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Computationally Driven Discovery of Novel p53 Y220C Stabilizers

Kang Wang[1], Xingyong Liu[1, *]

[1]School of Chemical Engineering, Sichuan University of Science & Engineering, Zigong 643000, China

First author: 1258288399@qq.com (Kang Wang)

* Corresponding author: liuxy@suse.edu.cn (Xingyong Liu)

Abstract

The p53 Y220C mutation is a critical cancer-driving factor, making the development of small-molecule stabilizers capable of restoring its function an urgent clinical need. To accelerate the discovery of novel p53 Y220C stabilizers, this study employed an innovative computer-aided drug design strategy. Using the highly active molecule Activator 7 as a template, we performed a systematic virtual screening of the Freedom Space compound library by leveraging the Scaffold Hopper module of InfiniSee software in combination with its three-dimensional pharmacophore and shape features. The workflow adopted a multi-stage filtering strategy: an initial rapid screening based on 3D shape and pharmacophore matching was followed by high-precision molecular docking and binding free-energy prediction using the crystal structure of the p53 Y220C–KG13 covalent complex as the receptor model.Through this rational design pipeline, a series of structurally novel hit compounds with favorable predicted binding profiles were successfully identified. Among them, Candidate 2 not only retained the hydrogen-bond interaction between the template and the key residue Thr150 but also formed an additional hydrogen-bond network with the mutation-core residues Cys220 and Gly154 through its unique architecture, exhibiting higher predicted binding affinity and specificity. This compound, based on a novel scaffold, lays a foundation for the development of a new generation of proprietary p53 Y220C stabilizers.In summary, this study established an efficient computational screening methodology and discovered structurally novel potential p53 Y220C stabilizers, providing a critical starting point for subsequent experimental validation and lead-compound optimization.

Keywords

p53 Y220C; Computer-Aided Drug Design; Virtual Screening; Scaffold Hopping; Molecular Docking

1. Introduction

1.1 p53 Y220C

p53 is one of the most critical tumor-suppressor genes, and its encoded protein plays a pivotal role in regulating the cell cycle, inducing apoptosis, and maintaining genomic stability. However, p53 mutants are exceedingly common in cancer and are associated with more than 50 different cancer types [1]. The full-length human p53 protein (FLp53) consists of 393 amino acids and is organized into five distinct domains: the N-terminal transactivation domain (TAD), the proline-rich domain (PRD), the central DNA-binding domain (DBD), the tetramerization domain (TD), and the C-terminal regulatory domain (CTD). The TAD of p53 comprises two subdomains, TAD1 and TAD2[2, 3]. In unstressed cells, p53 exists as a mixture of monomers, dimers, and tetramers, with dimers being predominant [4].

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When cells are exposed to genotoxic stress (e.g., DNA breaks, oncogene overexpression) or microenvironmental abnormalities (e.g., hypoxia, nutrient deprivation), approximately 80% of p53 proteins can mediate dimer–dimer interactions via the TD, ultimately forming tetrameric complexes composed of two dimers. Through its DBD, the tetramer recognizes p53-binding sites located in the promoters or enhancers of target genes, thereby modulating transcription [5]. Unlike the well-folded DBD, whose structure has been resolved, the TAD and CTD of p53 are intrinsically disordered, which facilitates their interactions with co-factors to fine-tune transcription [6]. These two domains are also the primary sites for post-translational modifications (PTMs) [7]. p53 is recruited to DNA via specific response elements (REs) composed of two decameric repeats, through which it exerts its transcriptional function [8]. p53 directly regulates the transcription of more than 300 target genes. When indirect targets are considered, p53 is believed to mediate the expression of thousands of genes [9]. Most reported targets are protein-coding genes, but p53 can also regulate various non-coding RNAs [10].

In almost all cancers, p53 is inactivated either by direct mutation or by disruption of its associated signaling pathways [11]. p53 is among the most frequently mutated genes in cancer, with over half of all cancers harboring p53 mutations [6, 12, 13]. Missense mutations represent the predominant type of p53 alteration, and the consequences of p53 mutations can be categorized into three distinct effects: (1) Loss of function (LOF): mutant p53 loses the activity of wild-type (WT) p53. For example, p53 mutants often exhibit impaired ability to induce cell-cycle arrest and apoptosis [14]. (2) Dominant-negative effect (DNE): mutant p53 interferes with the function of remaining WT p53. In human pluripotent stem cells, mutant p53 gains an advantage in accelerating self-renewal. However, such DNE may be less common in normal tissues because mutant p53 levels are generally kept low in normal cells [15]. (3) Gain of function (GOF): mutant p53 often acquires additional activities not observed in the absence of WT p53, typically through interactions with specific co-factors [5]. The GOF of mutant p53 is largely attributed to its high-level accumulation in cancer cells. For instance, mutant p53 can co-aggregate with WT p53 and other tumor suppressors such as p63 and p73[16, 17]. Mutant p53 can acquire ferroptosis-resistance activity, thereby promoting tumor growth. By modulating the expression of pro-metastatic targets, mutant p53 enhances the metastatic potential of cancer cells in various mouse models [18, 19]. The exact causes of p53 mutations are not fully understood but may involve environmental and chemical carcinogens such as ultraviolet radiation, aflatoxin, and tobacco smoke [20].

