Modeling of clastogenic activity and molecular docking at the binding sites of hif-1A-dna by 3-arylcoumarins

Modelación de la actividad clastogénica y acoplamiento molecular en los sitios de unión del HIF-1α-ADN de 3-arilcumarinas

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ABSTRACT

This study investigated the potential of 3-arylcoumarins to induce DNA damage and disrupt the interaction between the hypoxia-inducible factor-1α (HIF-1α) and ARNT, with the aim of hindering heterodimer formation. The clastogenic potential of 33 coumarins was assessed using ModesLab 2.0 program, and their binding affinity to HIF-1α:ARNT was modeled with MOE 2019.01 software. The results revealed that 14 of the evaluated coumarins exhibited clastogenic potential, with structural classification modeled from 10 series related to the substitution pattern. To summarize, the presence of methoxy and nitro groups in the new 3-arylcoumarins Anti-HIF-1 structure is directly linked to its ability to induce high clastogenicity. Additionally, all these compounds exhibited a full capacity for DNA binding.

Keywords: hypoxia-inducible factor1; 3-arylcoumarins; clastogenicity; molecular docking.

RESUMEN

El presente trabajo analiza el potencial de las 3-arilcumarinas para causar daño al ADN e interferir con la interacción entre el factor inducido por la hipoxia-1α (HIF-1α) y ARNT para el bloqueo de la formación de heterodímeros. El potencial clastogénico de 33 cumarinas se determinó con el programa ModesLab 2.0 y su afinidad de unión a HIF-1α:ARNT se modeló con el software MOE 2019.01. Los resultados mostraron que 14 de las cumarinas poseen potencial clastogénico, clasificación estructural modelada a partir de 10 series relacionadas con el patrón de sustitución. El estudio concluyó que la capacidad de causar una alta clastogenicidad está relacionada con la presencia de grupos metoxilo y nitro en número y posiciones específicas en la nueva estructura Anti-HIF-1 de las 3-arilcumarinas, las que mostraron en su totalidad capacidad de unión al ADN.

Palabras clave: factor inducido por hipoxia-1; 3-arilcumarinas; clastogenicidad; acoplamiento molecular.

Introduction

Cancer is one of the leading causes of mortality worldwide.[1] Hypoxia-inducible factor1 (HIF-1) plays a crucial role in cellular detection and adaptation to changes in oxygen levels, proving essential for cell survival and serving a central role in cellular adaptation to hypoxic stress. It establishes itself as a key regulator in the response of cancer cells to hypoxia.[2,3,4] Comprising an oxygen-sensitive α subunit (HIF-1α) and a constitutive β subunit (HIF-1β or ARNT).[5,6] HIF-1 activity is closely linked to cancer development and therapeutic resistance, thereby limiting the effectiveness of treatments. The clinically relevant activity of HIF-1 is evident in hypoxic/ischemic events manifested in various human cancers, such as breast cancer.[7]

HIF-1α is a critical regulator of genes related to cancer traits, and its stabilization in tumor hypoxia and oncogenic mutations is linked to poor prognosis and increased mortality in various cancers. Therefore, inhibiting its activity is a promising cancer treatment strategy.[5,8]

Cancer drugs like cytostatics intervene in signaling pathways and crucial cancer targets, such as DNA, inhibiting genetic material synthesis or causing irreparable damage.[9] There is evidence of the potential of various chemicals to cause damage related to genotoxicity and clastogenicity.[1,10,11,12] Identifying clastogenic agents is crucial for human health, as they induce

chromosomal breaks. These clastogenic agents can have chemical, physical, or biological origins. Chemicals range from medicinal substances, foods, derivatives or additives, pesticides, photodynamic dyes, organic solvents to metals.[10]

The capacity of certain substances to provoke genotoxic effects, which are connected to persistent human illnesses, emerges from their direct interaction with genetic material, causing DNA harm or chromosomal irregularities. [12] This category falls between the three forms of genetic damage outlined, which encompass the loss or acquisition of entire chromosomes, clastogenicity, and chromosomal rearrangements.[8]

