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Toxicity Mechanism of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as Opportunity for Development of New Targeted Therapies Targeting Aryl Hydrocarbon Receptors (AhR)—Molecular Docking Simulation Study

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Abstract

Background/Objectives: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a highly toxic environmental contaminant whose adverse biological effects are primarily mediated through activation of the aryl hydrocarbon receptor (AhR). Upon ligand binding, AhR undergoes conformational changes that initiate nuclear translocation and transcriptional activation of xenobiotic-responsive genes, contributing to toxicity, carcinogenesis, and dysregulated immune and metabolic responses. Understanding the molecular basis of AhR activation by TCDD is therefore critical for the rational development of targeted therapeutic strategies. **Methods**: In this study, molecular docking simulations were employed to characterize the interaction of TCDD and selected AhR antagonists (CH223191, BAY 2416964, GNF-351) with the ligand-binding domain of AhR, with particular emphasis on the canonical PAS-B domain. **Results**: Docking analyses identified the PAS-B cavity (pocket C1) as the most biologically relevant binding site for high-affinity ligands, consistent with experimental evidence. Comparative docking of known AhR antagonists revealed stable binding poses characterized by hydrophobic packing, π–π interactions, and hydrogen-bonding networks that competitively block agonist access and prevent receptor activation. These findings support a competitive antagonism mechanism as a viable approach to counteract TCDD-induced AhR signaling. **Conclusions**: Collectively, this in silico study provides mechanistic insight into TCDD toxicity at the molecular level and highlights AhR antagonism as a promising strategy for the development of targeted therapies against dioxin-related pathologies.

Keywords: TCDD; AhR; PAS-B; molecular docking; competitive antagonist; targeted therapy

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1. Introduction

Dioxins are highly resistant to degradation across various environmental media [1,2], known as a persistent organic pollutant (POPs) that bioaccumulates in the food and feed chain. Halogenated compounds, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), exhibit particularly slow degradation due to their stable chemical structure. It is a byproduct of industrial processes and combustion, posing significant health risks due to its long biological half-life and high lipid solubility. Dioxins and related pollutants, including dibenzofurans, represent serious biological and ecological hazards. These risks stem primarily from their adverse effects on human and animal health, as both acute [3,4] and

long-term exposure [5] have been shown to impact populations globally. Classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) [6], TCDD's high toxicity is largely mediated through activation of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor. The AhR protein consists of 848 amino acids organized into four distinct domains: an N-terminal basic helix–loop–helix (bHLH) domain spanning residues 27–80; two Per-ARNT-Sim (PAS) domains, PAS-A (residues 111–181) and PAS-B (residues 275–342); and a C-terminal transactivation domain (TAD) extending from residues 490 to 805 [7]. Located at the N-terminal region, the bHLH domain mediates both DNA binding and dimerization with AhR-nuclear translocator protein (ARNT). The basic region recognizes the DNA consensus motif, while the helix-loop-helix component enables dimerization with other PAS proteins. This domain contains: a nuclear localization sequence (NLS) exposed upon ligand binding, allowing nuclear entry, and a nuclear export signal (NES), supporting cytoplasmic shuttling and recycling of AhR [8]. Following the bHLH domain, there are two homologous PAS domains: PAS-A contributes to dimerization specificity and ARNT interaction, and PAS-B serves as the ligand-binding domain (without direct involvement in the dimerization) and a high-affinity site for Heat Shock Protein 90 (HSP90), other structurally diverse ligands such as polycyclic aromatic hydrocarbons (PAHs), and kynurenine [7,9]. The TAD contains glutamine-rich motifs that recruit transcriptional co-activators like metazoan-specific acetyltransferase (p300/CBP) and steroid receptor coactivator-1 (SRC-1), essential for chromatin remodelling and transcription. It is relatively unstructured and species-variable [10]. This tripartite structure—bHLH, PAS-A/PAS-B, TAD—makes AhR a versatile environmental sensor and transcriptional effector [11,12].

AhR resides in the cytoplasm as part of a heterodimeric complex with two HSP90 family proteins, p23 and co-chaperone XAP2. HSP90 associates with the bHLH and PAS-B domains and likely conceals the receptor's NLS. Upon ligand binding, AhR undergoes a conformational change that exposes the NLS, enabling the complex to translocate into the nucleus. There, ARNT replaces the proteins bound to AhR, forming an AhR/ARNT heterodimer. This complex binds to xenobiotic-responsive elements (XRE) in the enhancer regions of target genes, such as CYP1A1, recruits transcriptional coactivators via the transactivation domains (TADs) of both AhR and ARNT, and initiates gene transcription [13] (Denison et al., 2011).

Beyond its classical role in xenobiotic metabolism, AhR has emerged as a critical regulator of metabolic, immune, and developmental pathways [14]. For the integration of endogenous and external small molecules into transcriptional responses, it serves as a cellular hub [15]. TCDD-AhR interactions are implicated in a wide range of toxic effects, particularly carcinogenicity and metabolic dysfunctions such as hepatotoxicity, glucose intolerance, and energy imbalance [16–18]. Apart from AhR, receptor-targeted drug development has been a major focus of the pharmaceutical industry for treating numerous diseases [19]. By contrast, the development of AhR-targeted therapies has lagged considerably. This delay largely stems from AhR's original identification as the receptor responsible for binding TCDD and mediating the toxic effects of TCDD and related halogenated aromatic (HA) environmental pollutants [20]. Its association with severe toxicity, documented in environmental exposure incidents and human poisonings [21,22], created a long-standing perception that impeded unbiased drug development efforts targeting AhR. This negative perception has begun to shift only with more recent findings demonstrating the essential involvement of AhR in numerous disease pathways, and with the discovery of a wide array of structurally diverse AhR ligands, encompassing industrial chemicals, plant bioactive components, microbial metabolites, and pharmaceutical agents [14,18,23–25].