Approximately 30% of p53 missense mutations occur in the DBD, with six common hotspot mutations—R282, R273, R249, R248, G245, and R175—being most frequent. Other recurrent missense mutation sites include H179 and Y220[21]. All such mutations impair the thermal stability of p53 to varying degrees [21, 22]. Unlike most p53 mutants, the p53 Y220C mutation—where tyrosine (Y) at position 220 is replaced by cysteine (C)—creates a hydrophobic cavity on the surface of the protein distant from the DBD due to the smaller side chain of cysteine (Figure 1) [23]. This structural alteration destabilizes the p53 core domain, reducing its thermal stability by approximately 4 kcal/mol. In contrast, wild-type p53 is relatively stable, with a melting temperature around 44 °C [24-26]. Consequently, the p53 Y220C mutant cannot maintain its native conformation at physiological temperature, leading to loss of function and ultimately contributing to tumorigenesis [23]. The Y220C mutation is estimated to drive about 75,000 new cancer cases annually [3].

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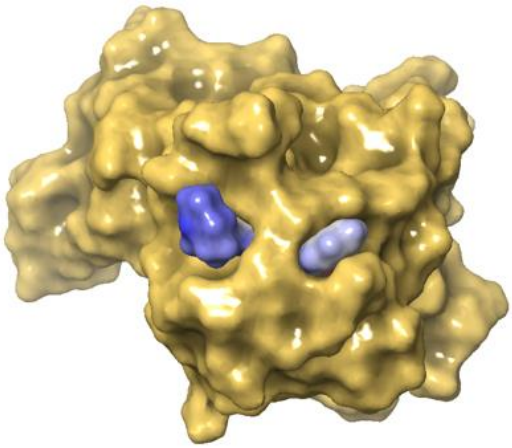

Figure. 1: p53 Y220C mutation region (PDB ID: 8DC8)

Because the cavity formed by Y220C is located away from the DBD, it offers a unique opportunity to develop small-molecule stabilizers that can rescue p53 Y220C function without interfering with its binding to natural substrates such as DNA [27, 28]. Such mutation-specific targeted stabilizers represent a promising personalized therapeutic strategy and hold significant potential for cancer treatment.

1.2 Research status of p53 Y220C small molecule drugs

In recent years, significant progress has been made in research targeting the p53 Y220C mutant. Researchers have successfully resolved the structures of p53 Y220C and its complexes with various ligands, providing an important theoretical foundation for structure-based drug development and leading to the successful identification of several small-molecule stabilizers for p53 Y220C (Figure. 2).

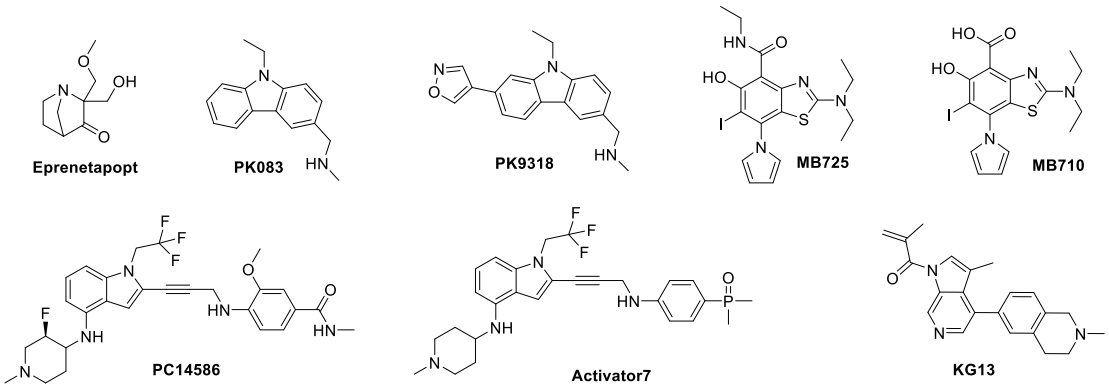

Figure. 2: Selected published p53 Y220C small molecule stabilizers

As a functional-restoring agent for mutant p53, APR-246 (eprenetapopt) was initially developed by Aprea Therapeutics in the early 2000s. Its mechanism of action involves the enzymatic conversion of the prodrug to the active metabolite methylene quinuclidinone (MQ) within cells. MQ covalently modifies the DNA-binding domain of mutant p53, promoting the refolding and stabilization of its misfolded β-sheet structure, thereby restoring transcriptional activity. Additionally, by binding to glutathione (GSH) or inhibiting antioxidant enzymes, APR-246 can induce oxidative stress and subsequently trigger apoptosis in cancer cells. As the first-in-class mutant p53 reactivator, APR-246 holds notable clinical significance [29].

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Employing a structure-based drug design strategy centered on p53 Y220C, the Boeckler team identified two small-molecule compounds, PK083 and PK9318. These compounds specifically bind to the Y220C mutant, stabilizing it in a wild-type-like conformation and subsequently inducing Y220C-dependent cell-cycle arrest and apoptosis [30]. In 2018, the Bauer research group discovered two aminobenzothiazole derivatives, MB710 and MB725, which bind tightly to the Y220C pocket, stabilize p53 Y220C, and demonstrate anti-cancer activity in p53 Y220C-harboring cell lines. Subsequently, a breakthrough was achieved in 2023 by the Guiley and Shokat teams at the University of California. By covalently targeting the cysteine residue at the Y220C site, they successfully developed the covalent small-molecule compound KG13. Co-crystal structure analysis of KG13 bound to p53 Y220C confirmed its covalent engagement with Y220C, restoring the mutant to a wild-type-like conformation. The compound showed excellent therapeutic efficacy in activity assays [31, 32].