The search for anticancer compounds has been a constant objective in the fields of molecular modeling and drug design. However, discovering selective antitumor compounds remains a significant challenge in cancer research. Given the aforementioned reasons, innovative approaches are required to effectively search for potential candidates for anticancer drugs.[13]In the case of designing new Anti-HIF-1 3-arylcoumarins, it is essential to conduct selective molecular modeling/screening to minimize potential risks and identify DNA binding sites. One fundamental toxicological criterion is chromosomal aberration or clastogenicity, a response to chemicals that can be harmful. Therefore, there is a clear need to generate automatic structural alerts to predict chromosomal aberration and other toxicological assessment criteria.[14]

A noteworthy previous study by Molina et al. focused on flavonoids, establishing a correlation between the pro-oxidant effect and the ability to cause clastogenic damage. This study provided structural alerts to prevent the future use of these compounds in phytomedicine or human nutrition.[15] Given that coumarins share a structural core similar to flavonoids, investigations regarding clastogenicity have also been conducted.[16] Previous chemoinformatic studies show a series of antioxidant coumarins, including 3-arylcoumarins, where clastogenic activity is predicted. The result yielded a preliminary interpretation of the structure/clastogenicity relationship, emphasizing the significance of hydroxyl groups in the coumarin scaffold at positions 7 and 8.[17] The aim of this *in silico* research is to study the clastogenic potential of new series of Anti-HIF-1 3-arylcoumarins and their relation to possible DNA binding sites associated with HIF-1 heterodimer formation.

Materials and methods

Clastogenicity

Weighted spectral moment calculations with physico-chemical properties were performed for the molecules of interest (see table 1) using the ModesLab 2.0 program. The purpose was to investigate the clastogenic activity of these compounds. The probability of being clastogenic or not was determined using the statistical model developed by Estrada and Molina, generated from the Statistics 7 program, employing the Linear Discriminant Analysis (LDA) technique. (14,15) Molecules with a percentage above 52,5 % are considered active; those below 48,5 % are inactive, with an indeterminate activity/inactivity area of 5% (range 48,5% - 52,5%), where the compound is defined as not classified (**NC**) according to the employed model.

Molecular Docking

In molecular docking, the crystal structures of HIF-1α:ARNT (PDB code: 4zpr) were utilized. The MOE 2019.01 tool was employed for molecular modeling under the following conditions: rigid receptor protocol, triangular matching matcher with slope 1 (London dG), and force field refinement with slope 2 (GBVI/WSA dG). The monomeric protein was extracted from the corresponding complexes, with HIF-1α extracted from the HIF-1α/ARNT complex (PDB code: 4zpr). A maximum of 5 poses were modeled for each compound.

Determination of atomic contacts between protein interfaces: The Cocomaps program (bioCOmplexesCOntact MAPS) was used to determine amino acid residues with atomic contacts closest to 6 Å on the protein surfaces.[18]

Determination of amino acid residues important for protein-protein interaction:

The web servers Robeta and Rosetta Backrub were employed to conduct a computational alanine scanning analysis. Residues were considered significant when the predicted binding energy variation (ΔΔGbind) was greater than or equal to 1.0 kcal mol-1, [19,20]as per the expression (Eq.1):

$$(\Delta\Delta Gbind=(\Delta GWTcomplex-\Delta GWTpartnerA-\Delta GWTpartnerB)-(\Delta GMUTcomplex-\Delta GMUTpartnerA-\Delta GMUTpartnerB)) \quad (1)$$

where WT stands for wild type and MUT represents the alanine mutation *in silico*.

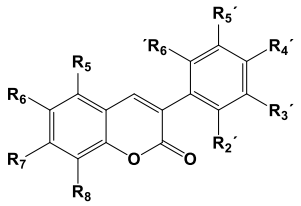
Visualization and presentation of complexes, poses, and interactions: the MOE 2019.01 program was used for visualizing and presenting the resulting complexes, poses, and interactions. This analysis included the visual evaluation of selected poses, obtained scores, and energy refinement for each pose.

