

Showcasing research from Professor Shandar Ahmad's laboratory, SciWhyLab, School of Computational and Integrative Science (SC&IS), Jawaharlal Nehru University (JNU), Delhi, India.

Protein-DNA interactions in disease and drug discovery

This review highlights disease-associated protein-DNA interactions as emerging drug targets, summarising therapeutic advances, clinical candidates, and computational and AI-driven strategies that enable their study and exploitation in genome regulation, disease mechanisms, and rational drug discovery.

Image reproduced by permission of Richa Mishra and Shandar Ahmad from *Chem. Commun.*, 2026, **62**, 3702.

As featured in:



See Richa Mishra and Shandar Ahmad, *Chem. Commun.*, 2026, **62**, 3702.



Cite this: *Chem. Commun.*, 2026, 62, 3702

Protein–DNA interactions in disease and drug discovery

Richa Mishra  and Shandar Ahmad *

Protein–DNA interactions (PDIs) are fundamental to all organisms and are often involved in the onset, progression, and severity of diseases or in defence against them. However, their use in drug targeting has remained challenging due to many reasons, including the electrostatic and non-specific interactions of the omnipresent DNA backbone. Nevertheless, PDIs, including regulatory transcriptional events and pathogen-sensing by hosts, have remained critical in drug discovery, and their potential as direct drug targets has been increasingly recognised. In this review article, we survey three key aspects of PDIs in humans, namely transcription, replication-and-repair, and genome organisation, whose misregulation has been implicated in various diseases, thereby highlighting PDIs as viable therapeutic targets. We provide a comprehensive list of targets and drugs used in drug discovery, that have reached the clinical trial or approval stage. We also review the computational methods, including AI-based approaches, that are powering these developments. We observe that, despite the general notion of PDIs being treated as undruggable, the literature shows that the time to use them as effective targets has come, as reflected by the growing number of candidate drugs across these categories.

Received 1st November 2025,
Accepted 22nd December 2025

DOI: 10.1039/d5cc06239j

rsc.li/chemcomm

1 Introduction

Protein–DNA interactions (PDIs) refer to the specific or non-specific binding of proteins to DNA sequences. They can affect a range of cellular functions, including gene expression, DNA replication, DNA repair, chromatin remodelling, and cell cycle

regulation.^{1,2} Disruption of PDIs is a hallmark of many diseases, particularly cancer, where dysregulation of transcription factors (TFs) such as c-Myc, p53, and nuclear factor kappa B (NF-κB), or DNA repair proteins like poly(ADP-ribose) polymerase (PARP) and RAD51, drives tumourigenesis and highlights PDIs as potential therapeutic targets.^{2–8}

PDIs have been widely studied using experimental and computational techniques.⁹ Some of the many questions surrounding PDIs relate to molecular specificity, detailed atomic

School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi, India. E-mail: shandar@jnu.ac.in



Richa Mishra

Richa Mishra is a PhD student at the School of Computational and Integrative Sciences, Jawaharlal Nehru University, India. She holds an MSc in Medicinal Chemistry and Drug Design, and her research interests include protein–DNA interactions, genomics, artificial intelligence and machine learning-based approaches, and structure-based drug discovery.



Shandar Ahmad

Shandar Ahmad is a professor at Jawaharlal Nehru University, New Delhi. He has extensively worked in the area of protein–DNA interactions, including sequence-based prediction of DNA-binding sites, the role of DNA shape in the recognition process, moonlighting prediction, binding specificity, conformational dynamics and integration of sequence, structure and gene expression in their annotations. He has also been involved in solving the topological organization of the MTB genome using the HiC technique and the role of genome organization in virulence.

interactions, identification of amino acid or nucleotide “binding sites”, interaction energetics, and the spatial and temporal order of binding partners.^{10–16} Identification of binding sites is of particular interest from a drug discovery perspective. In this regard, an early experimental method for the quantitative characterisation of PDIs was DNase I footprinting and titration, developed in the 1980s, which laid the groundwork for mapping DNA–protein binding sites.¹⁷ Since then, numerous methods have been developed to study these interactions at multiple levels. For example, electrophoretic mobility shift assays (EMSA) provide evidence of protein–DNA binding *in vitro*.¹⁸ Chromatin immunoprecipitation followed by sequencing (ChIP-seq) enables genome-wide mapping of binding events.¹⁹ Surface plasmon resonance (SPR) enables real-time measurement of biomolecular interactions and their kinetics at the ensemble level.²⁰ In contrast, single-molecule nanopore sensing detects PDIs by monitoring ionic current changes as biomolecules interact with or transit through a nanoscale pore, providing direct insight into binding kinetics, conformational dynamics, and molecular heterogeneity that are inaccessible to bulk methods.^{21–25} More elaborate atomistic insights come from structure-based approaches such as high-resolution X-ray crystallography²⁶ and nuclear magnetic resonance (NMR) spectroscopy, as well as²⁷ molecular assembly-level cryo-electron microscopy (cryo-EM).²⁸ High-throughput experimental methods include sequence-based techniques such as cleavage under targets and tagmentation (CUT&Tag)²⁹ and DNA affinity purification sequencing (DAP-seq),³⁰ as well as optical biosensing methods like bio-layer interferometry (BLI).³¹

Experimental data generated from these methods for disease-associated systems and their controls have been crucial in advancing our understanding of PDIs and their role in various cellular states, including disease onset, progression, and intervention.^{6,32–35}

However, obtaining such data is often complex for every biological context and cannot be effectively interpreted without a robust infrastructure to store, model, and predict biological meaning from them. Therefore, large biological databases have been developed to compile experimental results on PDIs. The Protein Data Bank (PDB)^{36,37} contains nearly 5000 human protein–DNA complexes, while Gene Ontology (GO)^{38,39} annotations list over 14000 nucleic acid-binding proteins (accessed *via* UniProt⁴⁰) (GO:0003676), including more than 9000 with DNA-binding activity (GO:0003677) and approximately 3500 with sequence-specific DNA-binding (GO:0043565). Despite this extensive coverage, many interactions lack detailed structural and mechanistic characterisation and therefore depend on computational approaches, which continue to be refined to enhance their predictive accuracy and biological interpretability.

Experimental data and computational analyses reveal that there are three general ways to modify PDIs, *e.g.* for a therapeutic intent. These include (1) designing molecules that bind to DNA-binding proteins, (2) directly engaging DNA, or (3) interfering with the protein–DNA complex.^{41–45} Each of these approaches presents unique challenges. For example, DNA-binding proteins often lack well-defined ligand-binding

pockets and were therefore long considered non-druggable.⁴⁶ On the other hand, compounds that interact directly with DNA, including intercalators, groove binders, and alkylating agents, usually display poor sequence selectivity and substantial systemic toxicity.⁴⁷ Finally, agents that trap transient DNA–protein complexes can cause widespread DNA damage and promote resistance.⁴⁸ Together, these challenges have contributed to the slow progress in targeting PDIs therapeutically, although the situation is gradually changing. For example, in the case of transcription factors, targeting domains other than the canonical DNA-binding region has been effective, as seen with estrogen and androgen receptor inhibitors such as fulvestrant and enzalutamide.^{49,50} Targeting *via* modes other than small molecules has also shown promise; for instance, the mini-protein Omomyc disrupts Myc–Max dimerisation and prevents DNA binding, effectively blocking transcriptional activity,⁵¹ while proteolysis targeting chimeras (PROTACs) degraders like bavdegalutamide (ARV-110) promote targeted degradation of DNA-binding proteins.⁵² The use of small molecules targeting replication or repair proteins through catalytic inhibition, such as olaparib and talazoparib, represents another successful approach.^{53,54} Chromatin modulators such as vorinostat and tazemetostat provide an epigenetic mode of modulation.^{55,56} Combination therapies are also being increasingly applied to mitigate off-target effects and enhance therapeutic selectivity.^{57–61} Thus, PDIs represent an important biomolecular system that holds great promise in solving the next generation of drug discovery problems. In this review, we discuss the current state of knowledge in the field of PDIs that forms the basis of this promise. We highlight the key molecular and structural principles governing protein–DNA recognition (Fig. 1), explore how dysregulation of PDIs contributes to human disease, and examine current and emerging strategies for their therapeutic targeting. We also outline key challenges associated with druggability, specificity, and resistance and discuss future perspectives on how advances in structural biology, chemical biology, and AI-driven modelling may enable more selective and effective modulation of PDIs. Building on this overview, the following section explores the structural features that determine how proteins recognise and bind to DNA.

1.1 Structural features governing protein–DNA recognition

Protein interactions with target DNA molecules can occur either specifically, depending on the DNA sequence and typically involving contacts between protein side chains and nucleotide bases (or sequence-dependent DNA shape features), or non-specifically, through interactions between protein side chains and the sugar–phosphate backbone of the DNA molecule. DNA-binding proteins such as TFs usually exhibit specific binding, whereas histones primarily display non-sequence-specific binding mediated by electrostatic contacts with the DNA backbone.^{69–71} The DNA replication process also displays a combination of both binding modes: initiator proteins bind specifically to origins of replication (ORI) sequences, while the subsequent machinery, including DNA polymerase and