On the clinical front, the PMV Pharma research team released Phase I clinical trial data for their selective p53 Y220C stabilizer, PC14586, in 2022. The results indicated favorable safety and tolerability profiles, making PC14586 the first p53 Y220C small-molecule stabilizer to receive FDA clearance for clinical trials in advanced solid tumors [33, 34]. In 2024, JAB-30355, an oral p53 Y220C small-molecule activator developed by Jacobio Pharmaceuticals, received clinical trial approval. It is intended for treating patients with locally advanced or metastatic solid tumors harboring the p53 Y220C mutation. Current preclinical data show that JAB-30355 exhibits high binding affinity for the p53 Y220C mutant protein and induces significant tumor regression across various cancer models, including gastric, ovarian, breast, and lung cancers [35].

In parallel, some agents have been found to selectively degrade the p53 Y220C mutant protein. For example, cabozantinib promotes its degradation via the ubiquitin-proteasome pathway, thereby inhibiting tumor growth. By competitively binding to the p53 Y220C mutant, cabozantinib sterically hinders the interaction between the UBL domain of the deubiquitinase USP7 and the β-sheet region of p53 Y220C. This blockade subsequently promotes CHIP (E3 ubiquitin ligase) -mediated proteasomal degradation of p53 Y220C [36].

Despite the reported progress on p53 Y220C stabilizers, several key scientific challenges in this field remain to be addressed: (1) The pharmacokinetic properties (absorption, distribution, metabolism, and excretion) of candidate compounds require systematic optimization to improve their bioavailability and in vivo stability. (2) The therapeutic efficacy against the p53 Y220C mutant is constrained by multiple factors, including mutant protein conformational stability and regulation of upstream/downstream signaling pathways, and whether tumor-suppressor function can be effectively restored remains uncertain. (3) Insufficient selectivity of some candidate drugs may lead to unintended off-target interactions, compromising treatment precision. (4) Safety assessments indicate that some therapeutic agents may induce dose-limiting toxicities during target engagement, necessitating more robust toxicological evaluation systems. The integration of artificial intelligence-aided drug design, computer-aided drug design technologies, and high-throughput screening platforms holds promise for providing breakthrough solutions to these limitations.

1.3 Advantages of Computational Screening Strategies

Traditional drug discovery approaches, such as high-throughput random screening, can cover broad chemical space but are inherently limited by high costs, long timelines, and low hit rates. In recent years, computationally driven rational design strategies have emerged as key enablers for accelerating lead-compound discovery. Among these, template-based virtual screening offers distinct advantages: (1) High-quality Starting Point and Improved Hit Rates: Using a known active molecule as a template allows direct inheritance of its validated

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pharmacophore features and binding mode. This focuses the search on the regions of chemical space most likely to contain active compounds, substantially increasing the efficiency and success rate of hit discovery. (2) Guided Structural Diversity: Through computational simulations—such as scaffold hopping and pharmacophore similarity searching—novel scaffolds that are structurally distinct (in 2D or 3D) from the template yet functionally equivalent can be systematically explored while preserving key interactions. This effectively bypasses existing patent barriers and expands chemical diversity. (3) Cost- and Time-Efficiency: Prior to synthesis and wet-lab validation, multi-stage computational filters—including molecular docking, binding-free-energy calculations, and drug-likeness predictions-enable the rapid elimination of unsuitable candidates, significantly reducing both development costs and timelines.

Building on these strategic advantages, this study aims to establish an efficient and rational discovery pipeline. We selected Activator 7, a potent activator of p53 Y220C with excellent activity (EC_{50} = 104 nM) and a well-defined binding mode, as the template molecule, providing an ideal starting point for computational design. By integrating structure-based molecular docking, pharmacophore modeling, and scaffold-hopping algorithms, we systematically explore small-molecule compounds that exhibit binding characteristics similar or superior to those of Activator 7 while possessing novel scaffolds.

2. Results

2.1 Chemical Structure Analysis of the Template Molecule

We first analyzed the chemical structure of the template molecule, Activator7. The core scaffold of Activator 7 is a pyrrolo[2,3-d] pyrimidine-a carefully designed heterocyclic system. Its structural features include: (1) The pyrrolopyrimidine skeleton is responsible for filling the surface hydrophobic cavity created by the Y220C mutation, forming stable stacking interactions via van der Waals forces with aromatic residues such as Phe270 and Trp146. (2) The molecule contains a basic aminopiperidine ring that can be protonated at physiological pH, enabling the formation of a salt bridge with acidic residues (e.g., Asp228) at the edge of the cavity, which contributes substantially to the binding energy. (3) A highly polar phosphine oxide group extends into the solvent-exposed region, forming a hydrogen-bond network with surrounding polar residues. (4) An alkyne-based rigid linker connects these modules, balancing conformational entropy penalty and spatial complementarity. The proposed binding mode of Activator 7 within the p53 Y220C protein is illustrated in Figure 3.

