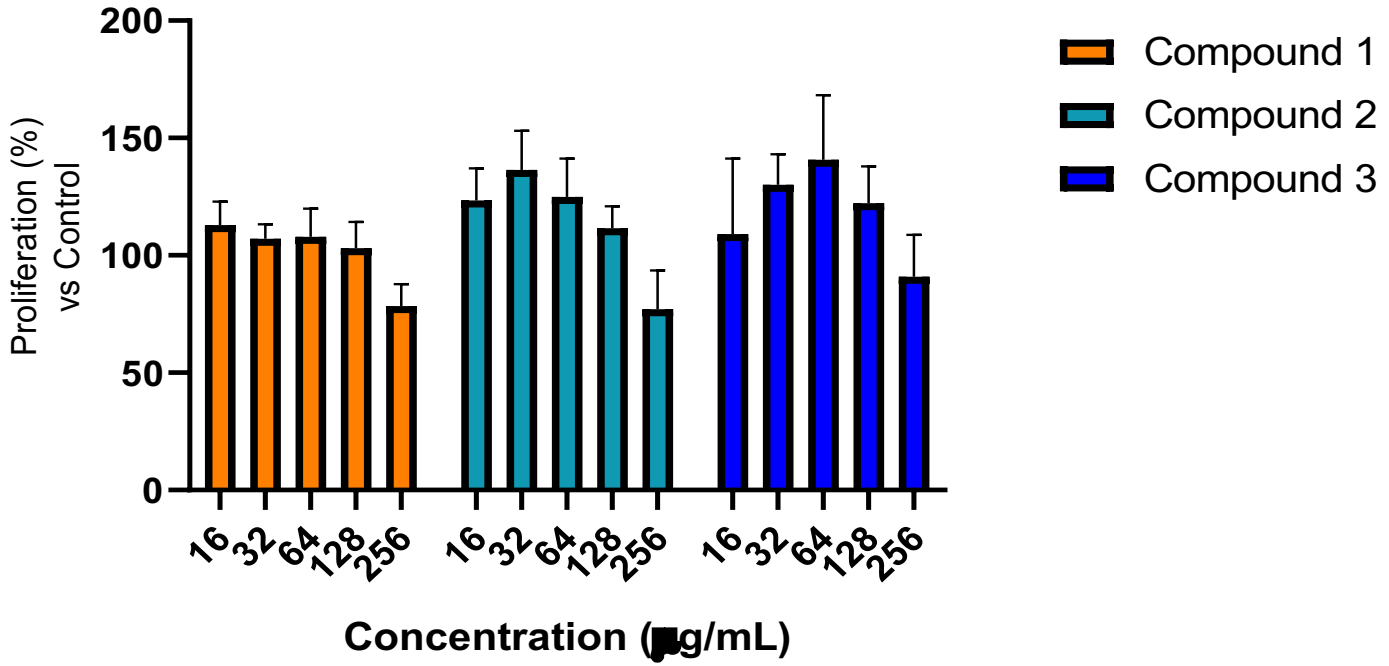
Graphic 2. Antiproliferative effects on MCF-7 tumor cells under normoxic conditions (72h).

Comp **1** IC_{50} = 159.32 µg/mL
Comp **2** IC_{50} = 188.73 µg/mL
Comp **3** IC_{50} = 116.99 µg/mL