Results and discussion

Clastogenic potential of 3-arylcoumarins

Cumarins are polyphenolic structures, and the possibility of binding to DNA has been reported, suggesting their potential to cause chromosomal aberrations. The results of the clastogenicity analysis, expressed as the percentage probability of clastogenicity, are detailed in table 1. Fourteen compounds were identified as clastogenic, namely **CMR**: **4**, **5**, **6**, **8**, **13**, **14**, **15**, **18**, **19**, **20**, **21**, **24**, **25**, and **29**. Among these, **CMR13** exhibited the highest percentage of clastogenicity, while **CMR25** showed the lowest percentage among those before mentioned. These findings provide valuable information regarding the specific coumarins' ability to induce damage to genetic material and suggest areas of interest for further investigations into their potential impact on chromosomal stability. The values obtained from the percentages of DNA binding (% DNA binding) (Table 1) show that these compounds have an affinity for it, results that are analyzed in the molecular docking modeling study in the HIF-1α:ARNT:DNA heterodimer.

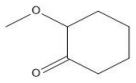
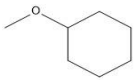
Table 1. Compounds derived from 3-arylcoumarins.



Comp	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$	% Prob Clastog	% Bind DNA
CMR1	Ac	H	Ac	H	H	Ac	H	H	H	38.5	60.0
CMR2	Ac	H	Ac	H	H	Ac	Ac	H	H	**51.3 NC**	40.0
CMR3	Ac	H	Ac	H	H	H	H	H	H	29.9	60.0
CMR4	H	OH	H	H	OCH_3	H	OCH_3	H	H	88.3	60.0
CMR5	H	Ac	H	H	OCH_3	H	OCH_3	H	H	87.8	75.0
CMR6	H	Ac	H	H	H	OCH_3	H	OCH_3	H	86.9	60.0
CMR7	H	S1	H	H	H	Br	H	H	H	13.1	40.0
CMR8	H	Ac	H	H	H	OCH_3	OCH_3	OCH_3	H	97.8	40.0

CMR9	H	CH_3	H	Br	H	OH	H	OH	H	29.9	60.0
CMR10	H	H	H	H	H	NH_2	H	H	H	32.5	40.0
CMR11	H	CH_3	H	H	H	Br	OCH_3	Br	H	**51.8 NC**	20.0
CMR12	H	CH_3	H	H	H	Br	OH	OH	H	32.3	20.0
CMR13	H	OH	H	H	H	OCH_3	OCH_3	OCH_3	H	97.9	60.0
CMR14	H	Ac	H	H	OCH_3	H	H	H	H	58.6	40.0
CMR15	H	CH_2Cl	H	H	H	H	OCH_3	H	H	60.0	60.0
CMR16	H	H	H	H	H	H	CH_2Br	H	H	37.3	80.0
CMR17	H	H	H	H	CH_2Br	H	H	H	H	38.1	20.0
CMR18	H	NO_2	H	H	H	H	NO_2	H	H	75.4	40.0
CMR19	H	OCH_3	H	H	H	NH_2	H	H	H	70.9	40.0
CMR20	H	OH	H	H	H	OCH_3	H	H	H	57.5	60.0
CMR21	H	Br	H	OH	H	OCH_3	H	H	H	64.1	60.0
CMR22	H	H	H	OH	H	CH_3	H	H	H	20.6	60.0
CMR23	H	H	H	H	CH_3	H	H	H	H	12,9	40.0
CMR24	H	Ac	H	H	H	OCH_3	OCH_3	H	H	88.2	40.0
CMR25	H	NH_2	H	H	H	NH_2	H	H	H	57.1	50.0
CMR26	H	Ac	H	H	Br	H	H	H	H	22.7	33.3
CMR27	H	CH_3	H	H	H	H	H	Br	OCH_3	**48.9 NC**	40.0
CMR28	H	S2	H	H	H	H	CH_3	H	H	10.5	40.0
CMR29	H	OCH_3	H	H	H	H	NO_2	H	H	78.6	60.0
CMR30	H	H	CH_2NO_2	H	H	H	H	H	H	39.2	40.0
CMR31	H	Br	H	OH	H	OH	H	H	H	35.2	100.0
CMR32	CH_2COCH_3	H	OCH_2-$COCH_3$	H	H	H	H	H	H	8.3	40.0
CMR33	H	H	OCH_2-$COCH_3$	H	H	H	H	H	H	18.6	60.0

Comp = Compound, % Prob Clastog = percentage of clastogenic probability,% Bind DNA = percentage of active poses, shaded in gray are the active ones with values exceeding 52.5%;compounds not classified are denoted as NC and highlighted in bold.

