

Molecular Dynamics Simulations in Cancer Drug Design: From Atomic-Level Insights to Precision Therapeutics: A Review



Shamsuddin Sultan Khan ^{1*}

Abstract

There is, perhaps, a quiet shift underway in cancer drug discovery—one that is less about discovering new molecules and more about understanding how they truly behave within the living, moving architecture of biology. Traditional approaches, while powerful, often rely on static representations of proteins, inadvertently overlooking the dynamic nature of molecular interactions. This review explores how Molecular Dynamics (MD) simulations are beginning to reshape this perspective, offering a more fluid and time-resolved understanding of drug–target interactions. By synthesizing findings across key oncological systems—including the Bcl-2 family, the p53–MDM2/MDMX regulatory axis, and mutant TP53–BRCA1 complexes—this study highlights how MD enables the identification of transient conformations, cryptic binding pockets, and residue-level interaction patterns that are otherwise inaccessible. These insights appear particularly relevant in addressing challenges of selectivity and resistance, where small structural differences can have disproportionately large functional consequences. At the same time, integrating MD with complementary techniques such as MM-PBSA free energy calculations and fragment-based drug discovery frameworks offers a more comprehensive, multi-scale approach to therapeutic design. Yet, despite these advances, uncertainties remain—especially regarding

sampling limitations and translational predictability. Taken together, this review suggests that MD simulations are not merely computational tools but evolving conceptual frameworks, gradually shifting drug discovery from static observation toward dynamic, precision-driven intervention. **Keywords:** Molecular Dynamics; Cancer Drug Design; Bcl-2 Family; Protein–Protein Interactions; Precision Therapeutics

Keywords

Molecular Dynamics; Cancer Drug Design; Bcl-2 Family; Protein–Protein Interactions; Precision Therapeutics

1. Introduction

The landscape of modern drug discovery—particularly in oncology—seems to sit at an uneasy intersection of promise and limitation. On one hand, technological advancements have undeniably accelerated our ability to interrogate biological systems; on the other, the process of translating these insights into clinically viable therapeutics remains stubbornly slow, costly, and uncertain. It is difficult not to wonder: why, despite such computational and experimental sophistication, do we still struggle to efficiently identify selective and durable anticancer agents? Perhaps the answer lies not in the absence of tools, but in how we conceptualize biological complexity

Significance | Reveals how molecular dynamics transforms drug design by capturing protein flexibility, enabling selective targeting, and advancing precision oncology therapeutics.

*Correspondence.

Shamsuddin Sultan Khan, Neo7bioscience, W Commerce St Ste 2886, Dallas, TX 75208, USA. E-mail: jupitex@gmail.com,

Editor Vishwanadham Mandala, Ph.D. And accepted by the Editorial Board March 12, 2020 (received for review Jan 10, 2020)

Author Affiliation:

¹ Neo7bioscience, W Commerce St Ste 2886, Dallas, TX 75208, USA.

Please cite this article as:

Khan, S. S. (2020). "Molecular Dynamics Simulations in Cancer Drug Design: From Atomic-Level Insights to Precision Therapeutics: A Review", Bioinfo Chem, 2(1), 1-12, 10737

© 2020 Bioinfo Chem, a publication of Eman Research, USA.
This is an open access article under the CC BY-NC-ND license.
(<http://creativecommons.org/licenses/by-nc-nd/4.0/>)
(<https://publishing.emanresearch.org>)

itself.

Over the past few decades, drug discovery has gradually shifted away from largely empirical, trial-and-error approaches toward more rational, structure-guided strategies. This transition, driven by the increasing financial and temporal burden of pharmaceutical development, has positioned Computer-Aided Drug Design (CADD) as a central pillar of modern research (Alonso et al., 2006). Yet even with high-throughput screening (HTS) and combinatorial chemistry enabling the evaluation of vast compound libraries, the sheer magnitude of chemical space—often described as astronomically large—renders exhaustive experimental exploration impossible. In this context, computational frameworks do not merely assist; they redefine the boundaries of what is practically searchable (Mortier et al., 2015).

Still, the challenge is not only one of scale, but also of biological nuance. Drug discovery efforts are increasingly directed toward protein-protein interactions (PPIs), which play critical roles in cellular regulation but are notoriously difficult to target. Unlike enzyme active sites, PPIs typically present broad, shallow, and solvent-exposed interfaces—features that historically led to their classification as “undruggable.” And yet, over time, this notion has begun to soften. Careful structural and computational analyses have revealed the presence of discrete “hot spots”—localized regions contributing disproportionately to binding affinity—suggesting that these interfaces may, in fact, be pharmacologically tractable under the right conditions (Barelier et al., 2010).

Among the most illustrative examples of this complexity is the Bcl-2 family of proteins, which governs the intrinsic apoptotic pathway. These proteins—ranging from pro-survival members such as Bcl-2 and Bcl-xL to pro-apoptotic effectors—form a tightly regulated network that determines cellular fate. Dysregulation, particularly overexpression of anti-apoptotic proteins, is a hallmark of many cancers, allowing malignant cells to evade programmed cell death (Pinto et al., 2011). While inhibitors such as Navitoclax (ABT-263) have demonstrated clinical potential, their lack of selectivity—especially toward Bcl-xL—has led to significant adverse effects, including thrombocytopenia. This raises an important and perhaps unresolved question: what structural or dynamic features truly govern selectivity among highly homologous protein targets?

A parallel challenge emerges in the p53 tumor suppressor pathway, often described—almost reverently—as the “guardian of the genome.” In a significant proportion of cancers, p53 function is not lost through mutation alone but is actively suppressed by regulatory proteins such as MDM2 and MDMX. These proteins bind to p53, inhibiting its transcriptional activity and promoting its degradation (Ding et al., 2005; Grasberger et al., 2005). Early efforts to disrupt this interaction, such as the development of Nutlin-3 analogs, showed promise but were ultimately limited by their inability to effectively target MDMX-overexpressing tumors. This has shifted attention toward dual inhibitors, yet designing such molecules for large, flexible PPI interfaces remains a formidable task (Barakat et al., 2010).