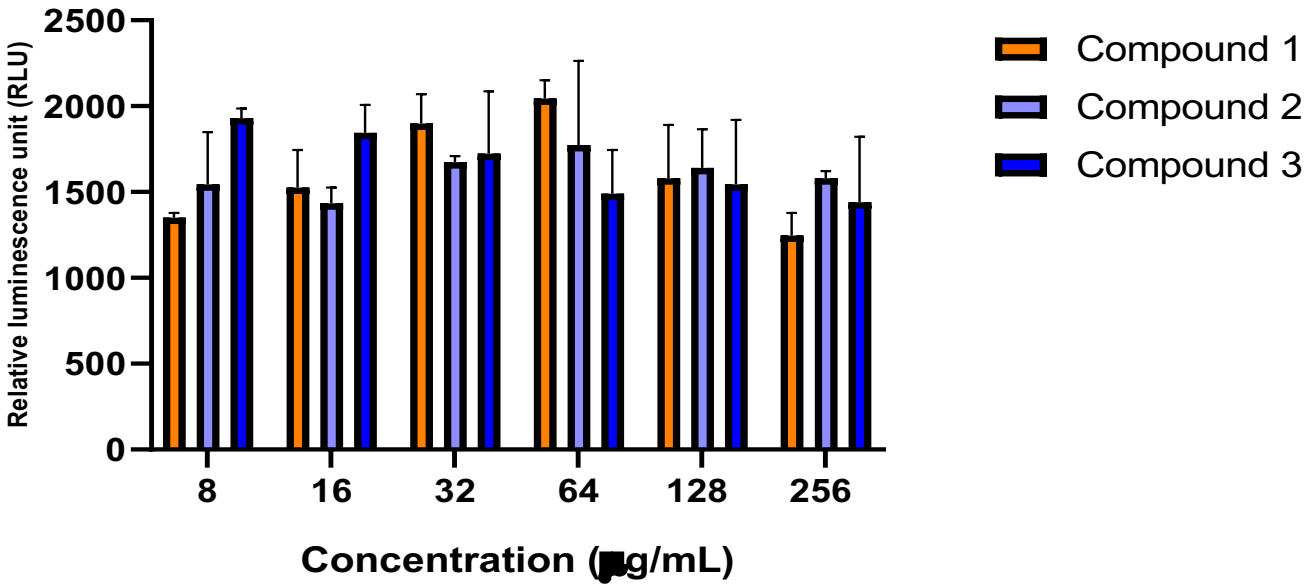


Graphic 3. Antiproliferative effects on MCF-7 tumor cells under hypoxic conditions (72h).

Comp **1** IC_{50} > 256 µg/mL
Comp **2** IC_{50} > 256 µg/mL
Comp **3** IC_{50} > 256 µg/mL



Graphic 4. Antiproliferative effects on MCF-7 tumor cells under hypoxic conditions induced by $CoCl_2$ (72h).



C(X) (µg/mL)	Compound 1			Compound 2			Compound 3		
	Mean	% CV	N	Mean	% CV	N	Mean	% CV	N
64	2045	5,18	2	1775	27,49	2	1490	17,08	2
128	1580	19,69	2	1640	13,80	2	1545	24,26	2
256	1247,5	10,50	2	1580	2,69	2	1440	26,52	2

Graphic 5. Luciferase activity on MCF-7 tumor cells under hypoxic conditions (18h).

Conclusions

- A series of three 3-arylcoumarin analogs were studied to determine antitumor activity profiles. Molecular docking studies were carried out between the compounds and specific monomers (HIF-1α and ARNT), which allowed to elucidate a possible mechanism of action from the potential binding mode of the ligands (compounds **1** and **3**) to the multiple sites of appropriate targets and how these may interfere with heterodimer formation (HIF-1α:ARNT).
- The compounds produce steric impediment by binding to the corresponding monomer by hydrogen bonding and by hydrophobic interactions. At the HIF-1α monomer, the three compounds bind to the DNA/bHLH interface, and with ARNT they interact differently at sites located in the bHLH, PAS-A and PAS-B domains. When the heterodimer is formed, the three compounds preferentially bind to the PAS-A/PAS-B interface of HIF-1α (inactive site).
- The coumarins evaluated showed different antiproliferative activities in the MCF-7 breast cancer cell line. Under normoxic conditions, the best activity was found for compound **3** (IC_{50} = 128.19 µg/mL), followed by compound **2** (IC_{50} = 132.63 µg/mL) and compound **1** (IC_{50} = 177.74 µg/mL). Under hypoxic conditions, the best activity was found for compound **3** (IC_{50} = 116.99 µg/mL), followed by compound **1** (IC_{50} = 159.32 µg/mL) and compound **2** (IC_{50} = 188.73 µg/mL). The evaluated compounds did not significantly affect proliferation in the MCF-7 cell line under the influence of $CoCL_2$.

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