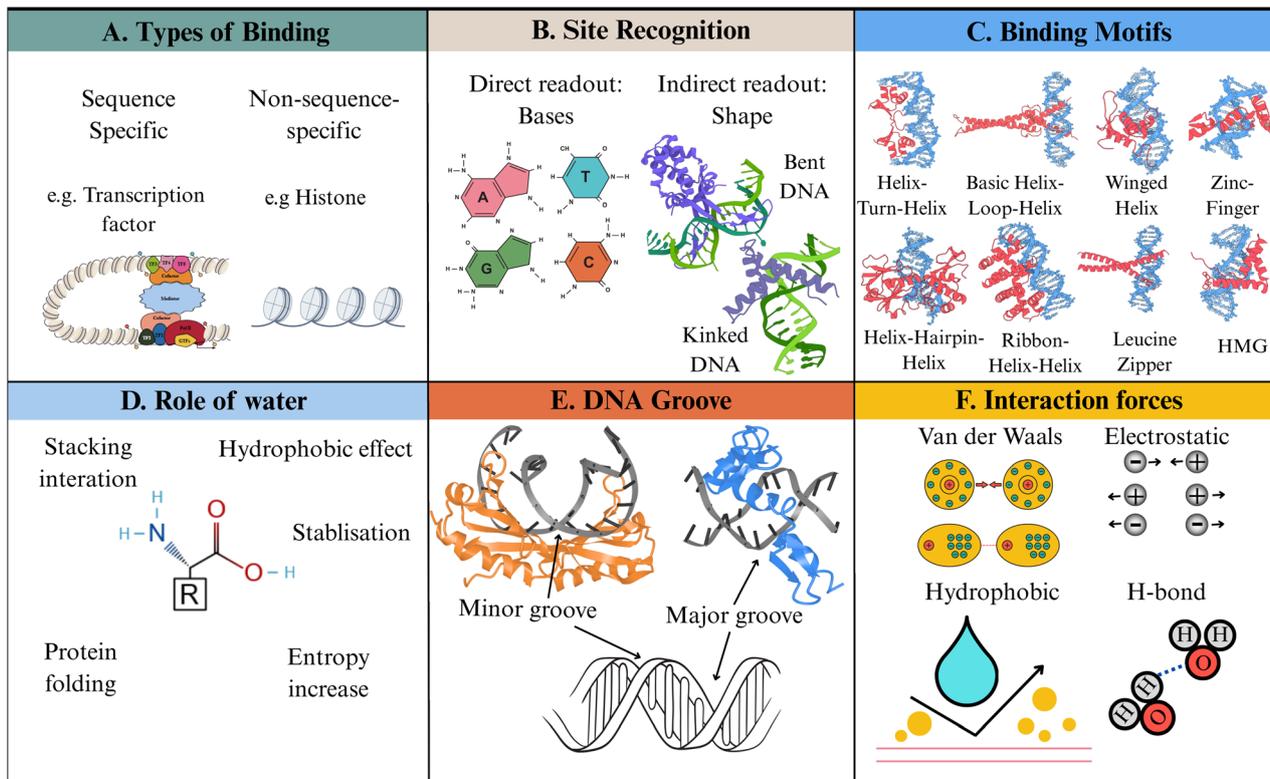


Fig. 1 Structural elements of protein–DNA interactions. (A) Types of binding modes. (B) Site recognition: direct readout through base-specific contacts in the major groove, and indirect readout through DNA shape, flexibility, and groove geometry (PDB: 1P51,⁶² 1CKT⁶³). (C) Binding motifs: schematic illustrations of common DNA-binding motifs. (D) Role of water: water-mediated stabilisation *via* bridging hydrogen bonds, hydrophobic effects, stacking interactions, and entropic contributions. (E) DNA grooves: minor-groove binding (PDB: 1CDW)⁶⁴ and major-groove binding (PDB: 1AAY).⁶⁵ (F) Interaction forces: key forces stabilising protein–DNA complexes. Created with icons from Biolcons⁶⁶ and Servier Medical Art (smart.servier.com), licensed under CC BY 4.0; protein visualisations generated *via* The Protein Imager⁶⁷ and Mol*.⁶⁸

helicase, associates with particular DNA structures (such as the replication fork) rather than specific sequences.⁷²

Role of DNA shape or structure. Genomic binding sites are recognised by proteins either through base sequences (direct readout) or the three-dimensional shape of the DNA (indirect or shape readout).^{73,74} In site-specific binding, the protein finds the sequence and binds to the part of the DNA structure that complements its shape.^{75,76} These nucleotide sequences determine the overall genome topography, including the local kinks, grooves and surface electrostatic charges.^{76,77} These interactions are primarily mediated by the van der Waals and electrostatic forces.^{76,78} In most cases, this site-specific binding is achieved through a combination of sequence (direct) and shape (indirect) readout mechanisms.⁷⁹ In sequence-specific binding, the protein recognises “contact bases” of the DNA through its side chains or backbone, while “non-contact” bases contribute to the overall DNA conformation required for recognition.⁸⁰ This recognition is not straightforward, as one cannot simply look at the protein sequence and predict the DNA sequence it will bind to. Both molecules are dynamic in nature, so the protein searches for a site on the DNA that is flexible enough to bend as needed by the protein, making the interaction thermodynamically favourable (Fig. 1). This flexibility allows DNA to often deviate from its canonical B-form and exist in different

forms, such as Z-DNA or G-quadruplexes (G4s), depending on various environmental conditions and local sequences.⁸¹

For a protein (typically a TF or DNA-sensing immune response factor) to specifically bind to DNA, it must read the bases paired within the double helix.⁸² The **major groove** of DNA is deep, wide, and highly exposed, thereby featuring an easily recognisable pattern of hydrogen-bond donors and acceptors created by the bases. In contrast to the minor groove, this enhances the specificity of binding because hydrogen bonds can be more easily formed between the DNA bases and the protein’s side chains. In the **minor groove**, which is shallow and narrow, binding occurs through electrostatic interactions and sequence-dependent shape complementarity, often influenced by a higher AT content. The high negative potential attracts positively charged arginine and lysine residues on the protein molecule, although arginine is more enriched (60%) compared to lysine (22%) because it forms more hydrogen bond contacts.⁸³ Recent studies show that DNA-binding specificity depends on groove recognition and balanced hydrogen-bonding with both DNA strands.⁷⁰ Some proteins also show flexibility in groove-binding preference upon post-translational modifications, for example, protamines bind preferentially to the minor groove in their unphosphorylated state, but the binding shifts to the major groove after phosphorylation.⁸⁴

Given that these binding interactions take place in an aqueous environment, water plays an essential role in PDIs.⁸⁵ Water molecules in DNA form various arrangements in the major and minor grooves; during binding, some of these water molecules are displaced, contributing to the **hydrophobic effect**, where the release of ordered water molecules increases the system entropy, making the interaction thermodynamically favourable and stabilising the complex. The specific groove hydration patterns and base-stacking interactions significantly contribute to the stability of different DNA conformations such as A-, B-, Z-DNA or G4. The hydrophobic effect also influences protein folding, as hydrophobic amino acids (such as leucine, isoleucine, and phenylalanine) tend to be located inside the protein core, whereas hydrophilic amino acids generally face outward toward the aqueous environment.^{82,85,86}

Role of protein structure. Proteins rely on structural features called DNA-binding motifs, which are designed to identify specific DNA sequences or structural patterns (Fig. 1).⁸⁷ Examples include helix-turn-helix (HTH; Cro repressor), helix-loop-helix (HLH; MyoD), basic helix-loop-helix (bHLH; Max), leucine zipper (bZIP; CREB), zinc finger (p53), β -scaffold (TATA-binding protein), homeodomain (PAX6), winged helix (HNF3), HMG box (HMGB1), helix-hairpin-helix (Endonuclease III) and ribbon-helix-helix (Arc repressor).⁸⁸ Several databases have been developed to create a comprehensive set of sequence patterns and motifs in DNA-binding proteins.^{89–94} The HTH motif, including “winged” forms, binds the DNA major groove *via* a recognition helix, as in TF families such as Cro and Repressor.⁸⁸ Zinc-coordinating motifs, such as $\beta\beta\alpha$ -zinc-fingers, hormone receptors, and loop-sheet-helix families, are stabilised by the tetrahedral coordination of zinc ions involving conserved cysteine and histidine residues, as exemplified by p53.^{87,95}

Zipper-type motifs have α -helices from two subunits which “zip” together *via* hydrophobic interactions. In leucine zippers, leucine residues occur at every seventh position in a coiled-coil segment, with the N-terminal basic region extending into the DNA major groove. In contrast, in helix-loop-helix proteins, the DNA-binding and dimerisation helices are separated by a flexible loop.^{88,96}

Histones and high-mobility group (HMG) proteins also use helices for DNA binding and are generally not sequence-specific.⁸⁸ While the former is primarily involved in DNA packaging, the latter plays a variety of regulatory roles.⁷² Other motifs, such as the helix-hairpin-helix (involved in base excision and mismatch repair) and the ribbon-helix-helix (found in bacterial Arc repressors), also contribute to DNA recognition during repair and regulation.^{72,88}

DNA-binding motifs are widespread in TFs; for example, the CREB family contains leucine zippers, whereas helix-loop-helix motifs can be found in the mouse and human forms of Max, human USF proteins, and others.⁷²

While structural features enable precise protein–DNA recognition under normal conditions, mutations or alterations in these features can disrupt interactions and lead to disease, as discussed in the following section.

2 Disease-causing dysregulation of PDIs

Mutations in proteins or their target genomic DNA that alter PDIs can disrupt normal gene regulation and genome organisation, leading to detrimental effects on cellular processes such as transcription, replication, and DNA repair. Such disruptions are linked to various diseases.⁶ For example, missense mutations in DNA-binding proteins have been associated with severe conditions including cancer and neurodevelopmental disorders.⁹⁷ Studying the effects of these mutations is, therefore, critical for understanding disease mechanisms and developing targeted treatment strategies. The following section explores diseases associated with DNA-binding proteins as well as DNA itself, including non-canonical structures. We have grouped PDI-associated diseases into those involving (1) transcription factors, (2) replication and repair, (3) non-canonical DNA elements, and (4) genome packaging and architectural integrity. Specific examples of disease-causing disruptions in these systems are outlined below.

2.1 Diseases linked to transcription factors (TFs)

TFs regulate gene expression by binding to specific DNA sequences and coordinating transcriptional programs essential for cellular identity, development, and environmental responsiveness. Disruption of TF–DNA interactions can have profound pathological consequences, including developmental disorders, inflammatory and metabolic conditions, and cancer.⁹⁸

Developmental TFs are particularly sensitive to perturbations in DNA binding. The paired box (PAX) family of TFs, including PAX3, PAX4, PAX5, PAX6, and PAX7, plays a central role in organogenesis. These transcriptional regulators contain a paired domain (PD) for DNA binding, consisting of an HTH motif that inserts into the major groove of DNA. Some PAX proteins, such as PAX3 and PAX6, also possess an additional homeodomain that provides an extra DNA contact point, increasing binding stability and specificity. PAX homeodomains favour TAAT-containing motifs and can bind paired TAAT sites cooperatively, enabling recognition of regulatory sequences that are usually inaccessible to other homeodomain factors.⁹⁹ The functional consequences of PAX6 mutations depend on whether they destabilise the paired domain or, instead, introduce more subtle alterations in DNA-binding affinity or specificity. Such disruptions in PAX–DNA interactions can misregulate key gene networks, leading to developmental disorders such as Waardenburg syndrome, renal-coloboma syndrome, and ocular defects, including aniridia and cataracts.² Aberrant PAX–DNA interactions are also implicated in tumourigenesis, contributing to diseases such as alveolar rhabdomyosarcoma, renal cell carcinoma, and melanoma.^{2,99}

Signal-responsive TFs also represent an important class of disease-associated PDIs. Signal transducer and activator of transcription 3 (STAT3) regulates gene expression by binding to interferon-responsive DNA elements. STAT3 specifically recognises the consensus DNA sequence (*TTCNNGAA*), and its DNA-binding affinity is enhanced by cytokine-induced

phosphorylation that promotes STAT3 dimerisation.¹⁰⁰ Structurally, STAT3 consists of a DNA-binding domain (DBD), an Src homology 2 (SH2) domain, a transactivation domain, and a coiled-coil domain (CCD). As a signalling molecule, dysregulation of STAT3 disrupts multiple cellular processes, including altered chondrocyte metabolism in osteoarthritis, Interleukin-6 (IL-6)-driven inflammation in atherosclerosis, and the dysregulation of fibrosis-related genes in myocardial fibrosis. Because STAT3 activation depends on cytokine signalling, aberrant STAT3 activity is also linked to asthma, autoimmune diseases, Alzheimer's disease, and breast cancer.¹⁰¹