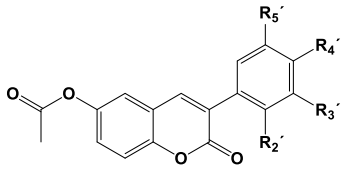
S1=  S2= 

From a structural perspective, 3-arylcoumarins were stratified into 10 series based on the substituents present in the coumarin scaffold and aryl ring (see tables 2-11). Significant patterns related to clastogenicity were observed within these series, as follows:

Series 1 (table 2): All coumarins with at least one OCH_3 group as a substituent exhibited some degree of clastogenicity, and this increased proportionally with the number of OCH_3 substituents.

Compounds with two methoxy groups in *meta* and *para* positions (cumulative) on the aryl ring tended to enhance clastogenic activity.

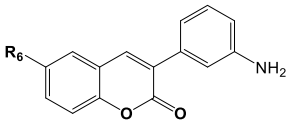
Table 2. Compounds derived from 3-arylcoumarins in Serie 1.



Comp	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	% Prob Clastog
CMR26	Br	H	H	H	22,7
CMR5	OCH_3	H	OCH_3	H	87,8
CMR24	H	OCH_3	OCH_3	H	88,2
CMR14	OCH_3	H	H	H	58,6
CMR6	H	OCH_3	H	OCH_3	86,9
CMR8	H	OCH_3	OCH_3	OCH_3	97,8

Series 2 (table 3): The addition of an amino or methoxy group in this series increases the risk of clastogenicity in coumarins. Clastogenic compounds with amino and methoxy groups (strong electron-donating groups) may exhibit differences in the probability percentage due to the pair of unshared electron pairs and lower electronegativity of oxygen compared to amino groups.

Table 3. Compounds derived from 3-arylcoumarins in Serie 2.

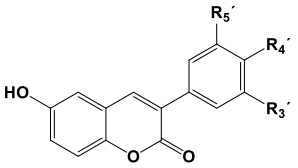


Comp	R_6	% Prob Clastog
CMR25	NH_2	57,1
CMR10	H	32,5
CMR19	OCH_3	70,9

Series 3 (table 4): The presence and increase in the number of methoxy substituents in the aryl ring correlated with an increase in the probability percentage of causing clastogenic damage. The methoxy group in compound CMR4 confers a high probability of clastogenicity, possibly associated with its position at 4' (para position of the aryl ring). This location appears to influence its ability to interact with DNA. Compounds with isolated methoxy groups (3', 5') show a

decrease in the probability percentage of being clastogenic and an increase in the percentage of DNA binding; in contrast to the observed trend when these methoxy groups are accumulated (3',4' or 4',5').

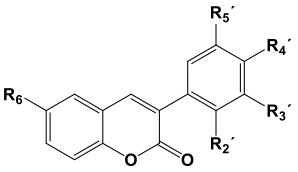
Table 4. Compounds derived from 3-arylcoumarins in Serie 3.



Comp	$R_{3'}$	$R_{4'}$	$R_{5'}$	% Prob Clastog
CMR4	H	OCH_3	H	88,3
CMR5	H	OCH_3	H	87,8
CMR20	OCH_3	H	H	57,5
CMR6	OCH_3	H	OCH_3	86,9
CMR24	OCH_3	OCH_3	H	88,2
CMR8	OCH_3	OCH_3	OCH_3	97,8
CMR13	OCH_3	OCH_3	OCH_3	97,9

Series 4 (table 5): While structures in the series were classified as clastogenic, the substitution of at least one methoxy group in the aryl ring increased the clastogenicity percentage. The presence of weak electron-donating groups (CH_3, Br) in the *ortho*, *meta*, and *para* positions on the aryl ring tends to categorize the compound as not classified (NC).

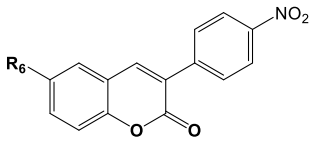
Table 5. Compounds derived from 3-arylcoumarins in Serie 4.