At this point, one begins to see a recurring theme: the inadequacy of static structural representations. Traditional structure-based drug design often relies on crystallographic snapshots—high-resolution, yes, but inherently limited in capturing the dynamic nature of proteins. Biological macromolecules are not rigid entities; they fluctuate, adapt, and occasionally reveal transient conformations that may be functionally significant. Molecular Dynamics (MD) simulations offer a way to access this hidden dimension. By modeling atomic movements over time, MD provides not just structures, but trajectories—pathways through conformational space that can illuminate mechanisms of binding, selectivity, and allosteric regulation (Hernández-Rodríguez et al., 2016).

In fact, one of the more compelling contributions of MD lies in its ability to identify “cryptic” binding sites—pockets that are absent in static structures but emerge during dynamic fluctuations. Such sites may prove crucial for targeting proteins previously considered intractable. When combined with analytical techniques such as Principal Component Analysis (PCA), MD can also reveal dominant motion patterns, offering insights into the collective behavior of protein domains (Wakui et al., 2018). This becomes particularly relevant in systems like Bcl-2 and Bcl-xL, where subtle conformational differences may dictate ligand specificity.

Beyond individual protein systems, MD has also been integrated into broader computational workflows. In Fragment-Based Drug Discovery (FBDD), for instance, small molecular fragments are screened against target proteins to identify weak but meaningful interactions. These fragments can then be optimized into higher-affinity compounds—a process that benefits greatly from dynamic

structural information (Amaro et al., 2008). Similarly, the Relaxed Complex Scheme (RCS) enables virtual screening across ensembles of protein conformations rather than a single structure, thereby increasing the likelihood of identifying viable hits (Barakat et al., 2010).

Another dimension of complexity arises when considering thermodynamic contributions to binding. Techniques such as MM-PBSA allow for the estimation of binding free energies by decomposing interactions into enthalpic and entropic components. While not without limitations, these methods provide a more nuanced understanding of ligand stability than traditional scoring functions (Mortier et al., 2015). When applied to systems such as the p53–MDM2/MDMX axis or mutant TP53–BRCA1 complexes, they can reveal subtle energetic differences that may underpin observed biological effects (Tiwari et al., 2017; You et al., 2016).

And yet, despite these advances, a degree of uncertainty persists. One might reasonably ask: are we truly capturing the determinants of selectivity and efficacy, or merely approximating them with increasingly sophisticated models? The answer, perhaps, lies in integration. No single method—whether docking, MD, or free energy calculation—can fully encapsulate the complexity of biological interactions. Instead, a hierarchical and multi-scale approach appears necessary, combining structural, dynamic, and thermodynamic perspectives into a coherent framework.

Against this backdrop, the present review seeks to explore not only the capabilities of modern computational techniques but also their limitations and interdependencies. Specifically, it aims to investigate how dynamic conformational landscapes influence inhibitor binding within the Bcl-2 family, whether ensemble-based screening can uncover dual inhibitors for MDM2 and MDMX, and how thermodynamic analyses can elucidate stability in complex systems such as mutant TP53–BRCA1 interactions. In doing so, the study attempts—perhaps cautiously—to move toward a more integrated understanding of drug-target interactions, one that acknowledges both the promise and the inherent uncertainty of computational drug design.

2. Methodology

2.1 Study Design and Conceptual Framework

This study was designed as a narrative review, aiming to

synthesize and critically interpret existing literature on the application of Molecular Dynamics (MD) simulations in cancer drug design. Unlike systematic reviews, which follow rigid inclusion protocols and quantitative synthesis, a narrative approach allows for a more flexible, integrative exploration of emerging concepts, methodological diversity, and evolving computational strategies. This was particularly appropriate given the interdisciplinary nature of the topic, which spans computational chemistry, structural biology, and oncology (Hernández-Rodríguez et al., 2016).

The conceptual framework of this review was guided by three interconnected themes: (i) structural dynamics of protein targets, (ii) computational strategies for ligand design and screening, and (iii) translational implications for precision therapeutics. These themes were iteratively refined throughout the review process to ensure coherence between the introduction, analytical sections, and synthesized findings.

2.2 Literature Search Strategy

A comprehensive literature search was conducted using major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search focused on peer-reviewed articles published primarily between 2000 and 2024, with foundational studies included where necessary to provide methodological context.

Key search terms included combinations of: “Molecular Dynamics simulations,” “cancer drug design,” “Bcl-2 inhibitors,” “protein–protein interactions,” “MDM2/MDMX,” “fragment-based drug discovery,” “MM-PBSA,” and “computational toxicology.”

Boolean operators (AND, OR) were applied to refine search results. For example:

- “Molecular Dynamics AND cancer drug design”
- “Bcl-xL AND inhibitor selectivity AND MD simulations”

Priority was given to studies that combined computational modeling with experimental validation, as well as those introducing novel simulation methodologies or therapeutic insights (Alonso et al., 2006; Mortier et al., 2015).

2.3 Inclusion and Exclusion Criteria

The inclusion criteria were defined to ensure relevance and scientific rigor:

Inclusion criteria:

- Studies focusing on Molecular Dynamics simulations in drug discovery
- Research addressing oncological targets, particularly Bcl-2 family proteins, MDM2/MDMX, and TP53-related systems
- Articles discussing binding energetics, conformational dynamics, or computational screening approaches
- Peer-reviewed journal articles written in English

Exclusion criteria:

- Studies lacking computational or structural analysis
- Articles focusing solely on experimental pharmacology without mechanistic insights
- Conference abstracts, editorials, and non-peer-reviewed sources

This selective approach allowed for a focused yet sufficiently broad representation of the field, capturing both methodological advancements and application-driven studies.