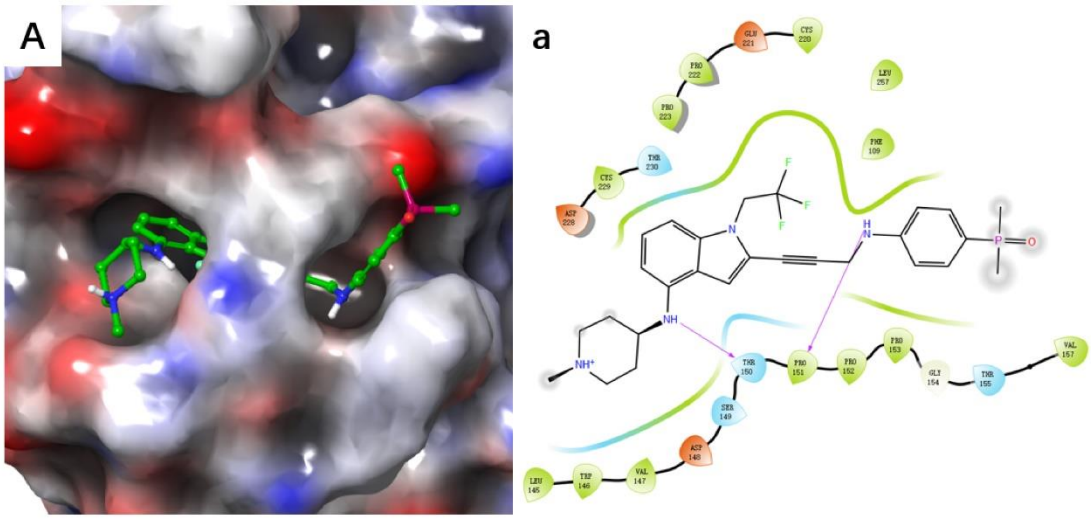

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Figure. 3: The potential binding mode of p53 Y220C and Activator7. **(A, a)** The 2D and 3D graph of p53 Y220C and Activator7 interaction mode.

2.2 Virtual Screening of Template Molecules

Using the Scaffold Hopper module within the InfiniSee platform, we conducted a systematic virtual screening campaign with the known active molecule Activator 7 as the template, aiming to identify novel-scaffold hit compounds. The specific workflow was as follows:

2.2.1 Template Preparation and Input

The two-dimensional structure (SMILES format) or three-dimensional structure file of the active template molecule Activator 7 was imported into the InfiniSee software. In the Scaffold Hopper module, it was explicitly designated as the "query template." The software automatically analyzed and extracted its core pharmacophore features and three-dimensional shape profile, which served as the benchmark for subsequent similarity comparison and scaffold-hopping.

2.2.2 Parameterized Scaffold-Hopping Settings

Key parameters were configured in the core parameter panel of Scaffold Hopper: "3D shape similarity" and "pharmacophore matching" were selected as dual-driven modes. Similarity thresholds were set to balance novelty and functional conservation. A crucial step was enabling the "scaffold-hopping" option and adjusting its intensity parameter, allowing the algorithm to perform discontinuous structural transformations on the core ring systems of Activator 7 while maintaining the spatial arrangement of key interaction points (e.g., hydrogen-bond donors/acceptors, aromatic centers).

2.2.3 Docking Library and Virtual Screening Execution

The built-in "Freedom Space" compound library of InfiniSee was selected as the screening source. This library contains a rich collection of virtual compounds with favorable drug-likeness and synthetic feasibility. After loading the library, the screening process was initiated. The software automatically executed the following computational sequence: first, a rapid initial screening based on shape and pharmacophore matching was performed to broadly select tens of thousands of molecules from the library that potentially share a similar mode of action with the template; subsequently, the initial hits were further filtered using Lipinski's Rule of Five and Veber's Rules. Lipinski's rules mainly include: no more than 5 hydrogen-bond donors; no more than 10 hydrogen-bond acceptors; a calculated LogP not exceeding 5; molecular weight below 500; and no more than 10 rotatable bonds. Veber's rules mainly include: no more than 10 rotatable bonds; a total of hydrogen-bond donors and acceptors not exceeding 12; and a polar surface area (PSA) ≤ 140 $Å^2$.

2.2.4 Result Analysis and Candidate Compound Selection

After the computation was completed, the InfiniSee platform output a list containing predicted binding scores, three-dimensional superposition diagrams, and key interaction analyses. The results were ranked and manually reviewed according to the following multi-criteria: (1) predicted binding affinity equal to or better than that of the template; (2) conservative matching with the core pharmacophore of the template, but with significant differences in the two-dimensional molecular scaffold (e.g., ring systems, linkers); (3) favorable interactions with key target residues (e.g., Cys220). Finally, from the highest-scoring clusters, several molecules with clearly novel structures and reasonable computational binding modes were carefully selected and defined as potential novel-scaffold hit compounds for subsequent synthesis and experimental validation.