Comp	R_6	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	% Prob Clastog
CMR27	CH_3	OCH_3	Br	H	H	**48,9 NC**
CMR9	CH_3	H	OH	H	OH	29,9
CMR11	CH_3	H	Br	OCH_3	Br	**51,8 NC**
CMR12	H	H	OH	OH	Br	32,3

Series 5 (table 6): Both compounds exhibited high percentages of clastogenicity. Substituting a nitro group with a methoxy group at position R6 of the coumarin scaffold increased this percentage, also raising the percentage of DNA binding.

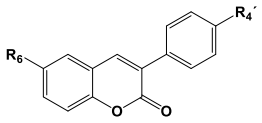
Table 6. Compounds derived from 3-arylcoumarins in Serie 5.



Comp	R_6	% Prob Clastog
CMR18	NO_2	75,4
CMR29	OCH_3	78,6

Series 6 (table 7): Only the compound with a methoxy group in the aryl ring in this series (CMR15) was considered clastogenic.

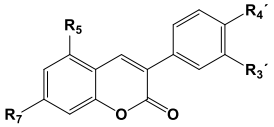
Table 7. Compounds derived from 3-arylcoumarins in Serie 6.



Comp	R_6	$R_{4'}$	% Prob Clastog
CMR15	CH_2Cl	OCH_3	60,0
CMR30	CH_2NO_2	H	39,2

Series 7 (table 8): Compounds in this series were non-clastogenic. This suggests that acetyl groups and other related substituents containing a carbonyl group in their structure, which are electron-acceptor in nature, are inactive according to the employed model; at least up to the limit of four acetyl groups in the structure **(CMR2)**.

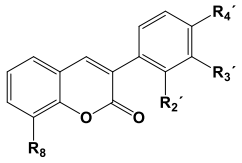
Table 8. Compounds derived from 3-arylcoumarins in Serie 7.



Comp	R_5	R_7	$R_{3'}$	$R_{4'}$	% Prob Clastog
CMR3	Ac	Ac	H	H	29.9
CMR2	Ac	Ac	Ac	Ac	**51.3 NC**
CMR1	Ac	Ac	Ac	H	38,5
CMR32	CH_2COCH_3	Ac	H	H	8,3
CMR33	H	OCH_2COCH_3	H	H	18,6

Series 8 (table 9): Compounds predicted in the series with the presence of the substituents OH, CH_3, and CH_2Br turned out to be inactive.

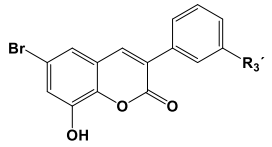
Table 9. Compounds derived from 3-arylcoumarins in Serie 8.



Comp	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	% Prob Clastog
CMR16	H	H	H	CH_2Br	37,3
CMR22	OH	H	CH_3	H	20,6
CMR17	H	CH_2Br	H	H	38,1
CMR23	H	CH_3	H	H	12,9

Series 9 (Table 10): Substituting a hydroxyl Group with a methoxy group in the same position on the aryl ring increased the probability of being clastogenic and transformed a non-clastogenic coumarin into a clastogenic one.

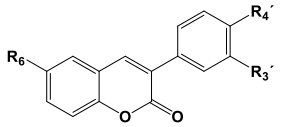
Table 10. Compounds derived from 3-arylcoumarins in Serie 9.



Comp	$R_{3'}$	% Prob Clastog
CMR21	OCH_3	64,1
CMR31	OH	35,2

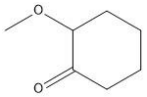
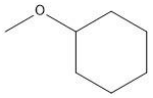
Series 10 (Table 11): Compounds in the series were non-clastogenic, demonstrating that the substituents Br and CH_3 on the aryl ring do not cause clastogenic damage.

Table 11. Compounds derived from 3-arylcoumarins in Serie 10



Comp	R_6	$R_{3'}$	$R_{4'}$	% Prob Clastog
CMR7	S1	Br	H	13.1
CMR28	S2	H	CH_3	10.5

corresponding,

S1=  S2= 

In summary, the structural analysis of the probability of causing clastogenicity in compounds derived from 3-arylcoumarins highlights the following key findings:

Potentially Causative of Clastogenicity: Nitro and/or methoxy-type substituents on the aryl ring are associated with compounds classified as potentially clastogenic. The presence of these groups and the amino group at position R6 of the coumarin scaffold can also confer clastogenicity to the compound.