2.4 Data Extraction and Thematic Synthesis

Relevant data from selected studies were extracted manually and organized into thematic categories. Key information included:

- Target proteins and biological pathways
- Simulation methods (e.g., classical MD, accelerated MD, cosolvent MD)
- Binding energy calculations (e.g., MM-PBSA, MM-GBSA)
- Structural insights (e.g., cryptic binding sites, conformational transitions)
- Pharmacokinetic and ADMET considerations

Rather than performing a quantitative meta-analysis, the extracted data were synthesized qualitatively to identify recurring patterns, methodological trends, and conceptual gaps. For example, studies on Bcl-xL consistently highlighted the role of dynamic binding pockets and residue-level interactions in determining selectivity, while research on the p53–MDM2/MDMX axis emphasized structural adaptability and dual-target inhibition strategies (Barakat et al., 2010; Wakui et al., 2018).

2.5 Integration of Computational Approaches

A distinguishing feature of this review is the emphasis on integrative computational methodologies. Selected studies were evaluated not only individually but also in terms of how different techniques complement each other. For instance:

- MD simulations were considered alongside docking and virtual screening methods
- Free energy calculations (MM-PBSA) were analyzed in conjunction with structural stability metrics such as RMSD and RMSF
- Fragment-Based Drug Discovery (FBDD) approaches were examined in relation to dynamic conformational sampling (Amaro et al., 2008; Barelier et al., 2010)

This integrative perspective enabled a more comprehensive understanding of how computational tools collectively contribute to rational drug design, rather than functioning in isolation.

2.6 Critical Evaluation and Interpretation

The selected literature was critically evaluated with attention to methodological strengths, limitations, and reproducibility. Particular emphasis was placed on:

- Simulation timescales and sampling adequacy
- Reliability of force fields and scoring functions
- Consistency between computational predictions and experimental outcomes

Where discrepancies were observed, these were discussed to highlight potential sources of uncertainty. For example, while MM-PBSA methods provide useful approximations of binding free energy, they may not fully capture entropic contributions or solvent effects (Kollman et al., 2000). Similarly, MD simulations, despite their power, remain limited by computational cost and sampling constraints.

2.7 Limitations of the Methodological Approach

As a narrative review, this study inherently lacks the statistical rigor of systematic reviews or meta-analyses. The selection of articles, while guided by clear criteria, may still introduce selection bias. Additionally, the qualitative synthesis approach relies on interpretative judgment, which may vary among researchers.

However, these limitations are balanced by the ability to

provide contextual depth and conceptual integration, which are essential for understanding complex, rapidly evolving fields such as computational oncology.

2.8 Summary of Methodological Approach

In summary, this narrative review employed a structured yet flexible methodology to explore the role of Molecular Dynamics simulations in cancer drug design. By combining targeted literature search, thematic synthesis, and critical evaluation, the study provides a comprehensive overview of current computational strategies and their translational relevance. The integration of structural, dynamic, and thermodynamic perspectives offers a multidimensional understanding of drug–target interactions, aligning with the broader objective of advancing precision therapeutics.

3. Bcl-xL Protein and Target-Selective Inhibition: A Dynamic and Evolving Paradigm

3.1 Reconsidering Apoptotic Control: Bcl-xL as a Biological Sentinel

Apoptosis, while often described in clear mechanistic terms, is perhaps better understood as a finely tuned negotiation—one that continuously balances survival and cellular self-destruction. At the center of this negotiation lies the Bcl-2 protein family, whose members collectively determine mitochondrial integrity and, ultimately, cellular fate. Among them, Bcl-xL emerges not merely as a participant but as a decisive regulator, a kind of molecular sentinel that restrains apoptosis by binding and neutralizing pro-apoptotic proteins such as BAX and BAK (Pinto et al., 2011).

Structurally, Bcl-xL is composed of a bundle of α -helices—typically eight or nine—that fold into a compact globular architecture. Within this fold resides the canonical BH3-binding groove, a hydrophobic cleft primarily formed by helices 2 through 5. This groove acts as a docking interface for BH3-only proteins, enabling Bcl-xL to sequester apoptotic activators and prevent mitochondrial outer membrane permeabilization. Under physiological conditions, this interaction is tightly regulated, contributing to cellular homeostasis.

Yet, this same mechanism becomes profoundly dysregulated in cancer. Overexpression of Bcl-xL has been documented across a range of malignancies, including hematological cancers and solid tumors, where it effectively shields cancer cells from apoptotic signals

(Oltsersdorf et al., 2005). One might reasonably ask whether this overexpression simply amplifies a normal function—or whether it fundamentally rewires apoptotic thresholds. Increasing evidence suggests the latter, with Bcl-xL also implicated in processes beyond apoptosis, including metabolic adaptation and resistance to chemotherapeutic stress. Such functional breadth complicates its therapeutic targeting, making it both an attractive and elusive candidate for drug design.

3.2 Beyond Static Models: The Enigma of Cryptic Binding Sites

For much of early drug discovery, proteins were conceptualized as static entities—rigid “locks” awaiting complementary “keys.” This framework, while useful, proves insufficient when applied to proteins like Bcl-xL. Its BH3-binding groove is not a permanently accessible cavity but rather a flexible, shape-shifting interface. Indeed, certain sub-pockets within this groove—particularly P1 and P2—remain partially or fully occluded in the apo state and only become accessible upon ligand binding or conformational rearrangement (Azam et al., 2014).