Two classes of AhR ligands with notable immunomodulatory properties are selective AhR modulators (SAhRMs) and rapidly metabolized AhR ligands (RMAhRLs). SAhRMs selectively and often tissue-specifically modulate AhR activity with reduced dioxin-like toxicity, while RMAhRLs activate AhR transiently due to rapid metabolic clearance, limiting systemic toxicity. The two groups mainly differ in transcriptional selectivity and pharmacokinetics: SAhRMs elicit sustained, targeted responses, whereas RMAhRLs produce short-lived yet effective activation. Examples of SAhRMs include SGA360, galangin, and indole-3-carbinol–derived metabolites such as indolo [3,2-b]carbazole (ICZ) and 3,3′-diindolylmethane (DIM) [25].

Building on these principles, this study aims to identify binding pockets involved in AhR-ARNT heterodimer formation and to assess, through molecular docking simulations, the inhibitory/modulatory potential of selected compounds. By moving beyond the traditionally characterized interaction sites, this work seeks to provide new structural insights into the mechanisms governing AhR signaling, highlighting a novel strategy for modulating AhR-ARNT interactions and expanding the current landscape of AhR-focused drug discovery.

2. Materials and Methods

2.1. AhR Model Preparation

A previously reported receptor model was obtained from the Protein Data Bank (RCSB PDB (RCSB.org), the US data center for the global Protein Data Bank (PDB) archive). This receptor model (PDB ID: 5NJ8) (https://www.rcsb.org/structure/5NJ8) (accessed on 20 August 2025) was used in complex with the synthetic AhR inhibitor CH-223191. Co-crystallization of the PAS-B domain of AhR with the synthetic inhibitor CH-223191 was performed in the original study to stabilize the receptor in its ligand-bound conformation, enabling X-ray structural analysis and identification of key amino acid residues involved in ligand recognition, which are critical for the rational design of novel inhibitors [7]. The resulting model exhibited acceptable structural quality, as indicated by z-score, Q-mean, and Ramachandran plot analyses. The receptor comprises four canonical domains—bHLH, PAS-A, PAS-B, and the Q-rich region—each contributing to signal transduction, with ligand binding occurring specifically within the PAS-B domain.

2.2. Ligand Model Preparation and Docking Analysis

Selected ligand structures were retrieved in MOL format from the MolView database (https://molview.org/) (© 2025 MolView). Both ligands and receptor structures were prepared and optimized for docking using AutoDock Tools [26]. Docking simulations were carried out with the CB-Dock2 web server (BioLip2 database (version of 2025.04.23)) (https://cadd.labshare.cn/cb-dock2/index.php) (accessed on 20 August 2025), which employs AutoDock Vina (the latest version (1.2.0) of AutoDock Vina). Blind docking was performed to identify ligand-binding domains and pockets, and binding affinities were evaluated based on Vina scores, which were used for ligand ranking. In the present study, the Vina score was used primarily as a relative metric to compare ligand poses and identify potential binding pockets within the AhR structure. The scoring function represents an approximate estimate of binding affinity rather than a precise thermodynamic free binding energy.

3. Results and Discussions

3.1. AhR Model

AhR model used, which represents four domains of the receptor, is shown in Figure 1. To identify potential binding pockets on AhR, blind docking was performed using TCDD,

a known high-affinity AhR ligand. Analysing all the docking simulation results, in concordance with previously published in the literature [27], we detected five cavities on the AhR structure potentially relevant for its inhibition, with different mechanisms, shown in Table 1. It should be noted that the aryl hydrocarbon receptor (AhR) is a cytosolic ligand-activated transcription factor rather than a transmembrane receptor; therefore, all predicted cavities are located within the cytosolic PAS-A and PAS-B domains and are accessible to ligands present in the cytoplasmic environment. Predicted binding cavities in the AhR identified by CB-Dock2 are shown in Figures 2 and 3.

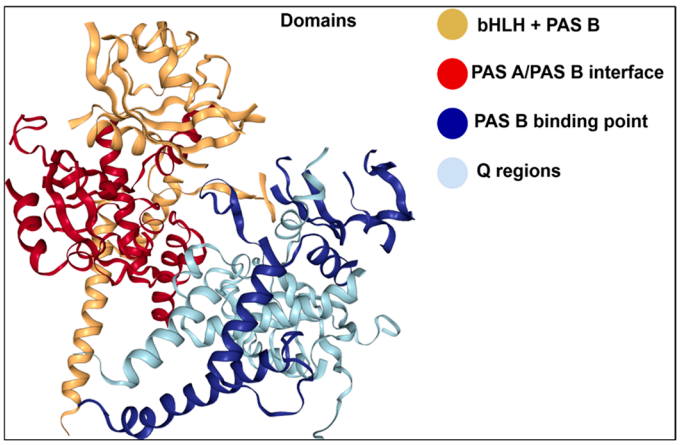

Figure 1. AhR model with all four domains of the receptor.

Table 1. Summary of binding cavities detected in the AhR structure using CB-Dock2 (Docking scores were calculated using AutoDock Vina).