Not Causative of Clastogenicity: Acetyl-type substituents (or other structurally similar groups containing a carbonyl group, e.g., **CMR32** and **CMR33**) at positions R5, R6, and R7 are not associated with clastogenicity. Compounds presenting specific substituents at positions R5, R6, and R7 of the aryl ring, such as Br (R2'), NH_2 (R3'), CH_2Br (R4'), or OH (R5'), also do not exhibit clastogenicity.

This structural analysis, stratified into 10 series, provides a detailed insight into the relationship between the structure of 3-arylcoumarins and their clastogenic potential. Methoxy and nitro substituents present in compounds from series 1, 3, 5, 6, and 9 (Tables 2, 4, 6, 7, and 10) are correlated with an increased probability of causing clastogenic damage. This finding aligns with reports by Molina *et al.*, 2005,[21] for benzocoumarin-type molecules that can bind to DNA. The presence of the methoxy group substituted at positions R6 and R7 of the coumarin scaffold generates derivatives with clastogenic potential (60.4 - 79.3 % clastogenicity).[21] These results provide clear structure-activity guidelines, allowing the identification of structural groups that may confer clastogenic risk and those that do not. This knowledge is essential for the safe and

effective design of compounds derived from 3-arylcoumarins in pharmacological and therapeutic applications.

Identification of Toxicophores

The determination of toxicophores is defined through structural alert rules, showing the contributions of common segment bonds to clastogenic activity.[21] The need for automated generation of structural alerts to predict clastogenic activity, among other toxicological criteria, is an important consideration.[14]

Figure 1 represents the proposed toxicophore structure for the studied 3-arylcoumarins, highlighting positions and substituents contributing to their clastogenicity. It is observed that the combination of electron-donating and -accepting groups, preferably strong ones, can favor DNA binding, thus affecting specific genes and cell division. The significance of position R8 in the coumarin scaffold is crucial, where the presence of the hydroxyl group is identified as a determining factor for clastogenicity. This finding is consistent with a previous study by Guardado *et al.* [17] The R8 position is considered critically relevant to prevent DNA damage (chromosomal aberration) in the new Anti-HIF-1 3-arylcoumarins.

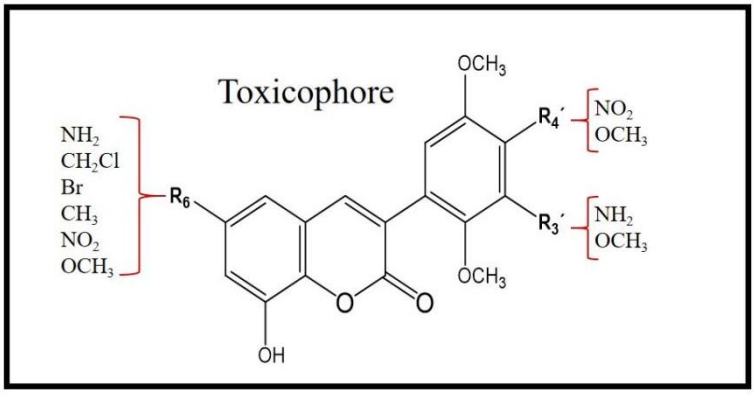


Fig. 1- Substituents at positions contributing to increased clastogenicity.

The structural series addressed in this research can be linked to a potential toxicophore for this type of coumarins in the future. The identification of this toxicophore provides valuable information for the future design of compounds derived from 3-arylcoumarins, enabling precise and strategic considerations to minimize the risk of clastogenicity and ensure safety in therapeutic applications related to HIF-1 inhibition.