This phenomenon introduces the notion of “cryptic” binding sites—transient pockets that are invisible in static crystallographic snapshots but emerge dynamically during protein motion. Such sites are not anomalies; rather, they appear to be intrinsic features of flexible proteins. In Bcl-xL, helices 3 and 4 play a particularly prominent role in mediating these transitions, undergoing subtle yet functionally significant rearrangements that reshape the binding landscape (Yang & Wang, 2011). The implications for drug design are profound. If key binding determinants are only intermittently accessible, then reliance on a single structural conformation—no matter how high its resolution—risks overlooking critical opportunities for ligand engagement. This realization has, perhaps inevitably, shifted attention toward dynamic modeling approaches capable of capturing the full spectrum of protein conformations.

3.3 The Long Road to Selectivity: Lessons from Early Inhibitors

The development of BH3-mimetic inhibitors marked a turning point in targeting the Bcl-2 family. Compounds such as ABT-737 and its orally bioavailable derivative Navitoclax (ABT-263) were designed to replicate the interactions of natural BH3 peptides, effectively displacing pro-apoptotic proteins from the binding groove (Oltsersdorf

et al., 2005). These agents demonstrated potent antitumor activity, validating the therapeutic potential of Bcl-2 family inhibition.

However, clinical success was tempered by significant toxicity. Navitoclax, while effective, exhibited dose-limiting thrombocytopenia due to its inhibition of Bcl-xL, a protein essential for platelet survival. This unexpected outcome highlighted a central challenge: achieving selectivity within a highly homologous protein family (Souers et al., 2013). The subsequent development of Venetoclax (ABT-199), a Bcl-2-selective inhibitor, represented a major advance. Structural and computational studies revealed that this selectivity arises from subtle differences in amino acid composition and interaction networks. For instance, the formation of a stable hydrogen bond with ASP103 in Bcl-2—absent or weakened in Bcl-xL due to the presence of GLU96—introduces a significant energetic penalty for off-target binding (Wakui et al., 2018). At first glance, such differences might appear minor. Yet, from a thermodynamic perspective, even a single hydrogen bond—or its absence—can shift binding affinities by several kilocalories per mole, enough to determine clinical selectivity. These findings underscore an important principle: in systems of high structural similarity, selectivity is often governed not by gross structural features but by subtle, dynamic interaction patterns.

3.4 Molecular Dynamics as a Computational Microscope

To interrogate these subtleties, researchers have increasingly turned to Molecular Dynamics (MD) simulations. Unlike static modeling approaches, MD captures the temporal evolution of biomolecular systems, providing insights into conformational flexibility, ligand binding pathways, and transient structural states (Hernández-Rodríguez et al., 2016).

In the context of Bcl-xL, MD simulations have revealed that ligand binding is not a simple two-state process. Rather, it often involves a “population shift,” where the protein transiently adopts a binding-competent conformation before ligand engagement stabilizes this state. This perspective challenges the traditional induced-fit model, suggesting instead a dynamic equilibrium of conformations that ligands selectively stabilize (Bekker et al., 2021).

Yet, standard MD simulations are not without limitations. Their ability to sample conformational space is constrained by timescale and energy barriers, often leading to

incomplete exploration of rare but functionally important states. To address this, enhanced sampling techniques have been developed. Accelerated MD (aMD), for example, introduces a biasing potential that facilitates transitions between energy minima, effectively broadening conformational sampling. Similarly, cosolvent MD employs small organic molecules to probe potential binding sites, revealing cryptic pockets that might otherwise remain hidden (Yang & Wang, 2011). Multicanonical MD (McMD) further extends this capability by enabling uniform sampling across a wide energy range, making it particularly useful for studying ligand binding mechanisms in flexible systems (Bekker et al., 2021). Together, these approaches transform MD from a descriptive tool into a predictive framework—one capable of guiding rational drug design.

3.5 Thermodynamic Landscapes: Quantifying Stability and Affinity

While structural insights are essential, they must ultimately be translated into quantitative measures of binding affinity and stability. This is where free energy calculations, particularly MM-PBSA and MM-GBSA methods, play a critical role. By decomposing binding interactions into enthalpic and entropic components, these approaches provide a more nuanced understanding of ligand–protein interactions (Kollman et al., 2000).

In Bcl-xL, such analyses have identified key “hot spot” residues—positions that contribute disproportionately to binding energy. Residues such as Phe97, Tyr101, and Arg139 consistently emerge as critical determinants of ligand affinity, forming a core interaction network that stabilizes inhibitor binding (Pinto et al., 2011). Interestingly, these residues often exhibit reduced flexibility upon ligand binding, as reflected in lower root mean square fluctuation (RMSF) values. Complementary metrics, including root mean square deviation (RMSD), further inform system stability by tracking structural deviations over time. Together, these parameters provide a multidimensional view of binding interactions, integrating structural, dynamic, and energetic perspectives.

3.6 Toward Next-Generation Strategies: Fragment-Based Drug Discovery

Despite significant progress, the design of selective Bcl-xL inhibitors remains an ongoing challenge. Increasingly, attention has turned to Fragment-Based Drug Discovery (FBDD) as a promising alternative to traditional high-

throughput screening. Rather than evaluating large, complex molecules, FBDD begins with small chemical fragments that bind weakly but specifically to target sites (Barelier et al., 2010).

These fragments serve as starting points for iterative optimization, guided by structural and computational insights. In the case of Bcl-xL, fragment-based approaches have been particularly effective in identifying ligands that engage specific sub-pockets within the BH3-binding groove. When combined with MD simulations and ensemble-based screening, FBDD allows for a more targeted exploration of chemical space, increasing the likelihood of identifying selective inhibitors. Recent advances, including High-Throughput Supervised MD (HT-SuMD), have further enhanced this approach by enabling the rapid screening of fragment libraries under dynamic conditions. Such methods not only improve hit identification but also provide mechanistic insights into binding pathways and conformational adaptation.