CurPocket ID	Vina Score	Cavity Volume (Å^3)	Center (x, y, z)	Docking Size (x, y, z)
C4	−7.5	1867	28, 16, 207	20, 20, 20
C1	−7.4	6499	29, 34, 207	35, 30, 28
C2	−6.7	3981	14, 41, 218	31, 27, 20
C3	−6.5	3416	1, 20, 216	35, 29, 20
C5	−4.8	1720	−7, 13, 193	27, 20, 26

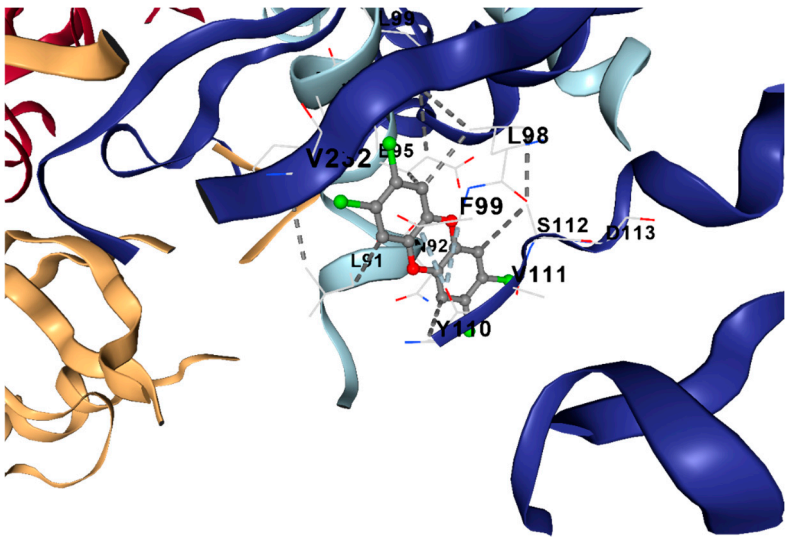

Figure 2. Docking pose of the ligand within Cavity C1, corresponding to the PAS-B domain of AhR (canonical ligand-binding domain).

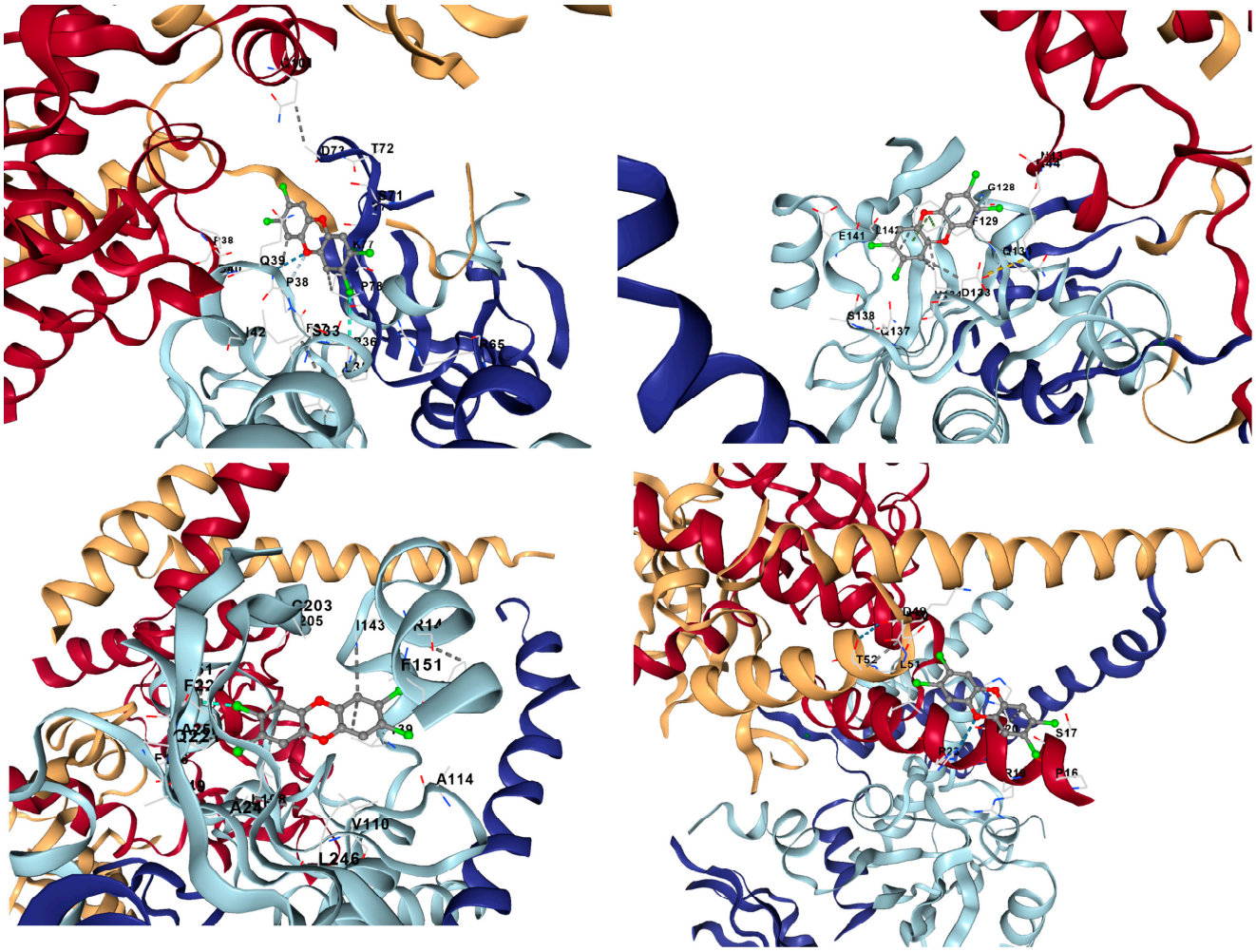

Figure 3. Predicted binding cavities in the AhR identified by CB-Dock2. The ligand docking poses are shown within distinct cavities (C2—top left; C3 top right; C4 bottom left and C5 bottom right). These cavities are primarily located on PAS-A or inter-domain surfaces and are interpreted as protein–protein interaction interfaces or pseudo-pockets rather than functional ligand-binding domains.