Molecular Docking Study

The identification of new small molecules capable of modulating HIF-1α activity is an active area of research. The goal is to influence its biological actions to promote or inhibit them, depending on the pathology to be treated. [2,5, 6, 8] The binding sites of 3-arylcoumarins at the binding interfaces of HIF-1α with DNA were determined through molecular docking. Figure 2 illustrates the set of potential active poses that block the formation of the HIF-1α:ARNT heterodimer at the previously mentioned interface.These coumarins demonstrate the ability to interact through steric hindrances, interfering with heterodimer formation. They establish hydrogen bonding and hydrophobic interactions with amino acid residues within a distance of less than 6 Å. This approach provides valuable insights into potential interaction sites between 3-arylcoumarins and HIF-1α:ARNT, laying a structural foundation for future drug design and therapeutic development studies, considering the prevalence of hydrogen bonding interactions with the electron-acceptor and electron-donor groups identified in the clastogenic study.

From their mode of non specific binding, it is observed that some of these compound shave the ability to bind to sites that are not crucial for the formation of the heterodimer at the bHLH-DNA interfaces. This feature could be responsible for the high probability of causing clastogenic damage. The inactive sites, located adjacent to the active site, are illustrated in detail in Figure 3.

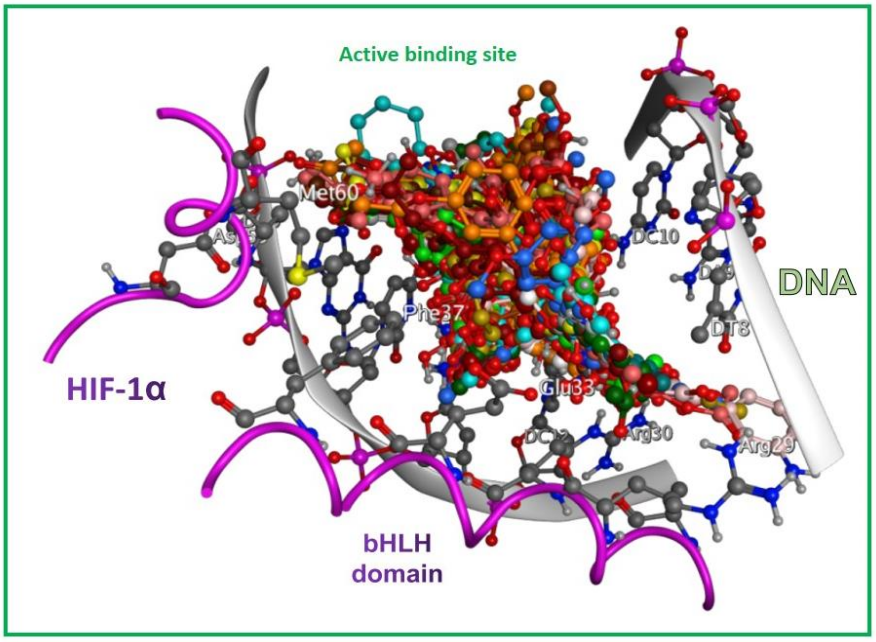


Fig. 2- Molecular docking of active 3-arylcoumarins at the bHLH (HIF-1α)/DNA (active binding site). PDB code: 4zpr.