3.7 Concluding Reflections: A Moving Target in Drug Design

In many ways, Bcl-xL epitomizes the challenges of modern drug discovery. It is not a static target but a dynamic system—one whose functional behavior is inseparable from its structural flexibility. Attempts to simplify this complexity, while historically necessary, now appear increasingly inadequate. The integration of molecular dynamics, thermodynamic analysis, and fragment-based strategies offers a more holistic framework for understanding and targeting such systems. Yet, even as these tools grow more sophisticated, a degree of uncertainty remains. Are we capturing the full spectrum of biologically relevant conformations? Or are there still hidden states—transient, elusive—that continue to evade our models? Perhaps the answer lies not in seeking definitive solutions but in embracing this uncertainty as an intrinsic feature of biological systems. In doing so, we move closer to a more nuanced, adaptive approach to drug design—one that recognizes proteins not as fixed structures, but as dynamic entities navigating a complex energy landscape.

4. Dynamic Determinants of Target Selectivity and Therapeutic Precision in Oncology

4.1 Subtle Determinants of Selectivity: Residue-Level Interaction Landscapes in Bcl-2 Family Proteins

The pursuit of selective inhibition within the Bcl-2 protein family has gradually shifted from a search for high-affinity compounds toward a more intricate question—what truly governs selectivity in structurally similar proteins? At first glance, Bcl-2 and Bcl-xL appear almost indistinguishable, sharing significant sequence identity and nearly identical three-dimensional folds. Yet, as the structural and thermodynamic profiles summarized in Table 1 reveal, the distinction between selective and non-selective inhibition is not only real but deeply rooted in subtle residue-level interactions.

As shown in Table 1, broad-spectrum inhibitors such as ABT-263 (Navitoclax) and ABT-737 demonstrate strong binding affinities across both Bcl-2 and Bcl-xL targets, engaging conserved residues like Tyr108 and Phe97 (Wakui et al., 2018). However, this apparent potency is accompanied by relatively high conformational variability, reflected in elevated RMSD values. Such fluctuations suggest that while these inhibitors bind effectively, they do not stabilize a single dominant conformational state—an observation that may help explain their off-target toxicities, including thrombocytopenia (Wakui et al., 2018).

A more refined picture emerges when considering selective inhibitors like Venetoclax (ABT-199). As highlighted in Table 1, Venetoclax achieves specificity for Bcl-2 through a persistent hydrogen bond with Asp103, a residue that plays a central anchoring role (Wakui et al., 2018). Intriguingly, when this interaction is translated to Bcl-xL, the analogous residue—Glu96—fails to support equivalent binding stability. The additional methylene group in glutamate subtly alters the geometry of the interaction, often forcing reliance on water-mediated hydrogen bonding, which is thermodynamically less favorable (Wakui et al., 2018).

It is tempting to view this as a minor structural nuance. Yet, as thermodynamic analyses suggest, such differences can introduce binding penalties of considerable magnitude, effectively dictating selectivity. These findings reinforce the idea that selectivity is rarely governed by large structural divergences, but rather by the cumulative effect of small, dynamic interaction mismatches.

4.2 Navigating Redundancy: Dual Inhibition within the p53–MDM2/MDMX Axis

If selectivity within the Bcl-2 family is challenging, the p53 regulatory axis presents an even more nuanced

Table 1. Structural and Thermodynamic Profiles of Bcl-2 Family Inhibitors. This table summarizes key structural, binding, and stability parameters of representative Bcl-2 family inhibitors. Affinity values, interaction residues, and RMSD ranges provide insight into binding strength and conformational stability.

Compound	Primary Target	Affinity (nM)	PDB ID	H-Bond Residue	π -Interaction Residue	Mean RMSD (Å)	Reference
ABT-263	Bcl-2/Bcl-xL	<0.044	4LVT	Tyr108	Tyr202	3.39–6.82	Wakui et al. (2018)
ABT-737	Bcl-2/Bcl-xL	<0.010	2YXJ	Asp103	Phe97	2.15–2.49	Bekker et al. (2021)
ABT-199	Bcl-2	48.00	4LVT	Asp103	Phe104	2.15–2.49	Wakui et al. (2018)
A-1155463	Bcl-xL	<0.01	4QVX	Ser106	Phe105	1.48–1.60	Wakui et al. (2018)
WEHI-539	Bcl-xL	1.10	3ZLR	Arg139	Phe105	1.51–3.18	Bekker et al. (2021)
Compound 10	Bcl-xL	80.00	4QVX	Asn136	Tyr101	1.48–1.60	Wakui et al. (2018)
Navitoclax	Bcl-2/Bcl-xL	<0.044	4LVT	Tyr108	Tyr101	3.39–6.82	Wakui et al. (2018)
Venetoclax	Bcl-2	48.00	4LVT	Asp103	Tyr202	2.15–2.49	Wakui et al. (2018)
BH3I-1	Bcl-xL	7.00 (IC ₅₀)	N/A	Phe97	Tyr101	2.50	Pinto et al. (2011)

Table 2. Comparative Binding Energetics of MDM2 and MDMX Antagonists. Binding energies (BE) calculated using MM-PBSA or related methods highlight inhibitor specificity toward MDM2 and MDMX targets.