Cavity 1, with the second-highest Vina score (−7.4) and the largest volume (6499 Å^3, center ≈ 29, 34, 207), is located in the PAS-B domain near the Q-rich region. PAS-B serves as the primary ligand-binding domain (LBD) of AhR, accommodating TCDD, PAHs, indoles, and flavonoids. The key residues lining Cavity 1 include F117, Y118, F172, I176, H177, F181, F188, I190, and D191, forming mainly van der Waals and hydrophobic interactions (F117, F172, F181, F188) along with specific polar contacts (Y118, H177, D191). The pocket is accessible to small hydrophobic ligands in the cytosol and is directly involved in ligand recognition, dissociation from chaperones (HSP90, XAP2, p23), and subsequent heterodimerization with ARNT, consistent with established AhR-ligand binding data [28,29].

Cavity 2 has a Vina score of −6.7, a volume of 3981 Å^3, and is centered at (14, 41, 218). It is located at the interface between PAS-A and PAS-B, corresponding to the surface involved in interactions with cytosolic chaperone proteins such as HSP90 and p23. This region contributes to the stabilization of AhR and maintains PAS-B in a ligand-accessible conformation. Although partially solvent-accessible, the pocket primarily represents a protein–protein interaction surface, and its accessibility to small ligands may depend on conformational dynamics of the receptor–chaperone complex. Due to this transient nature, it is often considered a pseudo-pocket in docking analyses [30].

The next pocket, Cavity 3, has a Vina score of −6.5, a volume of 3416 Å^3, and is centered at (1, 20, 216). Located at the distal region of PAS-A near the hinge connecting the DNA-binding domain (DBD) and PAS-A, Cavity 3 forms part of the AhR–ARNT dimerization interface. Because this interface becomes occupied upon heterodimer formation with ARNT, it is largely inaccessible in the active dimeric state. The appearance of this pocket in docking simulations likely reflects the monomeric receptor conformation prior to ARNT binding, where transient accessibility may exist. Consequently, this site represents a protein–protein interaction surface rather than a canonical ligand-binding pocket [31].

Cavity 4 with a Vina score of −7.5, a volume of 1867 Å^3, and center at (28, 16, 207), is located on the solvent-exposed surface of PAS-A domain, formed mainly by flexible loops and terminal helices. Although docking simulations produced a relatively high score, this pocket is shallow and open to the solvent, suggesting that it does not represent a physiologically relevant ligand-binding site. Instead, its appearance likely results from local conformational flexibility of surface residues, indicating a pseudo-pocket rather than a functional binding cavity [29].

Cavity 5, with a Vina score of −4.8, a volume of 1720 Å^3, and center at (−7, 13, 193), represents a small surface depression with limited structural depth and poor enclosure for ligand stabilization. Due to its small size and lack of functional residues involved in ligand recognition, this cavity is unlikely to support stable ligand binding and is therefore considered structurally non-relevant for AhR activation or inhibition.

3.2. Ligand Models

3.2.1. CH223191—AhR Inhibitor with Antidote Potential Against TCDD Poisoning

CH-223191 (Figure 4) is a synthetic AhR antagonist designed to selectively inhibit the toxic effects of dioxin-like compounds. Its IUPAC name is 2-methyl-2H-pyrazole-3-carboxylic acid [2-methyl-4-oxo-3-(3-trifluoromethylphenyl)-3,4-dihydroquinazolin-6-yl]amide, with a molecular formula of $C_{18}H_{14}F_3N_5O_2$ and a molecular weight of 389.33 g/mol. The compound features a quinazoline core substituted with a trifluoromethyl-phenyl group, along with polar pyrazole and amide moieties essential for AhR binding. CH-223191 functions as a potent and selective antagonist, effectively blocking ligand-induced AhR activation by TCDD without displaying agonist activity [32].

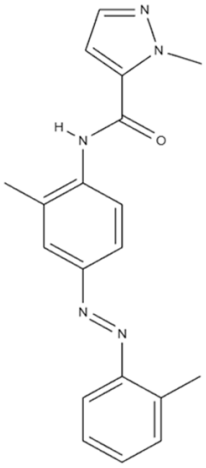

Figure 4. Chemical structure of CH223191 generated using MolView (© 2025 MolView) (https://molview.org).

Docking analysis indicates that CH223191 preferentially occupies the canonical ligand-binding region of the AhR, localized within the PAS-B domain (pocket C1). The PAS-B domain is well established as the primary binding site for high-affinity AhR agonists,

including TCDD [28,33]. The predicted binding pose shows strong complementarity between CH223191 and the hydrophobic–polar microenvironment of the PAS-B cavity. Multiple non-covalent interactions are observed, including π–π stacking between the quinazoline scaffold of CH223191 and aromatic residues within the PAS-B domain (e.g., Tyr and Phe), as well as van der Waals contacts with hydrophobic residues such as Val, Leu, and Ala. In addition, putative hydrogen bonds involving the amide and pyrazole moieties of CH223191 and polar side chains likely contribute to binding stability.