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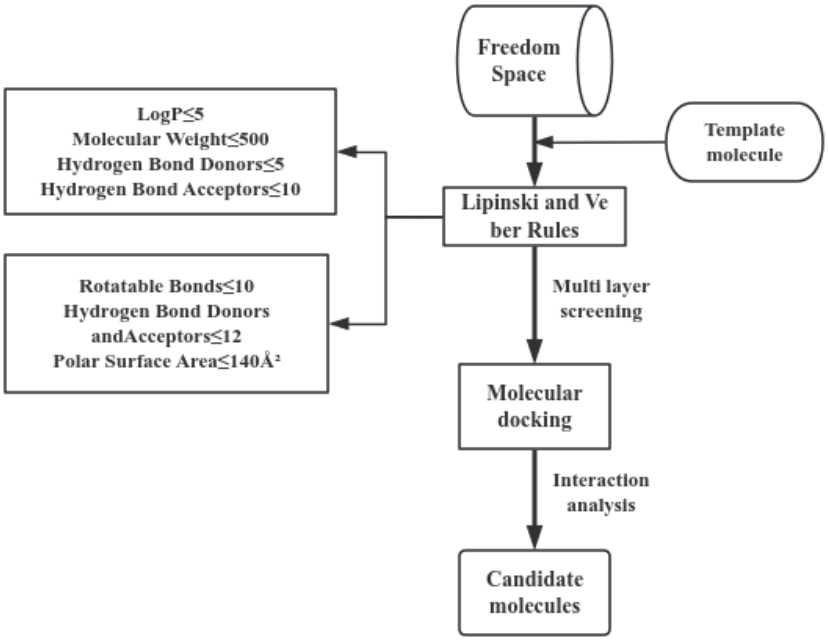
This workflow fully leverages the computational efficiency of InfiniSee and the chemical diversity of the "Freedom Space" library, enabling rapid and targeted exploration of structurally novel active compound space starting from a single known active molecule.

2.3 Virtual screening workflow



2.4 Identification of Hit Compounds

Through the design and construction described above, we obtained the two-dimensional chemical structures of candidate compounds 1, 2, 3, and 4 (Figure 4). These candidates were then subjected to molecular docking with the p53 Y220C protein using Schrödinger Maestro software for subsequent comparative analysis.

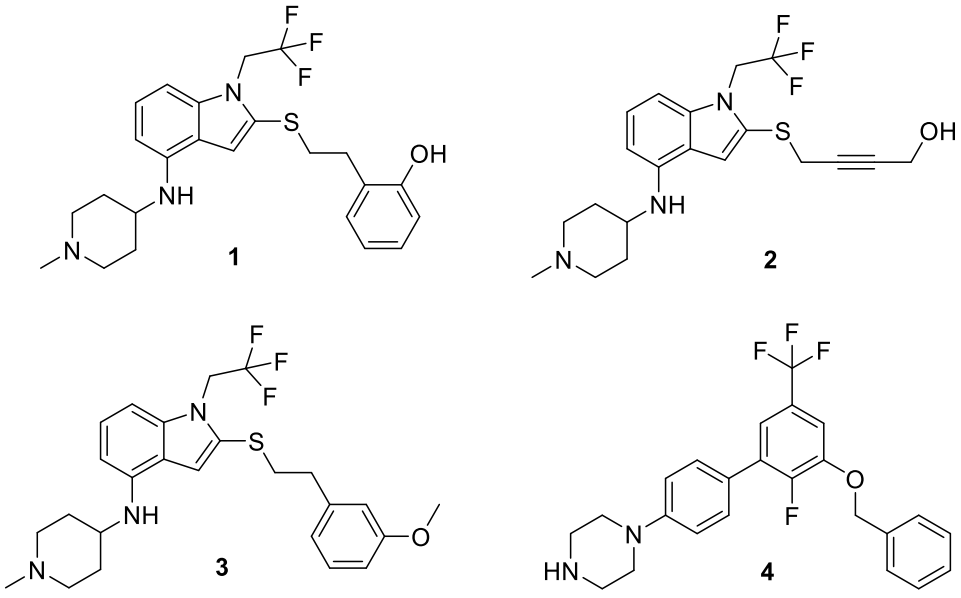


Figure. 4: Compound structures of candidate compounds 1, 2, 3, and 4

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Docking analysis revealed that all four candidate compounds (1~4) stably bound to the target's active pocket. Among them, candidate 2 achieved a docking score of -8.6 kcal/mol, which is significantly better than the template molecule Activator 7 (-7.2 kcal/mol), indicating a stronger predicted binding affinity. Key interaction analysis (Figure 5) showed that candidate 2 not only formed a stable hydrogen bond with the same critical residue THR150 as Activator 7, but also engaged in additional hydrogen-bond

interactions with CYS220 and GLY154 via its introduced hydroxyl group, further enhancing binding stability. In summary, the computer-aided drug-design approach enabled the screening and evaluation of candidate compounds. Candidate 2, with its superior docking score and richer key interactions, demonstrates promising development potential and provides a reliable candidate molecule for subsequent activity-validation experiments.

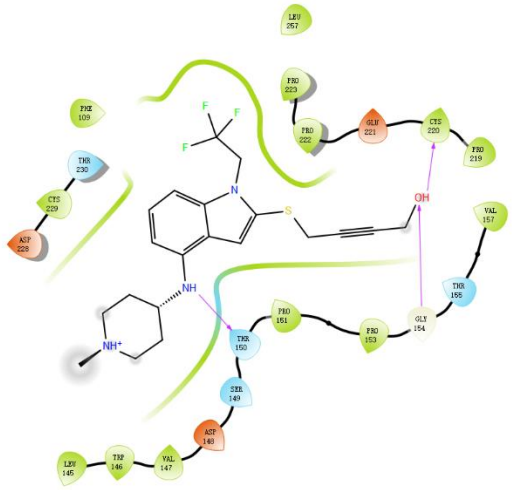

Figure. 5: The potential binding mode of p53 Y220C and candidate compound 2.