Nuclear hormone receptors constitute another important group of TFs whose disease associations arise from altered DNA binding. Estrogen receptor (ER) and androgen receptor (AR) bind hormone response elements composed of AGGTCA half-sites arranged as inverted or direct repeats. Ligand binding induces conformational changes that enhance DNA-binding affinity and coactivator recruitment.⁸⁸ IL-6 modulation of the AR coactivator p300 within the AR transcription complex is linked to prostate cancer. Androgens are also implicated in prostate diseases such as benign prostatic hyperplasia, while androgen deficiency is linked to muscular atrophy. ER-mediated transcription through estrogen response elements on DNA is involved in polycystic ovary syndrome, endometriosis, and, most importantly, breast and ovarian cancers. ER α is expressed in approximately 50–80% of breast cancer tissues.^{102,103}

Oncogenic TFs and tumour suppressors frequently drive cancer through dysregulated PDIs. The proto-oncogene *c-MYC* promotes proliferative gene expression by binding E-box motifs (CACGTG) as a heterodimer with Max, primarily in nucleosome-depleted chromatin regions. Disruption of the *c-Myc*–Max–DNA interaction has become an important therapeutic strategy in cancers such as breast and prostate cancer.^{4,5,72} Loss of p53 can worsen *c-Myc*–driven cancer progression. In breast cancer, combined p53 loss and *c-MYC* activation increase mitotic gene expression and expand cancer stem cell-like populations. p53 binds DNA as a tetramer through its central DNA-binding domain and recognises the consensus response element RRRCCWWGYYY ($R = A/G$, $W = A/T$, $Y = C/T$). p53's ability to activate target genes depends on this sequence and the surrounding chromatin context. Mutations that disrupt the DNA-binding domain or tetramerisation are common in cancer and prevent p53 from regulating its target genes, thereby contributing to tumour initiation and progression.¹⁰⁴ SOX2 binds the canonical SOX motif (A/T)TTGT through its HMG domain, inserting into the minor groove and bending DNA. In nucleosomes, SOX2 recognises a degenerate motif (A/T)TTNT, with affinity influenced by local chromatin structure. This enables SOX2 to act as a pioneer factor, maintain open chromatin, and support stem cell self-renewal. In breast cancer, SOX2 promotes cancer stem cell-like properties, tumour initiation, and therapy resistance. Depending on the context, it can function as either an oncogene or a tumour suppressor in cancers such as glioblastoma, lung, and squamous cell carcinoma.¹⁰⁵

Activator protein-1 (AP-1) binds to specific DNA sequences called TPA-responsive elements (TREs) *via* its basic leucine

zipper (bZIP) domains, mediating essential protein–DNA interactions for gene regulation.¹⁰⁶ The canonical AP-1 binding motif is the TRE consensus sequence 5'-TGAG/CTCA-3', and DNA-binding affinity and specificity depend on the exact Jun–Fos dimer composition, which positions the basic region in the major groove. Changes in AP-1 subunit makeup, expression, or modifications can alter its DNA-binding behaviour, driving cancers such as breast and lung cancers.¹⁰⁷ Disrupted AP-1 binding also affects the regulation of inflammatory cytokines, contributing to diseases such as rheumatoid arthritis and psoriasis.¹⁰⁸

In haematological malignancies, fusion transcription factors act as abnormal DNA-binding regulators. In acute myeloid leukaemia (AML), fusion proteins such as AML1/ETO (acute myeloid leukaemia 1–eight-twenty-one), PML/RAR (promyelocytic leukaemia–retinoic acid receptor alpha), and PLZF/RAR (promyelocytic leukaemia zinc finger–retinoic acid receptor alpha) aberrantly regulate transcription by activating stem cell renewal pathways, such as the Notch signalling pathway; while repressing DNA repair genes, including those involved in base excision repair (BER), leading to genomic instability and malignant transformation.¹⁰⁹

Finally, hypoxia-inducible factors (HIFs) represent a class of environmentally responsive TFs whose dysregulated DNA binding contributes to disease. The hypoxia pathway works *via* the stabilisation of the HIF- α subunit (such as HIF-1 α , HIF-2 α , or HIF-3 α), which dimerises with the constitutively expressed aryl hydrocarbon receptor nuclear translocator (HIF-1 β , also called ARNT). This complex formation is mediated by the basic helix-loop-helix (bHLH) motif and the Per-ARNT-Sim (PAS) domain, which also facilitates DNA binding. The heterodimer binds DNA at the hypoxia response element (HRE), a consensus sequence 5'-RCGCTG-3' ($R = A/G$). Its binding affinity and specificity are influenced by the flanking sequence and chromatin accessibility, as well as differences between HIF-1 α and HIF-2 α in target-gene preference. The stabilised complex then regulates transcription of target genes, mostly upregulating vascular endothelial growth factor (VEGF) and erythropoietin (EPO). Increased HIF levels are related to numerous diseases, such as ischaemic heart disease and heart failure, where HIF exerts a cardioprotective role.¹¹⁰ However, in chronic conditions, persistent HIF activation can lead to diseases such as glioblastoma multiforme (GBM), where HIF promotes tumour growth by activating VEGF in the tumour microenvironment,¹¹¹ or in von Hippel–Lindau (VHL)–associated cancers like renal cell carcinoma.¹¹² Overexpression of HIF is also linked to chemoresistance, including cisplatin resistance.¹¹³

2.2 Diseases linked to replication and repair proteins

Multiple PDIs are also crucial for DNA repair processes in eukaryotes. For example, proteins such as replication protein A (RPA) and xeroderma pigmentosum complementation group A (XPA) must bind to damaged DNA to initiate nucleotide excision repair (NER) by stabilising unwound DNA and recruiting downstream excision factors. Mutations in these proteins disrupt their DNA-binding ability, leading to defective

DNA repair, accumulation of DNA lesions, and genomic instability, which can result in diseases such as xeroderma pigmentosum.¹¹⁴

RPA is also essential for stabilising ssDNA during replication and repair. Dysfunction or insufficient RPA activity leads to replication stress, impaired homologous recombination (HR), and genomic instability. RPA also assists the activity of DNA polymerases α and θ , helping replication progress smoothly. Defects in RPA are associated with increased susceptibility to cancers such as breast cancer. RPA exhaustion increases cellular sensitivity to DNA-damaging agents.¹¹⁵ For further details on targeting RPA in cancer therapy, see the section on targeting replication/repair protein in this article.

HR repairs double-strand DNA breaks in a template-dependent manner by searching for homologous sequences and performing strand exchange. As described above, RPA stabilises single-stranded DNA and helps recruit RAD51 to form repair filaments. HR works in coordination with interdependent pathways, including the Fanconi anaemia (FA) pathway. BRCA2 mediates RAD51 loading, linking HR with FA pathway repair; mutations in these proteins lead to cancer predisposition.⁸

In another example under this category of PDI, MSH6, a component of the mismatch repair system (MMR), aids in correcting errors during DNA replication. The MMR corrects base mismatches and small insertion/deletion loops, acting as a crucial proofreading mechanism. MSH6 partners with MSH2 to form the MutS α complex that specifically recognises these errors. The N-terminal PWWP domain of MSH6 contributes to its chromatin localization and mutations that cause pathological effects. For example, the S144I mutation in this protein leads to hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome.¹¹⁶ MMR mutations are also commonly found in patients with prostate cancer.¹¹⁷

Base excision repair (BER) is another important pathway which corrects small, non-helix-distorting lesions caused by deamination, oxidation, and alkylation and repairs single-strand breaks (SSBs). If left unrepaired, such lesions can interfere with DNA replication and transcription and increase cytotoxic stress.⁷² The BER pathway involves several key proteins. Damaged bases are first recognised and excised by DNA glycosylases such as OGG1; defects in OGG1 compromise oxidative damage repair and have been linked to cancer and neurodegeneration. Gap filling is carried out by DNA polymerases such as Pol β (and in some contexts Pol θ). If unrepaired, apurinic/apyrimidinic (AP) sites accumulate, leading to stalled replication forks. The final nick is sealed by DNA ligases (LIG1 or LIG3) that form a complex with XRCC1. Mutations in XRCC1 are associated with neurological disorders due to impaired SSB repair.^{118,119} Defects in BER contribute to genomic instability, cancer predisposition, and progressive neurodegenerative diseases. For example, mutations in MutY homolog (MYH) increase the risk of colorectal cancer.¹²⁰ Similarly, defects in PARP-mediated single-strand break repair contribute to genomic instability and cancer predisposition⁷ (see the section on targeting replication/repair protein).

The FA pathway helps in stabilising the genome. So far, about 23 genes have been recognised as part of this pathway. It is activated when a replication fork stalls due to an inter-strand cross-link (ICL). The FA core complex, including FANCA, FANCB, FANCC, and FANCE, is recruited to the site of the damage. The first event is the monoubiquitination of FANCD2 and FANCI by the core complex. A disruption in this process causes FA, a genetic disorder linked to bone marrow failure and an increased risk of tumours, solid tumours. The FA pathway primarily affects stem cells, such as hematopoietic stem cells, because faulty DNA repair is associated with DNA damage, which in turn activates the p53 protein. It also coordinates with other repair mechanisms, such as HR and NER. It recruits proteins like FANCD1/BRCA2 and FANCR/RAD51 to the site of the lesion, where they protect the DNA from excessive degradation by nucleases, including those involved in the WRN complex. This protection is essential for the later HR repair. The pathway also borrows the XPF-ERCC1 nuclease, a core enzymatic component of NER, to perform the initial incision that “unhooks” the cross-link.^{121,122}

Another interesting example in this group is that of the non-homologous end joining (NHEJ) pathway, which is a faster way to fix double-stranded DNA breaks because it does not need a homologous template. It initiates with the binding of the Ku protein at the breakage site, which then recruits the ATP-dependent DNA-PKcs protein. The DNA-PKcs then facilitates the arrival of other proteins, like Artemis, XRCC4, and DNA Ligase IV, to the site for repair. Defects in this pathway are linked to diseases such as severe combined immunodeficiency (SCID). NHEJ is a very error-prone mechanism. When hyperactive, it can lead to drug resistance by efficiently repairing the damage caused by chemotherapy and radiotherapy.¹²³

Topoisomerases and DNA helicases are some of the additional examples of PDIs involved in replication/repair pathways. Topoisomerases are essential enzymes that regulate the DNA topology. Topoisomerase I (Topo I) relieves supercoiling by introducing transient single-strand breaks, whereas Topoisomerase II (Topo II) introduces ATP-dependent double-strand breaks to allow strand passage and decatenation. Under normal conditions, these reactions preserve genome stability. However, mutations, chemical inhibition, or DNA damage can trap the enzymes as covalent protein–DNA cleavage complexes (TOPccs), leading to persistent DNA breaks and genomic instability.¹²⁴