Inhibitor	MDM2 BE (kcal/mol)	MDMX BE (kcal/mol)	Specificity	Sub-pocket Mimic	Scoring Method	Ranking	Reference
MI-219	-10.60	-5.30	Dual	Trp23	MM-PBSA	1	Barakat et al. (2010)
Nutlin-3	-9.30	-6.10	MDM2	Phe19	MM-PBSA	2	Barakat et al. (2010)
TDP665759	-9.50	-5.60	MDM2	Leu26	MM-PBSA	3	Barakat et al. (2010)
PMI Peptide	-12.80	-13.10	Dual	All Three	MM-PBSA	Top	Barakat et al. (2010)
ZINC12503171	+2.10	-13.20	MDMX	Trp23	MM-PBSA	1	Barakat et al. (2010)
NSC#72254	+1.50	-13.10	MDMX	Phe19	MM-PBSA	2	Barakat et al. (2010)
Pub#20726118	+0.80	-12.90	MDMX	Leu26	MM-PBSA	3	Barakat et al. (2010)
CID_118439641	-78.42	N/A	MDM2	Trp23	XP Glide	1	Sirous et al. (2019)
CID_60452010	-71.47	N/A	MDM2	Phe19	MM-GBSA	2	Sirous et al. (2019)

problem—one of functional redundancy. MDM2 and MDMX, both negative regulators of p53, share overlapping yet distinct structural features, making selective targeting inherently complex. The comparative binding energetics presented in Table 2 offer a quantitative perspective on this challenge.

As illustrated in Table 2, classical inhibitors such as Nutlin-3 exhibit strong binding to MDM2 (9.30 kcal/mol) but significantly weaker affinity toward MDMX (6.10 kcal/mol) (Barakat et al., 2010). This discrepancy, while numerically modest, translates into substantial biological consequences, particularly in tumors where MDMX is overexpressed. The reduced efficacy of MDM2-specific inhibitors in such contexts underscores the necessity of dual-target strategies. Interestingly, dual inhibitors such as MI-219 and the PMI peptide demonstrate a more balanced energetic profile, maintaining favorable binding across both targets (Barakat et al., 2010). As Table 2 indicates, the PMI peptide achieves near-equivalent binding energies for MDM2 and MDMX, suggesting that effective dual inhibition requires structural adaptability—an ability to accommodate subtle differences in binding pocket geometry. One such difference lies in the MDMX binding cleft, which is narrower due to the inward orientation of residues such as Tyr99 (Barakat et al., 2010). This seemingly minor variation restricts ligand accommodation, favoring compounds that can mimic key p53 residues—particularly Trp23—while maintaining conformational flexibility (Grasberger et al., 2005).

Taken together, these findings suggest that successful dual inhibition is less about maximizing affinity and more about balancing structural compatibility across related yet distinct binding environments.

4.3 From Potency to Viability: Pharmacokinetics and the Druglikeness Threshold

While binding affinity and selectivity dominate early-stage drug discovery, they represent only part of the story. A compound that performs exceptionally well in silico may still fail clinically if its pharmacokinetic properties are unfavorable. This transition—from molecular interaction to systemic viability—is captured in Table 3, which summarizes key ADME descriptors for emerging Bcl-xL inhibitors.

As shown in Table 3, compounds such as Compound 24 exhibit promising interaction profiles but suffer from multiple Lipinski rule violations, including high molecular

weight and excessive flexibility (Azam et al., 2014). These properties, while beneficial for target engagement, often hinder membrane permeability and bioavailability.

In contrast, Compound 54 emerges as a more balanced candidate. With zero Lipinski violations, moderate polar surface area, and a favorable bioavailability score, it represents what might be considered a “sweet spot” in drug design (Azam et al., 2014). Notably, its structural simplicity does not compromise its ability to engage key residues, suggesting that optimal drug candidates are not necessarily the most complex, but rather the most balanced.

Similar trends are observed in the UBQF series, where pharmacophore-guided design enhances binding interactions but occasionally at the cost of increased molecular weight (Pinto et al., 2011). These trade-offs highlight a recurring tension in drug development: the need to reconcile molecular potency with pharmacokinetic practicality.

4.4 Stabilizing the Unstable: Targeting Mutant TP53–BRCA1 Complexes

Perhaps the most intriguing extension of these findings lies in the modulation of mutant protein complexes, particularly TP53–BRCA1 interactions. Unlike classical inhibition strategies, this approach seeks not to block function, but to restore or stabilize it—a subtle but significant shift in therapeutic philosophy.

The stability metrics presented in Table 4 provide compelling evidence for the potential of plant-derived alkaloids in this context. As shown, compounds such as Dicentrine exhibit markedly stronger binding to mutant TP53–BRCA1 complexes compared to their wild-type counterparts, with binding energies reaching 10.45 kcal/mol (Tiwari et al., 2017). Equally notable is the reduction in RMSD and RMSF values, indicating a more rigid and stable complex upon ligand binding. This stabilization appears to be anchored by key hotspot residues such as Asp1851, which serve as focal points for interaction (Tiwari et al., 2017). In contrast, synthetic inhibitors like Nutlin demonstrate weaker stabilization, accompanied by higher residual fluctuations. This suggests that natural compounds may possess structural features—perhaps greater conformational adaptability—that enable them to better accommodate the altered landscapes of mutant proteins. Such findings invite a broader question: could the future of oncology lie not

Table 3. Pharmacokinetic and Physicochemical ADME Descriptors of Novel Bcl-xL Leads. *Physicochemical properties relevant to drug-likeness, including Lipinski compliance and bioavailability scores.*

Compound ID	MW (g/mol)	Rotatable Bonds	H-Bond Acceptors	H-Bond Donors	TPSA (Å ²)	Lipinski Violations	Bioavailability Score	Reference
Compound 24	632.83	14	5	2	83.83	2	0.56	Azam et al. (2014)
Compound 46	614.28	0	2	0	34.14	2	0.17	Azam et al. (2014)
Compound 50	616.64	2	6	0	122.09	2	0.17	Azam et al. (2014)
Compound 54	488.49	0	4	2	74.60	0	0.55	Azam et al. (2014)
Compound 100	576.62	8	7	2	167.13	1	0.55	Azam et al. (2014)
UBQF14	750.00	12	8	2	110.00	1	0.55	Pinto et al. (2011)
UBQF17	680.00	10	6	0	95.00	1	0.55	Pinto et al. (2011)
Nutlin-2	581.50	7	5	1	80.00	1	0.55	Sirous et al. (2019)
Venetoclax	868.40	15	11	3	150.00	2	0.55	Wakui et al. (2018)