3. Discussion

Through template-based virtual screening, we obtained candidate compounds 1, 2, 3, and 4. Candidate 2 fully retains the most critical interaction of Activator 7 with the target—most notably, the stable hydrogen bond with the side-chain hydroxyl of THR150. This interaction serves as the anchor that positions the template at the edge of the p53 Y220C binding pocket and maintains the correct binding pose. Inheriting this key hydrogen bond ensures that compound 2 possesses initial positioning and binding stability comparable to the template.

Beyond this, the major breakthrough of candidate 2 compared to the template lies in its successful introduction of additional polar interactions, which significantly enhances binding affinity and specificity. The deliberately introduced hydroxyl group of candidate 2 forms an extra hydrogen bond with the backbone or side chain of the mutation-core residue CYS220. This interaction is doubly significant: (1) Direct stabilization of the mutation site: CYS220 is central to the cavity created by the Y220C mutation. Direct hydrogen bonding acts as an additional anchor point at the "foundation" of the cavity, more directly and effectively stabilizing the locally destabilized structure. (2) Potential allosteric effect: This interaction may fine-tune the conformation around CYS220, further promoting the tightening of the entire cavity toward a wild-type-p53-like conformation. Moreover, the hydroxyl group of candidate 2 also forms a hydrogen bond with the backbone carbonyl oxygen of GLY154. Located in a flexible loop region of the binding pocket, this interaction helps stabilize the local conformation of this region, reduces the entropic penalty upon binding, and may optimize the pocket shape for better ligand accommodation.

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Through ingenious group introduction, candidate 2 has constructed a richer and more robust hydrogen-bond network while perfectly inheriting the advantageous binding features of the template. The direct interaction with the mutant residue CYS220, in particular, is the key structural basis for its predicted superiority over the template. This binding mode provides a clear blueprint for guiding subsequent rational optimization.

Nevertheless, although computational models indicate an optimized binding mode for candidate 2, the predictions carry inherent uncertainties and must be confirmed through rigorous experimental validation. First, limitations of computational simulations are unavoidable. Molecular docking and binding-free-energy calculations rely on the accuracy of force-field parameters and approximations of solvation and protein flexibility. Whether the predicted additional hydrogen-bond network can stably exist in a real physiological environment and within a dynamic protein structure requires experimental confirmation; the model may overestimate interaction strengths or overlook potential conformational conflicts.

Therefore, critical validation steps are indispensable: (1) Protein thermal shift (PTS) assay to directly measure the thermal-stabilization effect (ΔTm) of compound 2 on the p53 Y220C protein, verifying its actual stabilizing ability and quantifying its potency relative to the template. (2) Cellular-level functional validation in cell lines harboring the p53 Y220C mutation, assessing compound 2's ability to restore p53 transcriptional activity and induce cell-cycle arrest or apoptosis, thereby confirming its functional agonist activity and cell permeability.

In summary, computational results provide a powerful design guide but are not definitive proof. Only by combining PTS-validated binding stabilization with cell-based validation of functional restoration can the true potential of candidate 2 be confirmed and advanced to subsequent development stages.

4. Methods

4.1 Choice of Docking Protein

This study employed PDB ID: 8DC8 as the core receptor structure for docking. This structure represents the co-crystal complex of the p53 Y220C mutant with the covalent inhibitor KG13, accurately delineating the geometric and chemical features of the Y220C cavity. Systematic preprocessing of the protein was first performed using the Protein Preparation Wizard module in Schrödinger Maestro: all water molecules and irrelevant ligands were removed, missing residues were completed, and the protonation states of key residues (e.g., Asp228, Cys220, Arg248) were optimized under pH 7.4, along with refinement of the hydrogen-bond network. To address distinct research aims, differential treatments were applied: for non-covalent screening, Cys220 was restored to a standard thiol group and KG13 was removed to obtain a "clean" active pocket; for covalent-binding analysis, the covalent linkage was retained as a reference conformation. Subsequently, the binding pocket was defined centered on the centroid of the original ligand, and docking grids covering the entire hydrophobic cavity and surrounding polar regions were generated using Glide Grid Generation. Docking calculations were conducted with the Glide module, employing either standard-precision or extra-precision modes as required, and key residue side chains were allowed flexibility when necessary to improve prediction accuracy. To validate the reliability of the model, the known active molecule was docked into the processed structure, confirming that key salt-bridge and hydrogen-bond interactions could be reproduced.

4.2 Molecular docking

Protein preparation was first performed using the Protein Preparation Wizard module in Schrödinger Maestro. The process included checking for and adding missing amino acids and

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loop structures, hydrogenation, generating disulfide bonds, zero-order bonds, and metal ions, and desolvation by removing crystallographic free water molecules located >5 Å from the ligand. Hydrogen bonds in the receptor were optimized to correct overlapping hydrogen atoms, and water and irrelevant solvent molecules were removed. Finally, the receptor was refined using the OPLS3e force field and protonated at pH 7.0. The Receptor Grid Generation module was used to generate the grid file required for molecular docking.