Topo II α contributes to cell proliferation during replication and mitosis, while Topo II β regulates transcription and chromatin organization in non-dividing cells.¹²⁵ Dysregulated topoisomerase activity is implicated in multiple diseases, including cancer,¹²⁶ neurological disorders such as SCAN1 and ataxia telangiectasia,¹²⁴ autoimmunity in scleroderma,¹²⁷ and immunodeficiency in Hoffman syndrome caused by *TOP2B* mutations.¹²⁸ Moreover, Topo I has transcriptional roles beyond DNA relaxation, with links to subsets of autism spectrum disorders.¹²⁷

Finally, DNA helicases are enzymes that use energy from ATP to unwind the DNA double helix. This step is necessary for

processes such as DNA replication, repair, and transcription. Helicases also help bring other proteins, such as DNA polymerases, to the replication fork.¹²⁹ Mutations in the *BLM* gene, which encodes a member of the RecQ helicase family, cause Bloom syndrome. The BLM helicase works as part of a multi-protein complex that includes helicases, topoisomerases, and DNA repair factors. This Bloom syndrome complex has a central role in HR repair and in maintaining genome stability. Defects in BLM or its partner components impair DNA repair, increasing cancer risk (lymphomas, leukaemias, *etc.*)¹³⁰

2.3 Diseases linked to non-canonical DNA structures

Non-canonical DNA structures (including hairpin loops, G-quadruplexes (G4), left-handed Z-DNA and triplexes) are often hotspots for DNA breaks and genomic instability. These structures form at characteristic DNA sequences. G4 arise at G-rich runs (such as repeated GGG motifs). Z-DNA forms at alternating purine–pyrimidine sequences, especially (CG)_n. Triplex DNA appears at homopurine–homopyrimidine mirror repeats. Hairpin-loops form when inverted repeats or triplet repeats like, CAG or CTG, fold back into a stem–loop shape. These conformations differ from canonical B-DNA in their base pairing, helical geometry, or strand arrangement.¹³¹

Proteins that normally bind to the B-DNA may not recognise these non-canonical structures or may bind improperly, causing misregulation of gene expression and contributing to various cancers and neurodegenerative diseases. For instance, G4 regulate the transcription of oncogenes such as *c-MYC*, *KRAS*, and *BCL2*. Their stabilisation or misregulation has been linked to cancers, including breast, lung, and colon cancer, while G4 structures have also been implicated in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Autoimmune diseases, particularly Aicardi-Goutières syndrome, are associated with the improper processing of Z-DNA. Cruciform structures have been linked to malignancies such as sarcomas and leukaemia and are commonly found near chromosomal fragile spots. Triplex structures frequently develop at repeating DNA sequences; the expansion of these repeats is a characteristic of hereditary conditions such as myotonic dystrophy, Huntington's disease, and Friedreich's ataxia.¹³² Hairpin-loop structures slow or block replication fork progression and can impede the activity of all three major replicative polymerases (Pol α , Pol δ , and Pol ϵ), thereby promoting replication stress and repeat-length instability. Hairpin-associated instability contributes to diseases such as spinocerebellar ataxias (SCAs) and other trinucleotide-repeat disorders, in which stable stem–loop formation correlates with pathogenic repeat expansion.¹³³

Some diseases associated with non-B DNA structures appear across more than one category because certain repeat tracts can adopt multiple alternative conformations. For example, CGG repeats can form both hairpins and G-quadruplexes, whereas GAA repeats can form hairpins as well as H-DNA. These overlapping structural possibilities explain why similar disease mechanisms appear under different structural classes. Many of these conditions are collectively recognised as repeat

expansion disorders (REDs), including Huntington's disease, myotonic dystrophy, fragile X syndrome and Friedreich's ataxia. These structures are also processed differently by DNA repair pathways, particularly MMR and NER, which can misrecognise or inefficiently resolve them and thereby contribute to disease progression.^{131,132,134} Research on identifying novel quadruplex structures and addressing their misregulation is in a relatively early stage but holds great promise as these unique structures may provide the missing high specificity targets in PDIs on the DNA side (see Section 3.2, Targeting the DNA).^{135–140}

2.4 Diseases linked to structural and architectural proteins

Structural and architectural proteins shape how DNA is packaged and accessed, playing a key role in regulating gene activity. Histones, CCCTC-binding factor (CTCF), cohesins, high-mobility group (HMG) proteins, alongside other chromatin-associated regulators, collectively maintain genomic organisation and govern the availability of the DNA template.

Mutations in core histones can disrupt nucleosome assembly and transcription, leading to neurodevelopmental disorders such as Rahman, Bryant–Li–Bhoj, and Tessadori–Bicknell–van Haften syndromes. These conditions are marked by developmental delay, intellectual disability, seizures, abnormal growth, craniofacial differences, and problems affecting multiple organs.¹⁴¹

Architectural proteins, such as CTCF and cohesin regulate gene expression *via* long-distance chromatin interactions. Improper mediation of enhancer–promoter interactions leads to transcriptional dysregulation. Cohesin forms a ring-like complex that mediates chromatin loop extrusion during interphase, while CTCF binds DNA to control topologically associating domain (TAD) boundaries and halt cohesin movement, organising chromatin topology. Mutations in these proteins can lead to TAD boundary disruption and oncogene misregulation, as well as cohesinopathies such as Cornelia de Lange syndrome, and contribute to cancer development by disrupting genome organisation.¹⁴²

HMG proteins are architectural proteins that bind DNA and induce bends, loops, or other conformational changes, regulating transcription and chromatin accessibility.¹⁴³ HMGB1, for example, possesses three main domains: the A-box binds damaged DNA and facilitates repair, the B-box has cytokine activity and recruits immune receptors such as Toll-like receptors (TLRs) and RAGE, and the C-terminal tail binds the minor groove of DNA, bending it and helping TF recruitment and repair. During myocardial infarction, HMGB1 is released as a danger associated molecular pattern (DAMP) into the cytoplasm, triggering inflammation and apoptosis; consequently, elevated HMGB1 levels are associated with myocardial damage.¹⁴⁴ Similarly, in allergic rhinitis, HMGB1 released by nasal epithelial cells promotes immune responses, activating T cells and inflammatory pathways.¹⁴⁵ HMGB1 also plays a role in tumour suppression by facilitating p53 binding to DNA and influencing the NF- κ B pathway, which is involved in inflammation and cell survival. Dysregulation or mislocalisation of

HMGB1 can promote cancer cell proliferation, induce HIF-1 α expression, and support tumour cell survival.¹⁴⁶

Proteins like chromatin remodelers and histone modification enzymes regulate how histones interact with other proteins and DNA. Although they do not bind DNA directly, they perform epigenetic regulation, indirectly modulating chromatin accessibility and PDIs critical for transcription, replication, and repair.¹⁴⁷ Histone modifications, collectively described as the “histone code”, include methylation, acetylation, phosphorylation, sumoylation and ubiquitination.¹⁴⁸ Mutations in these histone modification enzymes (writers and erasers), such as lysine methyltransferases (KMTs), lysine demethylases (KDMs), acetyltransferases (KATs), and deacetylases (HDACs, SIRT6), cause disorders including Kabuki, Wiedemann–Steiner, Sotos, Rubinstein–Taybi, and Luscan–Lumish syndromes. For example, histone deacetylases (HDACs) are involved in transcriptional inactivation of tumour-suppressor genes, leading to cancers, blood disorders, solid tumours, and non-neoplastic disorders.¹⁴⁹ Targeting HDACs has hence become a significant therapeutic strategy.¹⁵⁰ Epigenetic readers recognise chemical modifications (tags) on histones or DNA and mediate interactions between these proteins and DNA, guiding the recruitment of other factors that regulate gene expression and chromatin structure.¹⁵¹ Among them, BRD4 is the most studied and has a role in cell cycle, inflammation, and cancer progression. Likewise, defects in chromatin remodeler complexes such as SWI/SNF, CHD, and INO80, responsible for repositioning nucleosomes, are linked to syndromes including Coffin–Siris, Snijders Blok–Campeau, Sifrim–Hitz–Weiss, and CHARGE. These conditions often share features such as intellectual disability, seizures, skeletal abnormalities, and craniofacial malformations.¹⁴¹

Although this review focuses on human PDIs, it is worth noting that similar recognition mechanisms occur in host-pathogen interactions. For example, Z-DNA binding protein 1 (ZBP1) recognises foreign DNA, and its dysregulation can trigger autoimmune conditions such as Aicardi–Goutieres syndrome.¹⁵² While important, these interactions are beyond the scope of this review. With this understanding of dysregulation, the next section addresses therapeutic strategies to target these interactions.

3 Drug targeting strategies for PDIs

Given the central role of PDIs in regulating gene expression, DNA repair, and other cellular processes, therapeutic strategies have been developed to disrupt or modulate these complexes. These strategies include (1) directly targeting DNA-binding proteins, (2) interfering with the DNA molecule itself, or (3) modulating the protein–DNA interface to inhibit or alter binding. Each offers distinct mechanisms and clinical implications as reviewed below.

3.1 DNA-binding proteins as drug targets

DNA-binding proteins form a diverse group that includes TFs, replication and repair proteins, histones, and architectural

proteins. This section outlines strategies for targeting these proteins to modulate PDIs.

3.1.1 Transcription factor inhibition. There are multiple strategies to target TFs to modulate PDIs, each presenting distinct challenges and clinical implications.¹⁵⁵ A summary of key drugs targeting transcription factors is provided in Table 1. The most direct approach to targeting TFs utilises small molecules or peptides that bind the TF’s DNA-binding domain (DBD), thereby preventing DNA interaction and transcriptional activity. Because this method targets the functional interface directly, it could theoretically minimise off-target effects; however, DBDs are often described as “undruggable” due to their flat, featureless and electrostatic surfaces, making high-affinity binding by external perturbagens very difficult.¹⁵⁶ Nonetheless, candidate drugs such as omomyc and eprenetapopt (APR-246), targeting the DBDs of c-Myc and p53 respectively, have been proposed but remain in preclinical or early clinical trials.^{51,157,158} An alternative to orthosteric DBD inhibition is their allosteric modulation, where drugs bind domains outside the DBD to indirectly prevent DNA-binding. Examples include STAT3IC and TTI-101, which bind the SH2 domain of STAT3. This blocks the protein–protein interaction required for dimerisation and subsequent DNA-binding. Furthermore, FDA-approved ER or AR inhibitors, such as fulvestrant and enzalutamide, target the ligand-binding domain (LBD) to block nuclear localisation, dimerisation, or DNA-binding.^{49,50,159–163} In this category, belzutifan represents a significant breakthrough (Fig. 2), as it is the first FDA-approved drug to target HIF. It is currently used to treat von Hippel–Lindau (VHL) disease-associated renal cell carcinoma (RCC), retinal haemangioblastomas, and advanced sporadic RCC.^{164,165} It is also being tested in combination therapy for advanced clear cell RCC in several clinical trials (*e.g.*, NCT04586231, NCT03634540, and NCT05239728).^{59,166,167}

Another approach to target DNA-binding proteins leverages targeted protein degradation, such as PROTACs. For instance, ARV-110 targets the AR, removing the TF entirely rather than merely inhibiting its DNA-binding capability. While these strategies involve direct interaction with the TF, many have not yet achieved full clinical approval across all TF classes.