Although several potential binding pockets were identified, functional inhibition of AhR is most effectively achieved through ligand occupancy of the PAS-B domain, specifically pocket C1, which represents the canonical ligand-binding site (Table 2). While pocket C4 exhibited the highest Vina score, it corresponds to a small cavity within the PAS-A domain and is interpreted as a surface-associated pseudo-pocket. The elevated docking score for this site likely reflects scoring-function bias rather than true biological relevance [31]. The inhibitory activity of CH223191 can be explained by a competitive antagonism mechanism at the PAS-B ligand-binding pocket (C1) (Figure 5). Docking results indicate a high affinity of CH223191 for the ligand-binding domain (LBD) within the PAS-B region of AhR, suggesting that the antagonist occupies this site and thereby prevents binding of the agonist TCDD. This interpretation is further supported by experimental evidence showing that CH223191 selectively inhibits ligand-induced AhR activation, particularly TCDD-mediated signaling, without exhibiting intrinsic agonist activity [34].

Table 2. Summary of blind docking analysis of CH223191, BAY 2416964, and GNF-351 against the AhR using CB-Dock2.

CurPocket ID	Cavity Volume (Å^3)	Center (x, y, z)	CH223191		BAY 2416964		GNF-351	
			Vina Score	Docking Size (x, y, z)	Vina Score	Docking Size (x, y, z)	Vina Score	Docking Size (x, y, z)
C4	1867	28, 16, 207	−9.1	24, 24, 24	−8.4	23, 23, 23	−10.0	24, 24, 24
C1	6499	29, 34, 207	−8.0	35, 30, 24	−7.5	35, 30, 23	−9.1	31, 24, 24
C2	3981	14, 41, 218	−8.0	31, 24, 24	−7.3	31, 23, 23	−8.2	35, 30, 24
C3	3416	1, 20, 216	−7.1	35, 24, 24	−6.6	35, 29, 23	−7.9	35, 24, 24
C5	1720	−7, 13, 193	−6.2	24, 24, 24	−6.2	23, 23, 23	−6.2	24, 24, 24

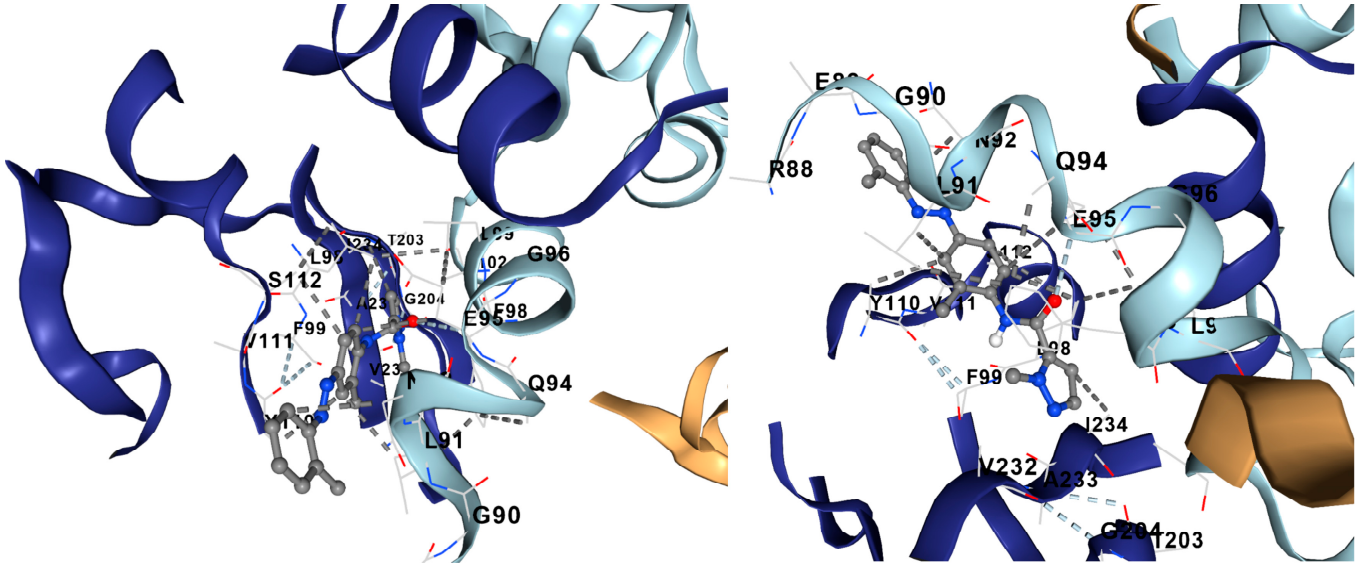

Figure 5. Docking pose of the ligand within Pocket C1 (place where inhibition of receptor occurs).

By preventing agonist binding within the PAS-B domain, CH223191 disrupts AhR signaling upstream of nuclear translocation and transcriptional activation. As a result, AhR remains in an inactive cytosolic complex with chaperone proteins, and the conformational changes required for ARNT heterodimerization do not occur. This blockade suppresses downstream target gene expression (e.g., CYP1A1) by preventing AhR–ARNT binding to XREs. This mechanism distinguishes CH223191 from SAhRMs, which may permit partial or context-dependent activation. Instead, CH223191 functions as a pure antagonist, supporting its potential utility as an antidotal agent against TCDD or related dioxins that act as AhR agonists.