Subsequently, the small-molecule compounds to be docked were imported into the software. Ligand preparation was performed using the LigPrep module, with energy minimization carried out using the OPLS_2005 force field. Protonation states were generated with Epik at pH 7.4, and three-dimensional conformations were generated.

Finally, the grid-point file was imported, and the small-molecule compounds were processed in extra-precision (XP) mode to generate 3D conformations. The resulting output provided the interaction patterns between the compounds and the protein.

5. Conclusion

Our work began with a clear rational starting point: the reported high-activity molecule Activator 7 served as the core template. Using the Scaffold Hopper module of InfiniSee software, guided by the template's three-dimensional pharmacophore and shape features, we systematically searched the Freedom Space compound library to identify molecules with novel scaffolds but equivalent function. The screening workflow employed a multi-level filtering strategy: initial rapid screening based on 3D shape similarity and pharmacophore matching, followed by precise binding-mode evaluation and binding-free-energy prediction of the initial hits via molecular docking. The final output was a prioritized list of compounds that balanced predicted binding affinity, conservation of key interactions, and structural novelty.

Through this computationally driven screening, we successfully identified several prospective hit compounds, with candidate 2 being particularly outstanding. Computational analysis revealed that this molecule not only fully retains the stable hydrogen bond between the template and the key residue Thr150, but its unique structural modification introduces additional hydrogen-bond interactions with the mutation-core residues Cys220 and Gly154. This evolution from a "single-point anchor" to a "networked-anchor" binding mode suggests potentially higher binding affinity and improved target specificity. Importantly, this compound is based on a completely novel scaffold, providing a critical entry point for circumventing existing patent barriers and expanding the chemical space of p53 Y220C stabilizers. Thus, these hit compounds, especially candidate 2, exhibit great potential to become high-potency, highly selective, and structurally novel next-generation allosteric stabilizers of p53 Y220C.

Although the computational predictions are encouraging, translating them into practical breakthroughs necessitates entry into the experimental validation phase. Our next steps will follow the closed-loop principle of "computation-guided, experiment-verified" research, specifically: (1) Chemical synthesis and characterization: Priority will be given to the chemical synthesis of candidate 2 and its key analogues, followed by rigorous structural confirmation and purity analysis using NMR, LC-MS, etc., to provide material for subsequent biological evaluation. (2) Biophysical validation: Protein thermal shift (PTS) analysis will be used to directly measure the thermal-stabilization effect (ΔTm) of the synthesized compounds on p53 Y220C, quantitatively verifying their ability to stabilize the mutant protein and comparing their potency with the template molecule Activator 7. (3) Cellular-functional validation: In tumor cell lines harboring the p53 Y220C mutation, reporter-gene assays, qPCR analysis of downstream target-gene expression, and cell-proliferation/apoptosis assays will

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be employed to comprehensively evaluate the compounds' ability to restore p53 transcriptional function and inhibit tumor-cell growth, while providing a preliminary assessment of cytotoxicity. (4) In-depth mechanistic exploration: Based on validation results, further studies such as surface-plasmon-resonance analysis of binding kinetics or molecular-dynamics simulations to investigate complex dynamic stability may be conducted to elucidate the mechanism of action in greater depth.

In summary, this study not only successfully applied advanced computational screening strategies to discover structurally novel hit compounds, but also laid a solid foundation for subsequent experimental translation and lead-compound optimization, marking a critical step toward the development of original anticancer drugs targeting the important therapeutic target p53 Y220C.

References

[1] MARTíNEZ-JIMéNEZ F, MUIñOS F, SENTíS I, et al. A compendium of mutational cancer driver genes [J]. Nature reviews Cancer, 2020, 20(10): 555-572.

[2] RAJ N, ATTARDI L D. The Transactivation Domains of the p53 Protein [J]. Cold Spring Harbor perspectives in medicine, 2017, 7(1).

[3] JOERGER A C, FERSHT A R. The tumor suppressor p53: from structures to drug discovery [J]. Cold Spring Harbor perspectives in biology, 2010, 2(6): a000919.

[4] GAGLIA G, GUAN Y, SHAH J V, et al. Activation and control of p53 tetramerization in individual living cells [J]. Proceedings of the National Academy of Sciences of the United States of America, 2013, 110(38): 15497-15501.

[5] LIU Y, SU Z, TAVANA O, et al. Understanding the complexity of p53 in a new era of tumor suppression [J]. Cancer cell, 2024, 42(6): 946-967.

[6] JOERGER A C, FERSHT A R. The p53 Pathway: Origins, Inactivation in Cancer, and Emerging Therapeutic Approaches [J]. Annual review of biochemistry, 2016, 85: 375-404.

[7] LIU Y, TAVANA O, GU W. p53 modifications: exquisite decorations of the powerful guardian [J]. Journal of molecular cell biology, 2019, 11(7): 564-577.

[8] HAFNER A, BULYK M L, JAMBHEKAR A, et al. The multiple mechanisms that regulate p53 activity and cell fate [J]. Nature reviews Molecular cell biology, 2019, 20(4): 199-210.

[9] FISCHER M. Census and evaluation of p53 target genes [J]. Oncogene, 2017, 36(28): 3943-3956.

[10] CHEN S, THORNE R F, ZHANG X D, et al. Non-coding RNAs, guardians of the p53 galaxy [J]. Seminars in cancer biology, 2021, 75: 72-83.