Due to the difficulties of direct or allosteric targeting of structures, the most clinically feasible method currently is indirect modulation. In this approach, drugs do not physically interact with the TF but instead influence upstream signalling pathways and alter TF phosphorylation, nuclear localisation, or activation, thereby affecting DNA-binding activity. Examples include CDK4/6 inhibitors such as palbociclib, ribociclib, and abemaciclib, which indirectly inhibit E2F TFs,^{168,169} and AKT inhibitors like capivasertib, which modulate FOXO TFs. These indirect approaches are FDA-approved in various oncology contexts and provide a practical route for therapeutically modulating PDIs.

3.1.2 Replication and repair protein inhibition. Targeting the proteins involved in DNA repair and replication machinery is one of the direct approaches to combat cancer. Tumour cells are particularly sensitive to the inhibition of these processes

Table 1 Drugs targeting DNA-binding proteins: transcription factors

Drug name	Target	Mechanism of action	Approval status	Drug class	Therapeutic area	Ref.
WBC100	Myc	Degrader: Induces proteasomal degradation of Myc by targeting the NLS1-basic-NLS. 2 region	Phase I trial (NCT05100251)	Molecular glue/degrader	Advanced solid tumours	170
OMO-103 (Omomyc)	Myc	DBD binding: Mini-protein interfering with Myc-Max dimerisation and DNA-binding, blocking transcriptional activity	Phase I/IIa trial (NCT04808362); Phase Ib trial (NCT06059001); Phase II trial (NCT06650514)	Mini protein	Solid tumours, metastatic PDAC, advanced high-grade osteosarcoma	51
IDP-121	Myc	Allosteric: Mini-protein interfering with Myc transcriptional activity	Phase I/II trial (NCT05908409)	Mini protein	Relapsed/refractory haematological malignancies	171
STATTIC	STAT3	Allosteric: STAT3 SH2-domain inhibitor, prevents dimerisation and DNA-binding	Preclinical	Small-molecule	T-ALL, solid tumours and haematologic malignancies	159 and 160
TTI-101	STAT3	Allosteric: Blocks STAT3 activation at the SH2 domain, preventing dimerisation and transcriptional activity	Phase I trial (NCT03195699); Phase II trial (NCT05440708)	Small-molecule	Advanced/metastatic solid tumours, hepatocellular carcinoma, ovarian cancer, gastric cancer	161
KT-333	STAT3	Degrader: Recruits STAT3 to E3 ubiquitin ligase for proteasomal degradation	Phase I trial (NCT05225584)	Heterobifunctional small-molecule degrader	Refractory lymphomas, LGL leukaemia, solid tumours	172
Vepdegestrant (ARV-471)	ER	Degrader: Recruits ER to an E3 ubiquitin ligase for proteasomal degradation	Phase III trial (NCT05654623)	PROTAC	ER-positive/HER2-negative advanced or metastatic breast cancer	61
Fulvestrant	ER	Allosteric: Binds LBD and induces proteasomal degradation	FDA-approved	SERD	Breast cancer	49 and 162
Enzalutamide	AR	Allosteric: Binds to LBD and prevents nuclear translocation and DNA-binding	FDA-approved	Anti-androgen	Prostate cancer	50 and 163
ARV-110	AR	Degrader: Recruits AR to cereblon E3 ligase for proteasomal degradation	Phase I/II trial (NCT03888612)	PROTAC	mCRPC	52
Eprenetapopt (APR-246)	p53	DBD binding: Covalently binds to mutant p53, restoring wild-type conformation	Phase III trial (NCT03745716)	Small-molecule	Myelodysplastic syndrome	157 and 158
Belzutifan	HIF-2 α	Allosteric: Binds to the PAS-B domain of HIF-2 α and prevents dimerisation with HIF-1 β , essential for DNA-binding and transcriptional activity	FDA approved	Small-molecule	VHL-associated tumours: RCC, CNS haemangioblastomas, or pNET	173

Abbreviations: ER, estrogen receptor; AR, androgen receptor; STAT3, signal transducer and activator of transcription 3; Myc, myelocytomatosis oncoprotein; HIF, hypoxia-inducible factor; p53, tumor protein p53; PROTAC, proteolysis-targeting chimera; SERD, selective estrogen receptor degrader; mCRPC, metastatic castration-resistant prostate cancer; PAS-B, Per-ARNT-Sim domain B; SH2, Src-homology 2; DBD, DNA-binding domain; NLS, nuclear localisation signal; LBD, ligand-binding domain; LGL, large granular lymphocyte; T-ALL, T-cell acute lymphoblastic leukaemia; PDAC, pancreatic ductal adenocarcinoma; VHL, von Hippel-Lindau; RCC, renal cell carcinoma; CNS, central nervous system; pNET, pancreatic neuroendocrine tumour; NCT, National Clinical Trial.

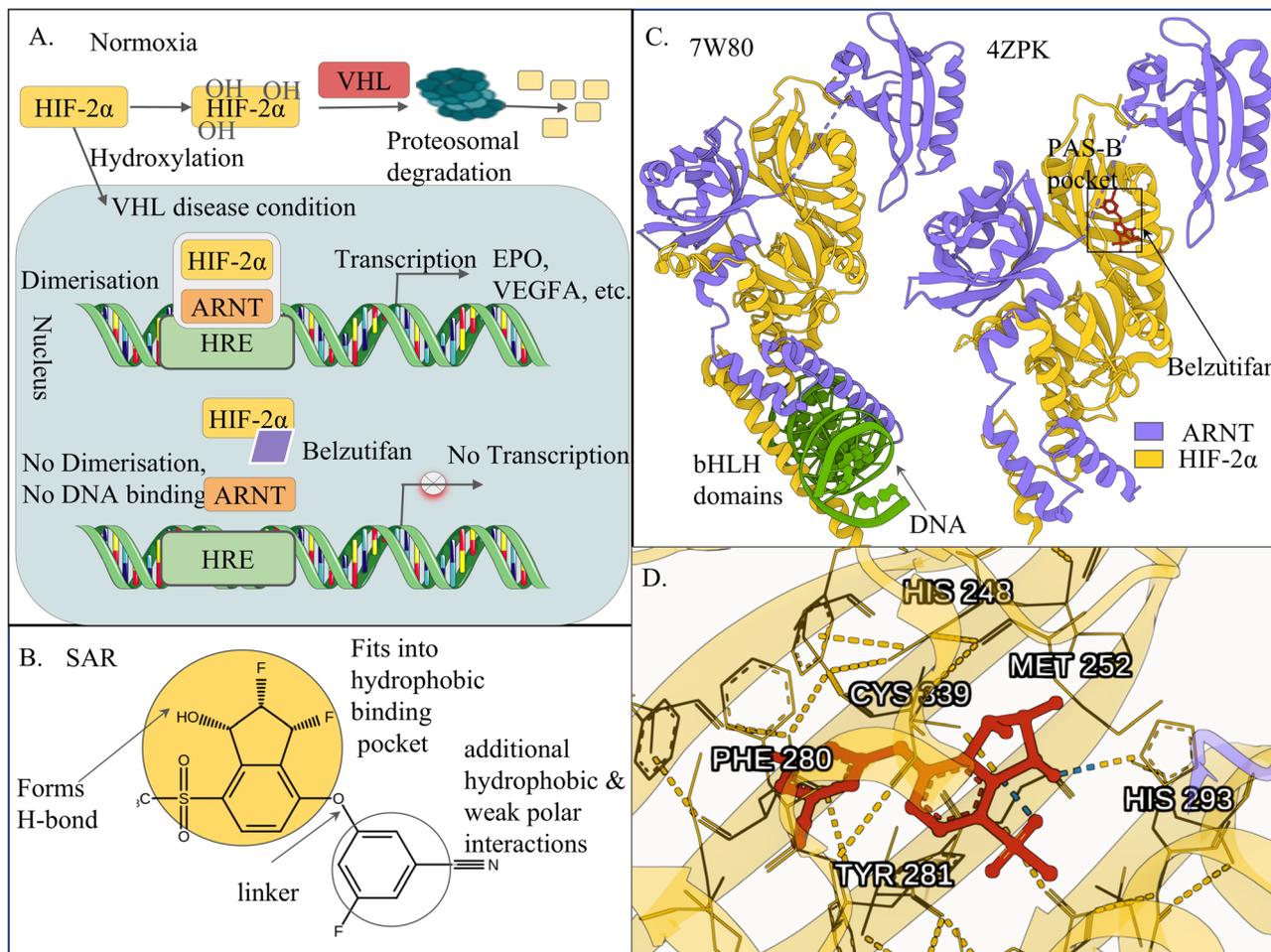


Fig. 2 Structural and mechanistic basis of hypoxia-inducible factor 2 α (HIF-2 α) inhibition by belzutifan. (A) Under normoxic and von Hippel–Lindau (VHL)-proficient conditions, HIF-2 α is hydroxylated, recognised by the VHL E3 ubiquitin ligase complex, and degraded. In hypoxia or VHL-deficient states, HIF-2 α accumulates, heterodimerises with the aryl hydrocarbon receptor nuclear translocator (ARNT), binds hypoxia-response elements (HREs), and activates transcription. Belzutifan binds the HIF-2 α PAS-B domain, disrupting HIF-2 α –ARNT dimerisation and preventing DNA binding. (B) Structure–activity relationship (SAR) of belzutifan highlighting chemical features required for selective PAS-B pocket engagement. (C) Structural comparison of DNA-bound HIF-2 α –ARNT (PDB: 4ZPK)¹⁵³ and belzutifan-bound HIF-2 α (PDB: 7W80),¹⁵⁴ showing that ligand binding stabilises a conformation incompatible with ARNT association and DNA binding. (D) Key interactions of belzutifan within the HIF-2 α PAS-B pocket, including Met252, whose side-chain reorientation stabilises a rigid, inactive PAS-B conformation. Created with icons from Servier Medical Art (smart.servier.com), licensed under CC BY 4.0; protein visualisations generated via Mol*,⁶⁸ MarvinSketch for chemical structures, Chemaxon (<https://www.chemaxon.com>).

due to their high proliferation and inherent genomic instability. Targeting these proteins would disrupt their proliferation, trigger cell death, or sensitise them towards DNA-damaging agents. Beyond cancer, targeting these proteins may be useful in other diseases where aberrant DNA repair contributes to pathology. This strategy does not come without challenges, as inhibition of essential repair and replication proteins can also disrupt normal cellular processes in healthy cells.

A summary of key drugs targeting replication and repair proteins is provided in Table 2.