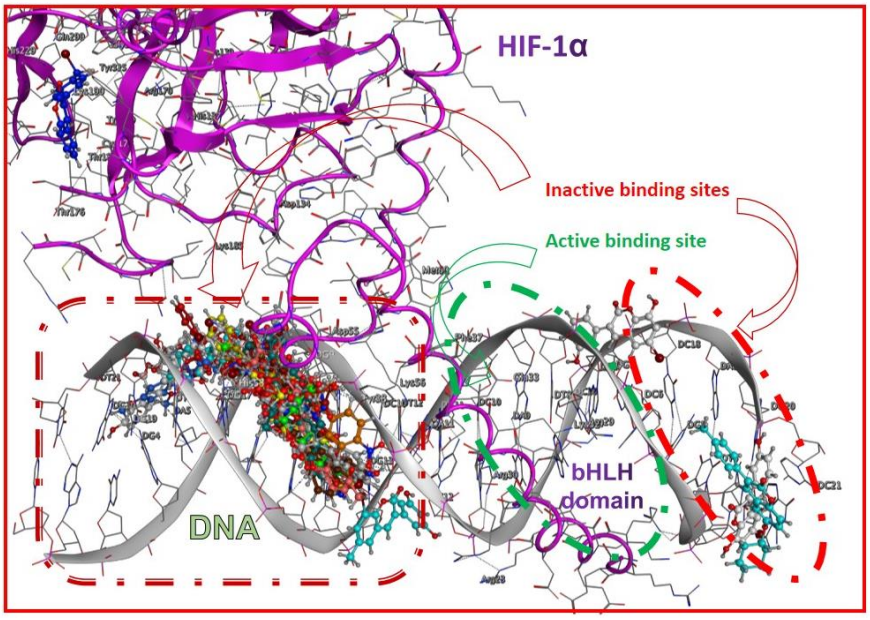


Fig. 3- Molecular docking of inactive 3-arylcoumarins at the bHLH (HIF-1α)/DNA Site. PDB 4zpr. Dashed green line circles: active binding site. Dashed circles and squares in red: inactive binding sites

These coumarin compounds can establish interactions with the nitrogenous bases present in the DNA structure, owing to the presence of the carbonyl group and highlighted substituents. The interaction can be hydrophobic, involving hydrogen bonds and covalent bonds, suggesting a potential risk of clastogenicity.The analysis reveals that 42,4 % of the compounds derived from 3-arylcoumarins exhibit some degree of clastogenicity, suggesting the possibility of damage associated with their ability to bind to DNA. The coumarin ring can establish various non-covalent interactions, such as hydrophobic, π-π, and electrostatic interactions, with the active site of various biomolecules. It can also form hydrogen bonds, van der Waals forces, among others. The presence of oxygen in the lactone ring adds a unique feature to these molecules, making them particularly suitable as ligands for supramolecular assemblies.[17,18,19,20,21,22] These interactions may have significant implications in cellular biology and biomolecular activity.

The presence of methoxy groups in the aryl ring of 3-arylcoumarins has been an aspect not explored previously. The relevance of this structural alert, stratifying the exploration of promising compounds, is emphasized.[17] In a recent study, structures containing substituent groups, including methoxy, were examined, as in the case of Rigosertib. This compound exhibited in vitro inhibitory capacity against PI3Kα and PI3Kβ isoforms, at concentrations

ranging from 1 to 10 µM. These results suggest that this substance acts as an antitumor agent through an alternative pathway.[23]

The common scaffold of coumarin, the benzo-α-pyrone, has demonstrated non-covalent interaction with various active sites, generating diverse biological activities in coumarins and their derivatives. Specific substituents such as nitro, bromo, and methyl have been identified in different positions of the coumarin scaffold. [23] In the studied coumarin derivatives, nitro, bromo, and methyl groups are prominent in different positions. The identification of structures in Series 6 and Series 9, where nitro and bromo substituents are located in position R6 of the coumarin nucleus, shows a structural relationship with previously reported active compounds.[24] This relationship is also observed with the presence of bromo in position R2' of the aryl ring. The importance of benzenesulfone coumarin derivatives as a structural subunit for the discovery of anticancer agents is highlighted.[23]

Furthermore, the synthesis and evaluation of coumarin derivatives designed to act on specific isoforms of the human carbonic anhydrase enzyme (hCA), abundant in various solid tumors, also underscores the presence of the nitro group in position R6 of the coumarin nucleus.[25] These elements reinforce the hypothesis of its importance as a relevant factor for antitumor activity.

The consideration of structural alerts related to clastogenicity and potential toxicophores in certain 3-arylcoumarins (see tables 2, 3, 4, 6, 7, and 10) aligns with the principle that antitumor compounds should not induce clastogenic damage. All studied molecules demonstrated the ability to bind to DNA.

Conclusions

This research offers valuable insights into the clastogenic potential of 3-arylcoumarins. The study identified several coumarin derivatives as clastogenic, highlighting the need for careful consideration during drug design. The structural analysis of 10 series revealed distinct patterns linking the presence of nitro and methoxy substituents with a high risk of clastogenicity, defining a significant toxicophore (structural alert). This research enhances our understanding of the potential genetic damage of 3-arylcoumarins and provides elements for designing safer, pharmacologically relevant compounds derived from these compounds, particularly those targeting HIF-1 inhibition. Careful evaluation is crucial to prevent potential risks such as teratogenicity or carcinogenicity and to ensure that selected promising molecules do not worsen the health of cancer patients.

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Conflict of interest

The authors declare no conflicts of interest in the submitted manuscript.

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