3.2.2. BAY 2416964—A Selective AhR Antagonist

BAY 2416964 (Figure 6) is a selective small-molecule antagonist of the AhR, developed by Bayer and currently under preclinical [25,35,36] and clinical investigation (NCT04999202, Phase 1) [37] as a modulator of AhR signaling and cancer immunotherapy candidate. Structurally, it features a quinazolinone-based aromatic core with a chlorophenyl substituent, a polar amide-linked side chain, and a heteroaromatic pyrazole moiety, conferring a balance of hydrophobic and hydrogen-bonding interactions. BAY 2416964 is considered a competitive antagonist, as it can directly occupy the AhR ligand-binding pocket and thereby prevent activation by agonists such as TCDD.

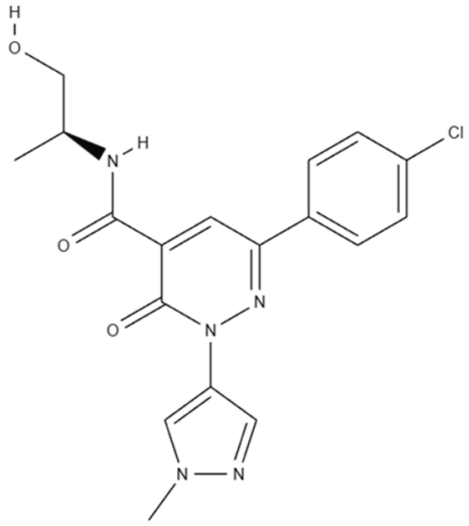

Figure 6. Chemical structure of BAY 2416964 generated using MolView (© 2025 MolView) (https://molview.org).

Molecular docking results (Table 2) indicate that although pocket C4 exhibits the highest predicted binding affinity (Vina score −8.4 kcal/mol), its small size and surface-exposed location suggest limited biological relevance. In contrast, pocket C1, despite a slightly lower affinity (−7.5 kcal/mol), represents the largest internal cavity (6499 $Å^3$) and corresponds to the canonical PAS-B ligand-binding domain (Figure 7). The binding pose within PAS-B shows stable accommodation of the ligand within a mixed hydrophobic–polar environment, consistent with the balanced physicochemical properties of the molecule. Binding stabilization is primarily mediated by π–π interactions involving the aromatic scaffold, van der Waals contacts with hydrophobic residues, and hydrogen-bond interactions with nearby polar side chains. This interaction profile supports a competitive antagonism mechanism, in which ligand occupancy of the PAS-B cavity prevents binding of agonists such as TCDD and thereby blocks the initial step of AhR activation [38].

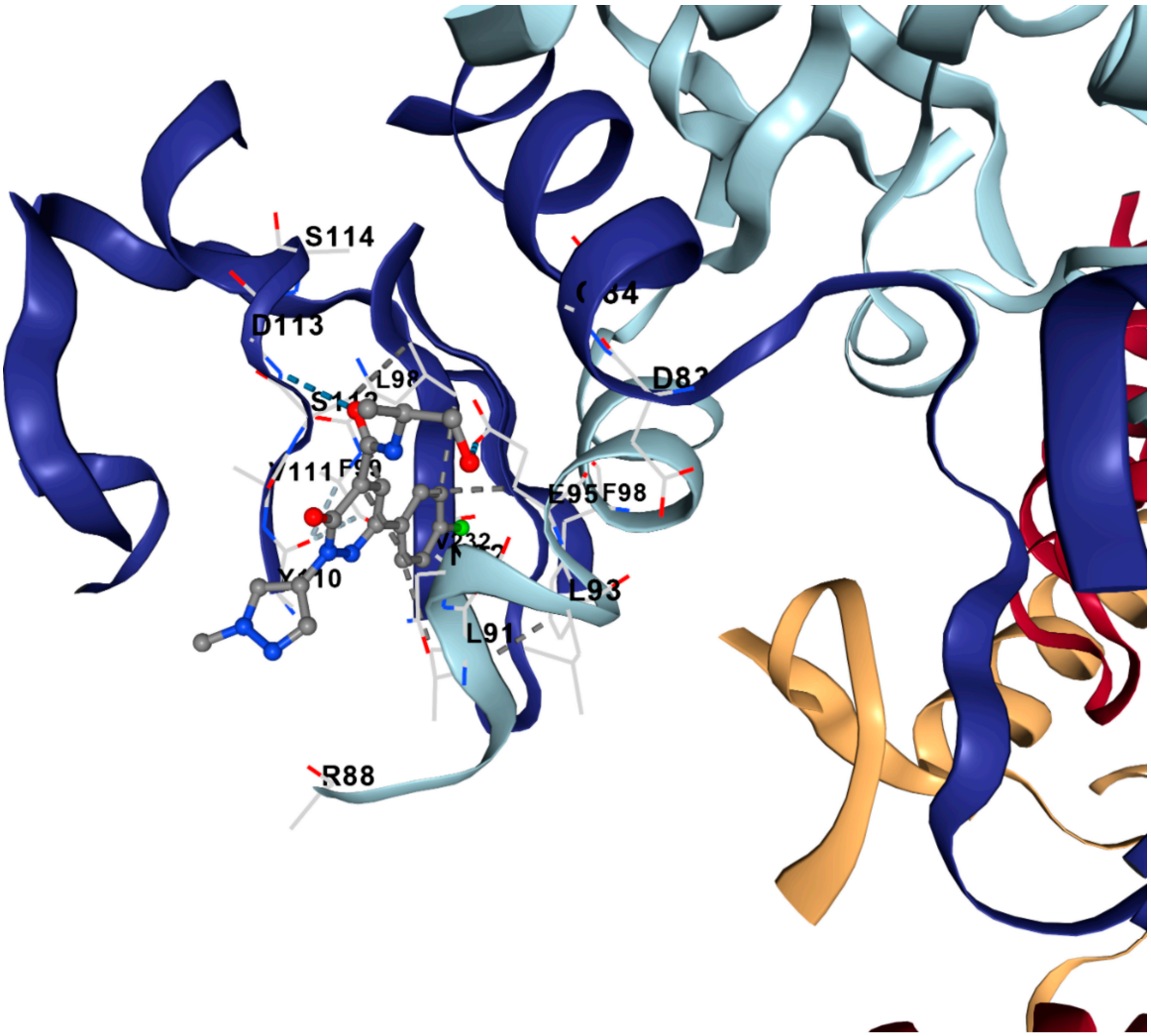

Figure 7. Docking pose of the ligand BAY 2416964 within Pocket C1.