Table 4. Stability Metrics of TP53–BRCA1 Modulation by Plant-Derived Alkaloids. *This table Comparative stability and binding efficiency of alkaloids in wild-type and mutant TP53–BRCA1 systems.*

Alkaloid	Complex Type	Binding Energy (kcal/mol)	Ki (μM)	Avg. RMSD (Å)	Avg. RMSF (Å)	Hotspot Residue	Reference
Dicentrine	Mutant	-10.45	1.10	1.25	0.85	Asp1851	Tiwari et al. (2017)
Dicentrine	Wild Type	-7.20	15.00	2.10	1.45	Tyr101	Tiwari et al. (2017)
Glaucine	Mutant	-9.80	2.50	1.50	0.95	GlnA104	Tiwari et al. (2017)
Glaucine	Wild Type	-6.50	45.00	2.45	1.80	Glu96	Tiwari et al. (2017)
Nutlin	Mutant	-8.10	12.00	1.85	1.15	Pro1856	Tiwari et al. (2017)
Nutlin	Wild Type	-6.90	22.00	2.30	1.65	Phe97	Tiwari et al. (2017)
Boldine	Mutant	-8.45	8.50	1.65	1.05	Asp1852	Tiwari et al. (2017)
Glaucine	Mutant	-9.80	2.50	1.50	0.95	GlnA104	Tiwari et al. (2017)
Amygdalin	AKT1	-8.92	0.28	3.82	2.10	Glu198	Al-Khafaji et al. (2020)

only in inhibiting aberrant proteins, but in rescuing their function through stabilization? While still speculative, the data presented in Table 4 suggest that this possibility warrants serious consideration.

4.5 Integrative Perspective: Toward Precision-Driven Drug Design

Bringing these strands together, a coherent picture begins to emerge—one in which drug discovery is no longer defined by isolated metrics, but by the integration of structural, dynamic, and pharmacokinetic insights. The collective evidence from Tables 1–4 suggests that therapeutic success depends on a delicate interplay between residue-level interactions, conformational flexibility, binding energetics, and systemic viability.

It is, perhaps, tempting to frame these advances as a linear progression toward precision medicine. Yet, the reality feels more iterative—marked by incremental refinements, occasional setbacks, and a growing appreciation for biological complexity. What is clear, however, is that computational approaches have moved from peripheral tools to central drivers of innovation. By elucidating the hydrogen-bonding persistence in Bcl-2 selectivity (Wakui et al., 2018), revealing the structural constraints of MDMX (Barakat et al., 2010), identifying pharmacokinetic “sweet spots” in novel scaffolds (Azam et al., 2014), and uncovering stabilization mechanisms in mutant protein complexes (Tiwari et al., 2017), these methods provide not just answers, but a framework for asking better questions.

5. Limitations

Despite the growing utility of Molecular Dynamics (MD) simulations in cancer drug design, several limitations remain that warrant careful consideration. One of the most persistent challenges lies in sampling efficiency—even long-timescale simulations may fail to capture rare but biologically critical conformational states. Additionally, the accuracy of MD outcomes is inherently dependent on the quality of force fields, which, while continuously improving, still rely on approximations that may not fully reflect complex biological environments. Another concern is the translation gap between computational predictions and experimental or clinical outcomes. Binding free energy calculations, such as MM-PBSA, provide valuable insights but may overlook entropic contributions or solvent effects. Furthermore, the narrative nature of this review introduces potential selection bias, as studies were chosen based on

thematic relevance rather than strict systematic criteria. Finally, computational cost and resource requirements can limit accessibility, particularly for large-scale or long-duration simulations, potentially constraining broader application.

6. Conclusion

In reflecting on the evolving role of Molecular Dynamics in cancer drug design, it becomes increasingly clear that the field is moving—perhaps cautiously—toward a more dynamic understanding of biology. Proteins are no longer viewed as static targets but as fluctuating systems, shaped by time, environment, and interaction. By integrating structural, thermodynamic, and pharmacokinetic insights, MD simulations offer a pathway toward more selective and adaptive therapeutics. Yet, the journey remains incomplete. The true potential of these approaches will likely depend on how effectively they can be integrated with experimental validation and clinical translation. Still, the direction is unmistakable—and, in many ways, quietly transformative.

Author Contributions

S.S.K. conceptualized the study, designed the review framework, conducted literature synthesis and analysis, interpreted the findings, and drafted, reviewed, and finalized the manuscript.

References

- Al-Khafaji, K., & Taskin Tok, T. (2020). Amygdalin as multi-target anticancer drug against targets of cell division cycle: Double docking and molecular dynamics simulation. *Journal of Biomolecular Structure and Dynamics*, 195, 105660.
- Alonso, H., Bliznyuk, A. A., & Gready, J. E. (2006). Combining docking and molecular dynamic simulations in drug design. *Medicinal Research Reviews*, 26(5), 531–568.
- Amaro, R. E., et al. (2008). Discovery of drug-like inhibitors of an essential RNA-editing ligase in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences*, 105(45), 17278–17283.
- Azam, S. S., Abro, A., Tanvir, F., & Parvaiz, N. (2014). Identification of unique binding site and molecular docking studies for structurally diverse Bcl-xL inhibitors. *Medicinal Chemistry Research*, 23, 3765–3783.
- Barakat, K., Mane, J., Friesen, D., & Tuszyński, J. (2010). Ensemble-based virtual screening reveals dual-inhibitors for the p53–MDM2/MDMX interactions. *Journal of Molecular Graphics and Modelling*, 28(6), 555–568.
- Barakat, K., Mane, J., Friesen, D., & Tuszyński, J. (2010). Ensemble-based virtual screening reveals dual-inhibitors for the p53–MDM2/MDMX interactions. *Journal of Molecular Graphics and Modelling*, 28(6), 555–568.