The most successful strategy for targeting DNA repair proteins has been the development of PARP inhibitors for *BRCA1/2*-deficient cancers, exemplified by the FDA-approved drugs olaparib and talazoparib. PARP1 is a protein that detects SSBs in DNA and helps repair them. Olaparib exploits between-pathway synthetic lethality, where in *BRCA*-mutant cells, the

HR pathway is defective and inhibition of PARP blocks the backup repair pathway, leading to cell death (Fig. 3). In contrast, normal cells remain largely unaffected because they retain their primary repair pathway.⁷ Talazoparib, in addition to catalytic inhibition, is a potent PARP trapper, stabilising PARP–DNA intermediates and preventing their resolution, an example of in-pathway synthetic lethality that enhances cytotoxicity.¹⁷⁶ However, resistance to PARP inhibitors in *BRCA*-deficient cancers has been reported. Some tumour cells restore HR function through secondary mutations that “fix” the defective *BRCA1/2* gene or by loss of p53-binding protein 1 (53BP1). For such resistance mechanisms to occur, the cells typically require intact HR-related domains (RING, BRCT, etc.).⁷

Overexpression of RAD51 contributes to PARP inhibitor resistance by enhancing HR. To target RAD51, small molecules like IBR2 and B02 are being studied, which are currently in

Table 2 Drugs targeting DNA-binding proteins: DNA replication and repair proteins

Drug name	Target	Mechanism of action	Approval status	Drug class	Therapeutic area	Ref.
IBR2	RAD51	Allosteric: Binds at the oligomerisation site, disrupting RAD51 polymer formation, impairing HR repair	Preclinical	Small-molecule	Breast cancer, CML, <i>etc.</i>	184
B02	RAD51	DBD: Binds to the DNA-binding surface, inhibits RAD51 strand invasion preventing HR-mediated repair, also used in combination with cisplatin	Preclinical	Small-molecule	Breast cancer, prostate cancer	185–187
Olaparib	PARP1/2	Catalytic: Competitive inhibition of PARP catalytic activity, blocks PARylation, prevents SSB repair	FDA-approved	Small-molecule	<i>BRCA</i> -mutated cancer	53 and 188
Talazoparib	PARP1/2	Catalytic: Inhibits and traps PARP, inducing DNA damage, especially in HR-deficient tumours	FDA-approved	Small-molecule	<i>BRCA</i> -mutated HER2-negative breast cancer	54 and 176
NERx329	RPA	DBD: Reversibly binds to the ssDNA-binding OB-fold domain of RPA, blocking its interaction with ssDNA and disrupting replication and repair	Preclinical	Small-molecule	NSCLC, <i>BRCA</i> -mutated cancers; potential to overcome platinum resistance	180
Peposertib (M3814)	DNA-PKcs	Catalytic: ATP-competitive inhibition of kinase activity, blocks NHEJ activation	Phase I trial (NCT04555577, NCT05868174), In combination therapies (NCT05687136)	Small-molecule	Solid tumours, glioblastoma, <i>etc.</i>	182 and 189

Abbreviations: HR, homologous recombination; DBD, DNA-binding domain; PARP, poly(ADP-ribose) polymerase; SSB, single-strand break; *BRCA*, breast cancer susceptibility gene; RPA, replication protein A; OB-fold, oligonucleotide/oligosaccharide-binding fold; ssDNA, single-stranded DNA; NHEJ, non-homologous end joining; NSCLC, non-small cell lung cancer; CML, chronic myeloid leukaemia.

preclinical trials. IBR2 disrupts RAD51 oligomerisation and prevents the formation of functional RAD51 filaments, while B02 blocks RAD51 binding to ssDNA and inhibits the strand exchange mediated by it in HR. B02 also makes the tumour cells more sensitive to other therapies like ionising radiation, mitomycin C and cisplatin. It has shown efficacy in triple-negative breast cancer (TNBC) models in mouse xenografts. Combination therapy of B02, PARP inhibitor and p38 inhibitor can overcome resistance in *BRCA1/2*-deficient cancers, by blocking DNA repair and associated survival pathways.^{7,177,178} Ongoing studies are exploring new chemical scaffolds inspired by these inhibitors to design next-generation RAD51-targeting molecules.¹⁷⁹

For RAD51 to work, it first needs RPA to bind and protect the ssDNA; then, RAD51 replaces RPA to initiate the HR process. RPA is also essential for the NER pathway, thus playing a dual role in DNA replication and repair. RPA can be targeted in two main ways: allosteric inhibition or directly targeting its ssDNA-binding domains, known as OB-folds, to prevent DNA-binding. For example, NERx329 is a small-molecule inhibitor in preclinical trials that has shown promising results by reversibly binding to these domains. This strategy is effective in non-small cell lung cancer (NSCLC). Furthermore, RPA inhibitors are effective in combination with PARP inhibitors for *BRCA*-mutated cancers and may potentially overcome resistance to platinum-based drugs in lung cancer.¹⁸⁰

The FA pathway is another important target in this context. In patients with FA, the pathway is defective, and restoring its function could be a potential therapeutic goal. For example, research is exploring small molecules that can reactivate FANCD2 monoubiquitination thereby bypassing the underlying genetic defect. Conversely, in certain cancers, the FA pathway is often hyperactive, enabling cancer cells to repair DNA damage caused by treatments like chemotherapy. Shutting down this

pathway could make these cancer cells more vulnerable. A key step for this therapeutic approach is the targeted inhibition of FANCD2 monoubiquitination. For instance, TAK243, an inhibitor of ubiquitin-activating enzymes (UAE), has been shown to inhibit this crucial step potently. While UAEs have a broad effect, this finding confirms that targeting a key component of the FA pathway is a viable strategy for developing new therapeutic agents. This approach could sensitise cancer cells to existing treatments, while separate efforts could develop activators to treat FA.¹²²

Beyond direct inhibition of repair proteins, another effective strategy is to utilise immunotherapy, particularly for cancers with defective DNA repair mechanisms. Immune checkpoint inhibitors are used as a treatment for cancers with a defective MMR pathway. These tumours have a high number of mutations, a state known as microsatellite instability-high (MSI-H). The presence of these mutations causes the tumour to produce abnormal proteins, which the immune system can recognise. This high mutational load makes these tumours susceptible to immunotherapy. Drugs like pembrolizumab, which is FDA-approved, are used. While not a direct therapeutic for the MMR defect, it causes the immune system to attack tumour cells. It is currently approved for various MSI-H cancers.¹⁸¹

The popular approach to target the NHEJ pathway is to inhibit the DNA-PKcs protein, with drugs like peposertib (currently in clinical trials). These ATP-competitive inhibitors bind to the protein's active site, preventing it from activating other proteins in the pathway. Importantly, these inhibitors block the enzymatic activity of DNA-PKcs after it has bound DNA, rather than preventing DNA-binding itself. This inhibition leaves the DNA damage unrepaired, making the cancer cells more vulnerable.¹⁸² In addition to targeting DNA-PKcs, other NHEJ proteins are also being investigated as therapeutic targets.

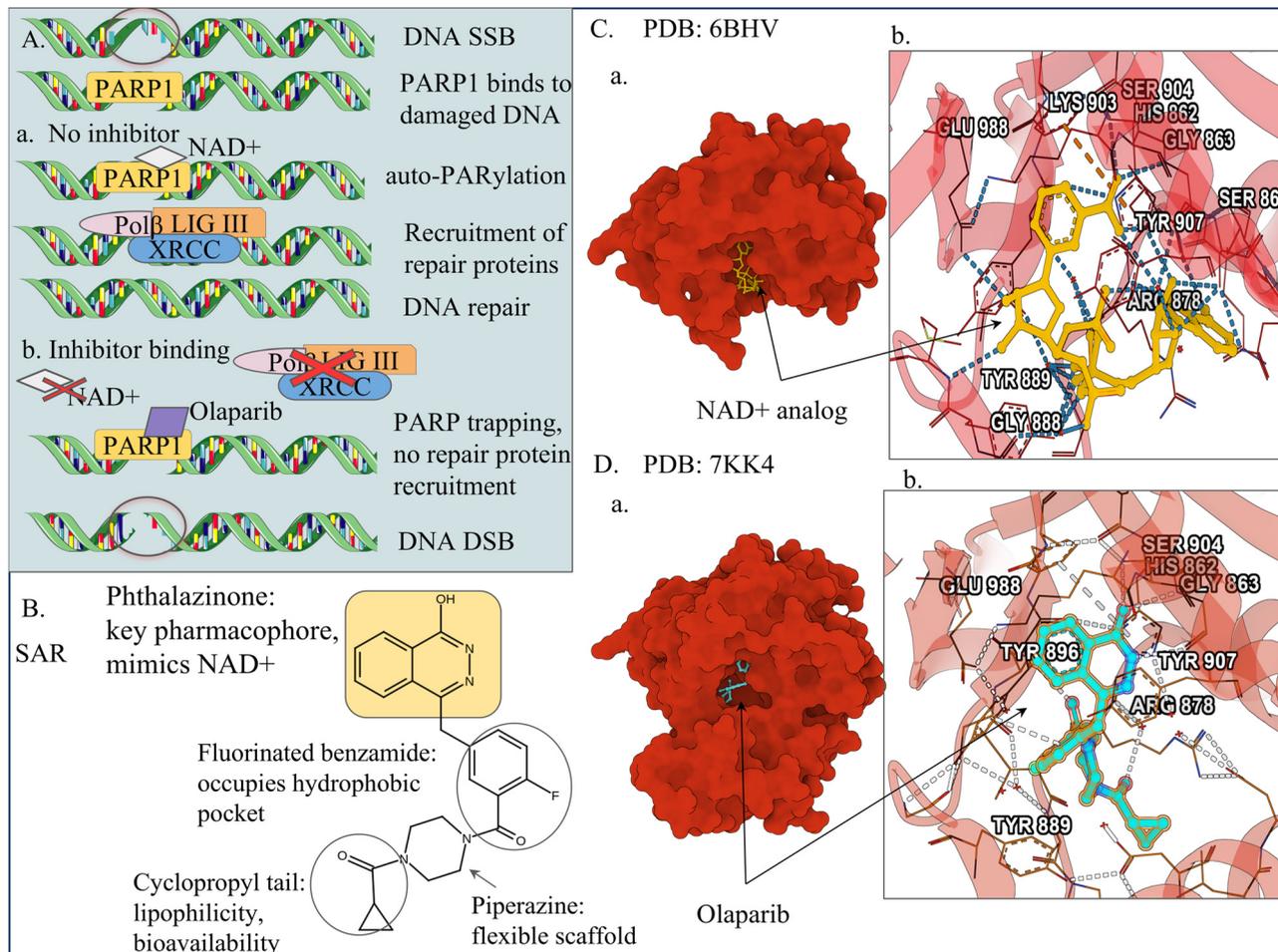


Fig. 3 Mechanistic and structural basis of PARP1 inhibition by olaparib. (A) Mechanism of action of olaparib. In the absence of inhibitor, poly(ADP-ribose) polymerase 1 (PARP1) binds to single-strand DNA breaks (SSBs), undergoes auto-poly(ADP-ribose)ylation using NAD⁺, recruits DNA repair factors, and facilitates SSB repair. In the presence of olaparib, PARP1 catalytic activity is inhibited and the enzyme becomes trapped on DNA, preventing repair, leading to replication fork collapse, accumulation of double-strand breaks (DSBs), and cell death. (B) Structure–activity relationship (SAR) of olaparib highlighting key chemical features required for high-affinity binding to the PARP1 catalytic domain and efficient PARP trapping. (C) Crystal structure of the PARP1 catalytic domain bound to an NAD⁺ analogue (PDB: 6BHV)¹⁷⁴ showing the overall protein surface, the NAD⁺-binding pocket, and key ligand–protein interactions. (D) Crystal structure of PARP1 bound to olaparib (PDB: 7KK4)¹⁷⁵ showing olaparib (blue) occupying the NAD⁺-binding site and representative ligand–protein interactions. Created with icons from Servier Medical Art (smart.servier.com), licensed under CC BY 4.0; protein visualisations generated via Mol*,⁶⁸ MarvinSketch chemical structures, and Chemaxon (<https://www.chemaxon.com>).