[11] OLIVIER M, EELES R, HOLLSTEIN M, et al. The IARC TP53 database: new online mutation analysis and recommendations to users [J]. Human mutation, 2002, 19(6): 607-614.

[12] BYKOV V J, WIMAN K G. Mutant p53 reactivation by small molecules makes its way to the clinic [J]. FEBS letters, 2014, 588(16): 2622-2627.

[13] BAUER M R, JOERGER A C, FERSHT A R. 2-Sulfonylpyrimidines: Mild alkylating agents with anticancer activity toward p53-compromised cells [J]. Proceedings of the National Academy of Sciences of the United States of America, 2016, 113(36): E5271-5280.

[14] KIM M P, LOZANO G. Mutant p53 partners in crime [J]. Cell death and differentiation, 2018, 25(1): 161-168.

[15] MERKLE F T, GHOSH S, KAMITAKI N, et al. Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations [J]. Nature, 2017, 545(7653): 229-233.

[16] LI J, GUO M, CHEN L, et al. p53 amyloid aggregation in cancer: function, mechanism, and therapy [J]. Experimental hematology & oncology, 2022, 11(1): 66.

[17] XU J, REUMERS J, COUCEIRO J R, et al. Gain of function of mutant p53 by coaggregation with multiple tumor suppressors [J]. Nature chemical biology, 2011, 7(5): 285-295.

ISSN: 3079-6709

[18] SU Z, KON N, YI J, et al. Specific regulation of BACH1 by the hotspot mutant p53(R175H) reveals a distinct gain-of-function mechanism [J]. Nature cancer, 2023, 4(4): 564-581.

[19] DIBRA D, XIONG S, MOYER S M, et al. Mutant p53 protects triple-negative breast adenocarcinomas from ferroptosis in vivo [J]. Science advances, 2024, 10(7): eadk1835.

[20] RIVLIN N, BROSH R, OREN M, et al. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis [J]. Genes & cancer, 2011, 2(4): 466-474.

[21] TUVAL A, STRANDGREN C, HELDIN A, et al. Pharmacological reactivation of p53 in the era of precision anticancer medicine [J]. Nature reviews Clinical oncology, 2024, 21(2): 106-120.

[22] HAINAUT P, PFEIFER G P. Somatic TP53 Mutations in the Era of Genome Sequencing [J]. Cold Spring Harbor perspectives in medicine, 2016, 6(11).

[23] BULLOCK A N, HENCKEL J, FERSHT A R. Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: definition of mutant states for rescue in cancer therapy [J]. Oncogene, 2000, 19(10): 1245-1256.

[24] BEJ A, RASQUINHA J A, MUKHERJEE S. Conformational Entropy as a Determinant of the Thermodynamic Stability of the p53 Core Domain [J]. Biochemistry, 2018, 57(44): 6265-6269.

[25] ANG H C, JOERGER A C, MAYER S, et al. Effects of common cancer mutations on stability and DNA binding of full-length p53 compared with isolated core domains [J]. The Journal of biological chemistry, 2006, 281(31): 21934-21941.

[26] FRIEDLER A, VEPRINTSEV D B, HANSSON L O, et al. Kinetic instability of p53 core domain mutants: implications for rescue by small molecules [J]. The Journal of biological chemistry, 2003, 278(26): 24108-24112.

[27] JOERGER A C, ANG H C, FERSHT A R. Structural basis for understanding oncogenic p53 mutations and designing rescue drugs [J]. Proceedings of the National Academy of Sciences of the United States of America, 2006, 103(41): 15056-15061.

[28] WILCKEN R, LIU X, ZIMMERMANN M O, et al. Halogen-enriched fragment libraries as leads for drug rescue of mutant p53 [J]. Journal of the American Chemical Society, 2012, 134(15): 6810-6818.

[29] MISHRA A, TAMARI R, DEZERN A E, et al. Eprenetapopt Plus Azacitidine After Allogeneic Hematopoietic Stem-Cell Transplantation for TP53-Mutant Acute Myeloid Leukemia and Myelodysplastic Syndromes [J]. Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2022, 40(34): 3985-3993.

[30] BOECKLER F M, JOERGER A C, JAGGI G, et al. Targeted rescue of a destabilized mutant of p53 by an in silico screened drug [J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(30): 10360-10365.

[31] LANE D P, VERMA C S. Covalent Rescue of Mutant p53 [J]. Cancer discovery, 2023, 13(1): 14-16.

[32] GUILEY K Z, SHOKAT K M. A Small Molecule Reacts with the p53 Somatic Mutant Y220C to Rescue Wild-type Thermal Stability [J]. Cancer discovery, 2023, 13(1): 56-69.

[33] Glimmers of Hope for Targeting p53 [J]. Cancer discovery, 2022, 12(8): Of5.

[34] MANFREDI J. The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor [J]. Genes & development, 2010, 24(15): 1580-1589.

[35] ZHENG Q, WANG P, LIANG C, et al. JAB-30355: a highly potent, orally bioavailable p53 Y220C reactivator [J]. 2023, 22(2): 127-144.

[36] LV F, ZHANG L, JI C, et al. Cabozantinib Selectively Induces Proteasomal Degradation of p53 Somatic Mutant Y220C and Impedes Tumor Growth [J]. The Journal of biological chemistry, 2025: 108167.