- Barelrier, S., Pons, J., Marcillat, O., Lancelin, J. M., & Krimm, I. (2010). Fragment-based deconstruction of Bcl-xL inhibitors. *Journal of Medicinal Chemistry*, 53(6), 2577–2588.
- Bekker, G. J., Fukuda, I., Higo, J., Fukunishi, Y., & Kamiya, N. (2021). Cryptic-site binding mechanism of medium-sized Bcl-xL inhibiting compounds elucidated by McMD-based dynamic docking simulations. *Scientific Reports*, 11(1), 1–15.
- Bekker, G. J., Fukuda, I., Higo, J., Fukunishi, Y., & Kamiya, N. (2021). Cryptic-site binding mechanism of medium-sized Bcl-xL inhibiting compounds elucidated by McMD-based dynamic docking simulations. *Scientific Reports*, 11(1), 1–15.
- Ding, K., Lu, Y., Nikolovska-Coleska, Z., Qiu, S., Ding, Y., Gao, W., ... & Wang, S. (2005). Structure-based design of potent non-peptide MDM2 inhibitors. *Journal of the American Chemical Society*, 127(29), 10130–10131.
- Grasberger, B. L., Lu, T., Schubert, C., Parks, D. J., Carver, T. E., Koblisch, H. K., ... & Bone, R. F. (2005). Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. *Journal of Medicinal Chemistry*, 48(4), 909–912.
- Hernández-Rodríguez, M., et al. (2016). Current tools and methods in Molecular Dynamics (MD) for drug design. *Current Medicinal Chemistry*, 23(32), 1–18.
- Hernández-Rodríguez, M., Rosales-Hernández, M. C., Mendieta-Wejebe, J. E., Martínez-Archundia, M., & Correa-Basurto, J. (2016). Current tools and methods in Molecular Dynamics (MD) for drug design. *Current Medicinal Chemistry*, 23(32), 1–18.
- Kollman, P. A., Massova, I., Reyes, C., Kuhn, B., Huo, S., Chong, L., ... & Case, D. A. (2000). Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. *Accounts of Chemical Research*, 33(12), 889–897.
- Lessene, G., Czabotar, P. E., Sleebs, B. E., Zobel, K., Lowes, K. N., Adams, J. M., ... & Fairbrother, W. J. (2013). Structure-guided design of a selective BCL-XL inhibitor. *Nature Chemical Biology*, 9, 390–397.
- Mortier, J., Rakers, C., Bermudez, M., Murgueitio, M. S., Riniker, S., & Wolber, G. (2015). The impact of molecular dynamics on drug design: applications for the characterization of ligand-macromolecule complexes. *Drug Discovery Today*, 20(6), 686–702.
- Oltersdorf, T., Elmore, S. W., Shoemaker, A. R., Armstrong, R. C., Augeri, D. J., Belli, B. A., ... & Rosenberg, S. H. (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*, 435, 677–681.
- Pinto, M., Orzaez, M. D. M., Delgado-Soler, L., Perez, J. J., & Rubio-Martinez, J. (2011). Rational design of new class of BH3-mimetics as inhibitors of the Bcl-xL protein. *Journal of Chemical Information and Modeling*, 51(5), 1242–1253.
- Pinto, M., Orzaez, M. D. M., Delgado-Soler, L., Perez, J. J., & Rubio-Martinez, J. (2011). Rational design of new class of BH3-mimetics as inhibitors of the Bcl-xL protein. *Journal of Chemical Information and Modeling*, 51(5), 1242–1253.
- Rew, Y., Sun, D., Gonzalez-Lopez De Turiso, F., Bartberger, M. D., Beck, H. P., Canon, J., ... & Olson, S. H. (2012). Structure-based design of novel inhibitors of the MDM2–p53 interaction. *Journal of Medicinal Chemistry*, 55(11), 4936–4954.
- Sirous, H., Fassihi, A., Brogi, S., Campiani, G., & Saghaie, L. (2019). Integrated virtual screening and molecular dynamics simulation revealed promising drug candidates of p53-MDM2 interaction. *Computational Biology and Chemistry*, 83, 107105.
- Souers, A. J., Levenson, J. D., Boghaert, E. R., Ackler, S. L., Catron, N. D., Chen, J., ... & Fairbrother, W. J. (2013). ABT-199 a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature Medicine*, 19, 202–208.
- Stewart, M. L., Fire, E., Keating, A. E., & Walensky, L. D. (2010). The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. *Nature Chemical Biology*, 6(8), 595–601.
- Tiwari, S., Awasthi, M., Singh, S., Pandey, V. P., & Dwivedi, U. N. (2017). Modulation of interaction of mutant TP53 and wild type BRCA1 by alkaloids: A computational approach towards targeting protein-protein interaction. *Journal of Biomolecular Structure and Dynamics*, 36(10), 1–13.
- Wakui, N., Yoshino, R., Yasuo, N., Ohue, M., & Sekijima, M. (2018). Exploring the selectivity of inhibitor complexes with Bcl-2 and Bcl-XL: A molecular dynamics simulation approach. *Journal of Molecular Graphics and Modelling*, 79, 166–174.
- Yang, C. Y., & Wang, S. (2011). Hydrophobic binding hot spots of Bcl-xL protein-protein interfaces by cosolvent molecular dynamics simulation. *ACS Medicinal Chemistry Letters*, 2(4), 280–284.
- You, W., Huang, Y. M., Kizhake, S., Natarajan, A., & Chang, C. E. A. (2016). Characterization of promiscuous binding of phosphor ligands to breast-cancer-gene 1 (BRCA1) C-terminal (BRCT). *PLOS Computational Biology*, 12(8), e1005057.