These include inhibitors for the Ku protein (*e.g.*, STL127705) to prevent initial DNA-binding, for Artemis (*e.g.*, Ebselen) to block DNA processing, and for DNA ligase IV (*e.g.*, L189) to stop the final sealing of the DNA break. However, most of these inhibitors are still in the preclinical stage, and further research is needed in this direction.¹⁸³

3.1.3 Targeting chromatin modulators and architectural proteins. Table 3 summarises representative drugs and emerging candidates that target chromatin modulators and architectural proteins involved in protein–DNA regulation. These agents act either by directly inhibiting structural proteins such as cohesin, CTCF, or HMGB1, or by indirectly modulating their function through epigenetic regulators such as HDACs and methyltransferases.

The design of drugs targeting cohesin and CTCF is still in the conceptual and experimental stage. For example, the first

cohesin-inhibiting peptide (CIP), which inhibits the ATPase activity of cohesin by binding to the Smc3 domain, has been developed, suggesting potential applications in cancer therapy. However, challenges remain in using peptides for tumour targeting due to specificity issues.¹⁹⁰ There is also potential for treating cohesin-mutated cancers by targeting cohesin components or their regulators, as well as associated transcriptional or signalling events and DNA damage repair systems. Candidates for these strategies include drugs such as glycyrrhizic acid, olaparib, talazoparib, cisplatin, and etoposide.¹⁹¹ CTCF epigenetically regulates gene expression and plays an important role in alternative splicing, with DNA methylation controlling its binding to targeted sequences.¹⁹² The development and persistence of CALM-AF10 leukaemia depend on CTCF, which exerts its regulatory effects through histone modifications.¹⁹³ In pancreatic ductal adenocarcinoma (PDAC),

Table 3 Drugs targeting DNA-binding proteins: chromatin modulators/architectural proteins

Drug name	Target	Mechanism of action	Approval status	Drug class	Therapeutic area	Ref.
Glycyrrhizin	HMGBl	Direct inhibitor of HMGBl; suppresses tumour proliferation and induces apoptosis <i>via</i> HMGB1-mediated DNA repair (NHEJ) in cancer models	Preclinical	Natural product	Colorectal and cervical cancer	195
Vorinostat	HDAC1,2,3,6	Inhibits HDACs, increasing histone acetylation, which loosens chromatin and helps reactivate suppressed genes	FDA-approved	Small-molecule (hydroxamic acid)	Cutaneous T-cell lymphoma	55
Romidepsin	Class I HDACs	Reduced intracellularly to free thiol which binds zinc ions at the HDAC active site, leading to histone hyperacetylation and chromatin relaxation and apoptosis induction	FDA-approved	Cyclic peptide (prodrug)	CTCL, PTCL	200
Panobinostat	Pan-HDAC	Binds zinc ions in the HDAC site leading to histone and tubulin acetylation, disrupting chromatin compaction and microtubule function and causing apoptosis	FDA-approved	Small-molecule (hydroxamic acid derivative)	Multiple myeloma (in combination therapy)	149 and 203
Givinostat	Pan-HDAC	Hydroxamate chelation of zinc ions, preventing histone deacetylation, leading to sustained histone acetylation, reduces fibrosis and inflammation in muscle tissue	FDA-approved	Small-molecule, hydroxamic acid derivative	Duchenne muscular dystrophy	206 and 213
Tazemetostat	EZH2 (H3K27 methyltransferase, PRC2 complex)	Inhibits EZH2 catalytic activity, preventing trimethylation of silenced tumour suppressor genes	FDA-approved	Small-molecule (HMT inhibitor)	Epithelioid sarcoma, follicular lymphoma	56 and 214
Pinometostat	DOT1L (H3K79 methyltransferase)	Selectively blocks DOT1L-mediated H3K79 methylation, essential for maintaining oncogenic transcription programs in MLL-rearranged leukaemias	Phase I/II trial (NCT03701295), Phase I trial (NCT01684150)	Small-molecule (HMT inhibitor)	MLL-rearranged leukaemia	208 and 209
Iadademstat	LSD1 (KDM1A histone demethylase)	Irreversibly inhibits LSD1, preventing the removal of H3K4me1/2 marks, restoring expression of differentiation genes and promoting maturation of leukemic blasts	Phase IIa trial: Eudract 2018-000482-36 (combination therapy), Phase II trial: Eudract 2018-000469-35 (combination therapy)	Small-molecule (KDM inhibitor)	AML, SCLC	207 and 215
Apabetalone	BRD4	Selectively inhibits the BD2 domain of BET proteins, preventing BRD4 binding to acetylated histones and preventing TF recruitment, leading to gene silencing	Phase III trial (NCT02586155) for type 2 diabetes	Small-molecule	Type 2 diabetes, CVD, long COVID	212 and 216

Abbreviations: HMGBl, high-mobility group box 1; HDAC, histone deacetylase; CTCL, cutaneous T-cell lymphoma; PTCL, peripheral T-cell lymphoma; EZH2, enhancer of zeste homolog 2; HMT, histone methyltransferase; PRC2, polycomb repressive complex 2; H3K27me3, histone H3 lysine 27 trimethylation; DOT1L, disruptor of telomeric silencing 1-like; H3K79, histone H3 lysine 79; MLL, mixed lineage leukaemia; LSD1 (KDM1A), lysine-specific demethylase 1A; H3K4me1/2, histone H3 lysine 4 mono-/di-methylation; AML, acute myeloid leukaemia; BRD4, bromodomain-containing protein 4; BET, bromodomain and extra-terminal family; BD2, bromodomain 2; NHEJ, non-homologous end joining; CVD, Cardiovascular disease.

CTCF has been identified as a potential therapeutic target for immunotherapy, and combination of curaxin with gemcitabine has been explored as a treatment strategy.¹⁹⁴

Structural proteins such as HMGB1 have also been extensively studied. Glycyrrhizin, a naturally derived compound from licorice, has been identified as a direct inhibitor. Studies in colorectal and cervical cancer models have demonstrated that glycyrrhizin suppresses tumour cell proliferation and induces apoptosis, potentially by modulating HMGB1's involvement in the NHEJ pathway, thereby affecting DNA repair processes.¹⁹⁵ In liver disease, glycyrrhizin exerts anti-inflammatory effects by targeting HMGB1's extracellular signalling and has been approved for this use in some countries; this represents a mechanism distinct from its modulation of HMGB1-related DNA interactions in cancer cells.¹⁹⁶

Building on these approaches, current efforts increasingly focus on epigenetic regulation as a complementary strategy to target chromatin-associated proteins. Since the activity of structural proteins, like cohesin, CTCF, and HMGB1, is influenced by histone modification and chromatin state, targeting histone-modifying enzymes and chromatin readers offers an indirect yet effective approach.

HDAC inhibitors are well-established drugs that increase histone acetylation, loosening chromatin and reactivating silenced genes. Vorinostat is the first FDA-approved HDAC inhibitor. It is suberoylanilide hydroxamic acid (SAHA) used for the treatment of cutaneous T-cell lymphoma (CTCL). It binds to both Class I and Class II HDACs, with a preference for the former.¹⁹⁷ It is currently under trial for other types of tumours, such as head and neck squamous cell carcinoma (HNSCC),¹⁹⁸ and breast cancer, both as a monotherapy¹⁹⁹ and in combination with drugs like olaparib.⁶⁰ Romidepsin, a cyclic peptide is used to treat CTCL, peripheral T-cell lymphoma (PTCL) and hepatocellular carcinoma.^{200–202} Panobinostat, a pan-HDAC inhibitor approved for multiple myeloma,²⁰³ can cross the blood–brain barrier, and studies are investigating its use in brain cancers^{204,205} and other solid tumours.¹⁴⁹ The mechanisms of all are explained in Table 3. Givinostat is a recently FDA-approved drug and the first non-steroidal medication for the treatment of Duchenne muscular dystrophy (DMD). DMD is characterised by the loss of dystrophin, which, through a series of processes, affects nitric oxide signalling that controls HDAC activity, leading to its hyperactivation and transcriptional repression of genes such as myogenic microRNAs and follistatin. These genes are important for muscle repair. By inhibiting HDAC2, givinostat is effective in the treatment of DMD.²⁰⁶

Histone methyltransferase inhibitors include tazemetostat, an FDA-approved small-molecule selective inhibitor of EZH2 KMT catalysis. It inhibits EZH2-mediated H3K27 methylation, thereby preventing gene repression. It is used for refractory follicular lymphomas and various solid tumours. Chromatin remodelers, like SWI/SNF, involved in gene transcription, are also linked to EZH2 activity when dysfunctional. Consistent with this, tazemetostat showed clinical activity in *SMARCB1/INI1*-deficient epithelioid sarcoma, resulting in FDA accelerated

approval in 2020.^{56,207} Pinometostat is another selective inhibitor that targets DOT1L, the H3K79 methyltransferase.²⁰⁸ It has shown activity in early-phase studies of acute leukaemias with *MLL* rearrangements and has been evaluated in Phase Ib/II trials, both as monotherapy and in combination with chemotherapy or azacitidine; however, the results show limited efficacy.^{208,209} On the other hand, FDA-approved revumenib, which also downregulates the same oncogenic genes *HoxA/MEIS1* as pinometostat, is a menin-KMT2A inhibitor essentially blocking protein–protein interaction and is used in AML therapy too, while pinometostat is also stated to increase the sensitivity to this inhibitor, and trials have been conducted for the combination therapy.^{209–211} Iadademstat, an LSD1 inhibitor, works opposite to tazemetostat and pinometostat, as it selectively inhibits a histone demethylase and reactivates cancer-suppressing genes. It is still in early clinical trial phases for relapsed AML, both as a single agent and in combination with azacitidine. Iadademstat has also shown activity in SCLC, with ongoing combination trials.²⁰⁷

Emerging approaches target epigenetic readers and chromatin remodelers. Apabetalone is a selective BET inhibitor that binds to the BD2 bromodomain and is currently in Phase III trials for cardiovascular and kidney diseases.²¹²

While the strategies above focus on modulating proteins that bind or organise DNA, another complementary approach is to directly target the DNA molecule itself, either to block protein binding or to modulate its structure, which is discussed in the following section.