3.2.3. GNF-351—A Selective AhR Antagonist

GNF 351 (Figure 8) is a highly selective and potent aryl hydrocarbon receptor (AhR) antagonist. It contains a rigid, nitrogen-rich tricyclic aromatic core that enables strong receptor binding without intrinsic agonist activity, even at high concentrations. The compound is highly lipophilic (logP ≈ 4.5), exhibits excellent solubility in organic solvents (≥125 mg/mL in DMSO), and has very poor aqueous solubility, resulting in minimal systemic absorption. In animal models, oral administration leads to negligible plasma and urinary exposure, with predominant fecal excretion and extensive Phase I metabolism mediated by cytochrome P450 enzymes [39]. Despite limited systemic distribution, these properties make GNF 351 well-suited for localized AhR inhibition, particularly in the gastrointestinal tract. Its low in vitro cytotoxicity further supports its potential for continued preclinical development [40].

The molecular docking model (Table 2, Figure 9) demonstrates high-affinity binding of GNF-351, a potent and selective AhR antagonist, within the PAS-B ligand-binding domain of the AhR. Consistent with experimental evidence, GNF-351 effectively blocks ligand-induced receptor activation without triggering downstream gene transcription [41]. Blind docking identified additional cavities, including pockets C4 (Vina score −10.0 kcal/mol) and C2 (−9.1 kcal/mol), with favorable predicted binding energies. However, these elevated scores likely reflect structural and algorithmic biases inherent to docking simulations rather than true functional relevance. In contrast, ligand binding within the canonical

PAS-B pocket (C1) is experimentally established as the primary determinant of AhR activation and, thus, represents the most biologically relevant site for functional inhibition. The predicted binding pose indicates that stabilization is primarily mediated by hydrophobic packing and π–π interactions with aromatic residues, complemented by polar interactions involving heteroatoms within the ligand scaffold. Occupation of the PAS-B cavity is therefore expected to competitively block binding of high-affinity agonists such as TCDD, preventing the conformational changes required for AhR activation, nuclear translocation, and downstream signaling. Collectively, these findings support the classification of GNF 351 as a potent AhR antagonist.

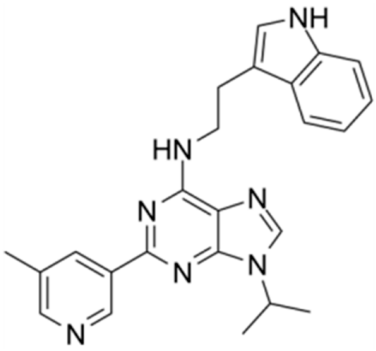

Figure 8. Chemical structure of GNF-351 generated using MolView (© 2025 MolView).

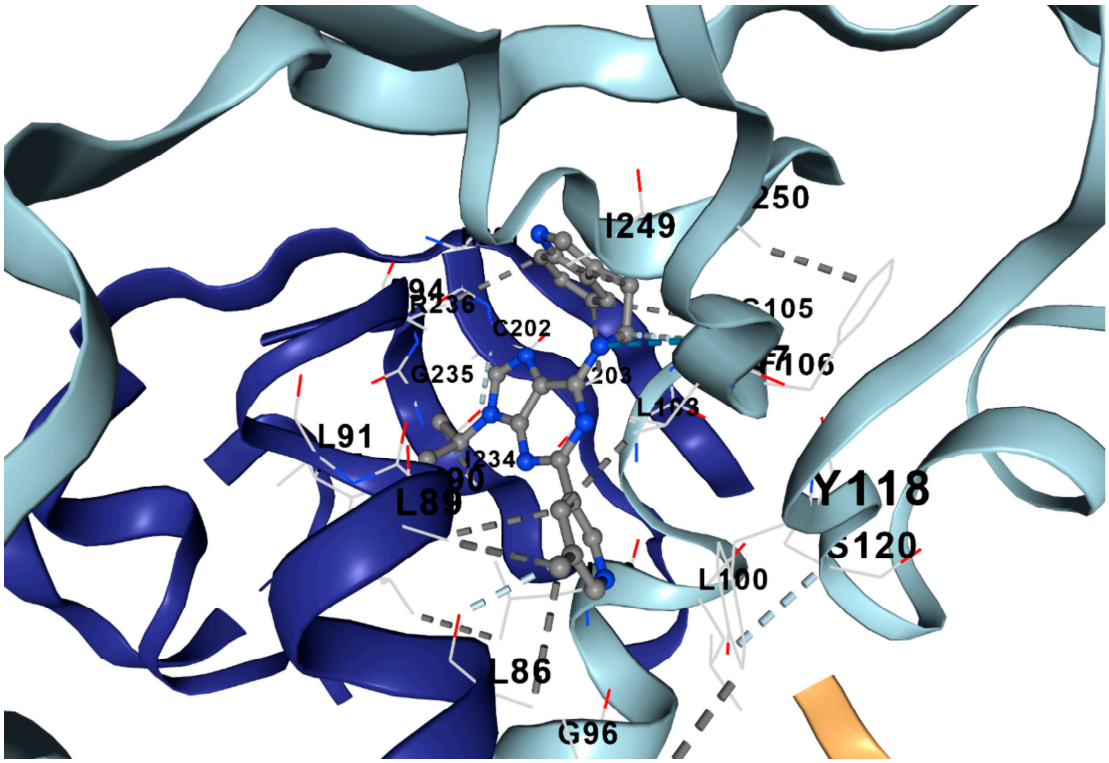

Figure 9. Docking pose of the ligand GNF-351 within Pocket C1.

Docking analyses consistently identified the canonical PAS-B cavity (pocket C1) as the most favorable and biologically relevant binding site for TCDD. This finding aligns well with experimental mutagenesis and ligand-binding studies that have established PAS-B as the critical domain responsible for high-affinity recognition of planar aromatic hydrocarbons [42,43]. The predominance of hydrophobic interactions observed in the docking poses reflects the highly lipophilic nature of TCDD and the nonpolar character of the PAS-B cavity. Such hydrophobic packing likely contributes to the remarkable stability

and persistence of the TCDD–AhR complex, which is a defining feature of dioxin toxicity. In addition, π–π interactions between the aromatic rings of TCDD and key residues within the binding pocket further stabilize the complex and may help explain the receptor's selectivity for planar, polyhalogenated ligands.

The comparative docking of known AhR antagonists revealed that chemically diverse molecules can effectively target the same PAS-B cavity while adopting distinct yet stable binding orientations. Despite differences in size and functional groups, CH223191, BAY 2416964, and GNF-351 exhibited overlapping interaction patterns within pocket C1, suggesting that competitive occupation of this cavity is sufficient to block agonist-induced receptor activation. The presence of hydrogen-bonding interactions in antagonist-bound complexes, which were less prominent in the TCDD-bound poses, may contribute to differences in binding kinetics or reversibility and could partially explain the antagonists' ability to prevent sustained AhR activation. These findings are consistent with experimental observations showing that such antagonists suppress AhR nuclear translocation and transcriptional activity without acting as partial agonists [44].

From a toxicological perspective, the results reinforce the concept that TCDD toxicity is fundamentally linked to its structural compatibility with the PAS-B domain rather than to covalent modification or irreversible receptor engagement. This distinction is important, as it implies that TCDD-induced signaling may be pharmacologically counteracted through rationally designed competitive inhibitors. The docking data support a model in which effective AhR antagonists must combine strong hydrophobic complementarity with specific polar interactions to achieve sufficient affinity while preventing the conformational rearrangements required for receptor activation.

Methodologically, this study highlights the strengths and limitations of molecular docking in investigating AhR–ligand interactions. While docking provides valuable hypotheses regarding binding modes and key interaction residues, it represents a static approximation of a dynamic system. The AhR PAS-B domain is known to exhibit conformational flexibility, which may influence ligand accommodation and binding stability. The docking energies (Vina scores) should be interpreted qualitatively, and more rigorous binding free energy calculations would be required for quantitative thermodynamic evaluation. Therefore, future work incorporating molecular dynamics simulations would be valuable for assessing binding persistence, conformational adaptability, and solvent effects. Computational docking studies are widely used in early-stage receptor–ligand investigations to prioritize potential binding sites and guide experimental design, particularly for receptors such as AhR, where structural and mechanistic information is still emerging. Additionally, experimental validation through binding assays, reporter gene studies, or structural techniques will be necessary to confirm the predicted interactions and biological relevance of the identified cavities.

Overall, the findings contribute to a growing body of evidence supporting AhR antagonism as a viable strategy for mitigating dioxin-induced toxicity. By clarifying how TCDD and structurally distinct antagonists engage the same ligand-binding domain, this study provides a structural framework for the rational design of new AhR inhibitors. Such compounds may have therapeutic potential not only in the context of accidental or occupational dioxin exposure but also in diseases characterized by aberrant AhR activation, including certain cancers, inflammatory disorders, and metabolic dysfunctions.

4. Conclusions

This study provides a detailed molecular-level perspective on the interaction between TCDD and the AhR, reinforcing the central role of the AhR PAS-B domain as the primary determinant of high-affinity ligand recognition and receptor activation.

Beyond elucidating the molecular basis of TCDD-induced AhR activation, this work highlights the utility of structure-based in silico approaches for dissecting receptor–ligand interactions in toxicology-relevant pathways. The insights gained here may inform the rational design and optimization of next-generation AhR antagonists with improved selectivity and efficacy. Nevertheless, the conclusions drawn from docking simulations require further validation through molecular dynamics simulations and experimental studies, including binding affinity measurements and functional assays. Overall, this study strengthens the case for AhR antagonism as a viable therapeutic strategy for mitigating dioxin-associated toxicity and related pathological outcomes.

Future investigations will extend these results by exploring the broader implications of AhR modulation across diverse biological contexts. Given the wide range of AhR functions, spanning environmental toxicology to pathophysiological and oncogenic processes, strategies aimed at counteracting canonical AhR signaling are of considerable interest. While the development of selective AhR modulators (SAhRMs) represents a promising approach to achieve tissue- and context-dependent modulation of AhR activity, predicting agonistic versus antagonistic outcomes remains a major challenge. Comprehensive evaluation through multiple bioassays, accounting for tissue-, organ-, and species-specific responses, is therefore required to identify ligands with optimal therapeutic potential. In this context, the AhR model developed in the present study provides a valuable framework for the rational design and evaluation of AhR-targeting compounds.

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