3.2 Targeting the DNA

Proteins recognise DNA through either a direct sequence-based readout mechanism, an indirect or shape-based readout mechanism, or a combination of both. Small-molecule drugs work on a similar principle, recognising binding sites through ionic and hydrogen bonding interactions, and indirectly through DNA shape complementarity, depending on the nucleotide sequence. A summary of key drugs targeting DNA instead of proteins for disrupting PDIs is provided in Table 4.

Binding can occur in two modes for drug–DNA interactions: covalent binding (alkylating agents), which is irreversible, and non-covalent binding (intercalators and groove binders), which is reversible.⁷⁴ Direct binding of small molecules is a valuable approach for targeting TFs and topoisomerases.^{47,48}

For example, cisplatin, an alkylating agent, is a square planar platinum(II) complex with two ammine and two chloride ligands.^{217,218} It forms covalent adducts at the N7 position of guanine, causing DNA crosslinking, bending, and unwinding; this inhibits replication and transcription by blocking the binding of TFs and stalling RNA polymerase.²¹⁹ While these lesions are normally substrates for the NER pathway, protein shielding of cisplatin adducts can hinder the recruitment of repair factors such as XPA, leading to persistent DNA damage (Fig. 4).^{220,221} Oxaliplatin, with a bulky diaminocyclohexane (DACH) and oxalate ligands, forms larger adducts and is mainly used in cisplatin-resistant colorectal cancers.^{218,222} Other new-

Table 4 Drugs binding to DNA

Drug	Target	Mechanism of action	Approval status	Drug class	Therapeutic area	Ref.
Cisplatin	N7 of guanines	Forms intra- & interstrand cross links; deforms DNA, blocking transcription and replication. Disrupts TBP, NF-Y, DNA/RNA polymerases, RPA; recruits HMGB1 to adducts; activates p53 in response to damage	FDA-approved	Small-molecule (Pt-based alkylating agent)	Testicular, ovarian, bladder, lung, head and neck cancer	266 and 267
Oxaliplatin	N7 of guanines	Forms bulky DACH-Pt intra- and interstrand crosslinks (preferentially forms H-bonds at the 3' G); deforms DNA; blocks DNA synthesis, transcription, and replication. Disrupts TBP, NF-Y, DNA/RNA polymerases, RPA, and repair proteins (MutS α); activates p53 and Bax	FDA-approved	Small-molecule (Pt-based alkylating agent)	Colorectal and oesophageal cancer	267 and 268
Trabectedin	N2 of guanine	DNA bending and adduct formation, blocks RNA polymerase II binding; inhibits activity of TFs like FUS-CHOP and EWS-FLI1	Phase III trial (NCT02672527, NCT01343277)	Small-molecule (natural product intercalator)	Metastatic liposarcoma, leiomyosarcoma, soft tissue sarcoma	224, 269 and 270
Melphalan	N7 of guanine and N3 of adenine	DNA crosslinks and adducts causing helix distortion, DNA damage and mutation. Affects DNA polymerase and replication proteins	FDA-approved	Small-molecule (nitrogen mustard, alkylating agent)	Multiple myeloma, ovarian cancer, lymphoma	271
Actinomycin D	GC-rich minor groove	Intercalates phenoxazone ring between bp, alters DNA structure, prevents binding of RNA polymerase and inhibits transcription; stabilises G4 structures in <i>c-MYC</i> promoter, <i>SOX2</i> downregulation	FDA-approved	Small-molecule (intercalator)	Rhabdomyosarcoma, choriocarcinoma, Ewing sarcoma, testicular cancer	243–245 and 272
Doxorubicin	DNA duplex	Intercalates and inhibits Topo II, causing DNA breaks, blocks transcription and replication by evicting histone	FDA-approved	Small-molecule (anthracycline intercalator)	Breast cancer, leukaemia, lymphoma, testicular, bladder, ovarian, lung cancer	47 and 273
Amsacrine (m-AMSA)	DNA duplex	DNA intercalator, stabilises the Topo II cleavage complex and prevents religation, causing DSBs, leading to apoptosis	Approved (selected countries)	Small-molecule (intercalator)	AML, Hodgkin's and non-Hodgkin's lymphomas	248 and 249
Voreloxin	DNA duplex, G4 DNA	Intercalates between bp to poison Topo II and induces site-selective DSBs, G2 cell cycle arrest, end-stacks on G-tetrads to suppress <i>c-MYC</i> / <i>BCL-2</i> expression	Phase II trials (NCT00408603, NCT00607997, NCT00252382, NCT00298896)	Small-molecule (quinolone-based intercalator)	AML, ovarian cancer, leukaemia, SCLC, solid tumours	250–252
Mithramycin	GC-rich minor groove	Inhibits TFs Sp1, EWS-FL1, histone modulation	Previously approved now discontinued, currently in trials for cancer	Small-molecule (non-covalent groove binder)	Osteosarcoma, Ewing sarcoma	237–239 and 274
CX-5461 (Pidnarulex)	G4 DNA	Stabilises G4 structures by π - π stacking on G-quartets, preferentially binding parallel promoter and telomeric G4s (e.g., <i>c-MYC</i> and <i>c-KIT</i>); traps Topo II, induces replication stress, R-loops & DNA damage; synthetic lethality in <i>BRCA1/2</i> - and HR-deficient cancers	Phase I/II trials (NCT04890613); FDA fast track designation	Small-molecule	Breast, ovarian and other <i>BRCA1/2</i> - or HR-deficient cancers	259

Abbreviations: Pt, platinum; TBP, TATA-binding protein; NF-Y, nuclear transcription factor Y; RPA, replication protein A; HMGB1, high-mobility group box 1; p53, tumour suppressor protein p53; Bax, *BCL-2*-associated X protein; *c-MYC*, cellular myelocytomatosis gene; MutS α , DNA mismatch repair complex MutS alpha; DACH, diamincyclohexane; bp, base pairs; G-4, G-quadruplex; Topo II, topoisomerase II; DSB, double-strand break; AML, acute myeloid leukaemia; SCLC, small-cell lung cancer; TF, transcription factor.

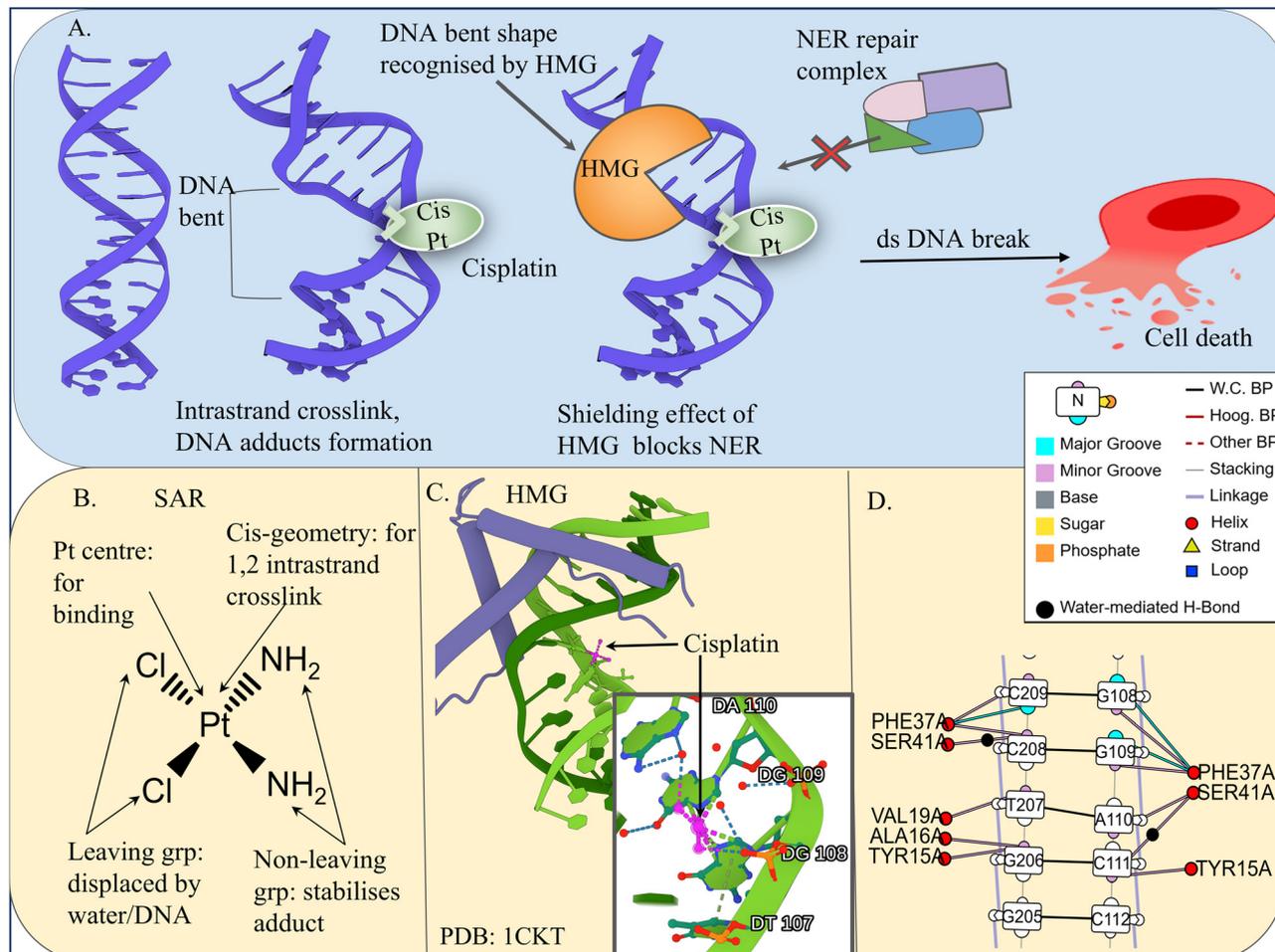


Fig. 4 Cisplatin–DNA interactions and structural basis of recognition. (A) Mechanism of action of cisplatin illustrating the formation of intra-crosslinks at the adjacent guanine position, and induction of pronounced DNA bending. The distorted DNA is preferentially recognised and shielded by HMG-domain proteins, thereby limiting access of the nucleotide excision repair (NER) machinery, promoting replication-associated DNA double-strand breaks (DSBs) and activation of apoptotic pathways. (B) Structure–activity relationship (SAR) of cisplatin highlighting the importance of the platinum centre, leaving groups, and coordination geometry for efficient DNA binding and cytotoxic activity. (C) Crystal structure of cisplatin bound to DNA (PDB: 1CKT) showing coordination to adjacent guanine bases dG108 and dG109, with additional stabilising interactions involving dA110 and dT107.⁶³ (D) NUCPLLOT representation of the cisplatin–DNA complex highlighting key contacts involving Tyr15, Ala16, Val19, Phe37, and Ser41. Visualisations generated via Mol*,⁶⁸ DNAProDB⁹³ and Web 3DNA 2.0.²²³ MarvinSketch was used for drawing chemical structures, Chemaxon (<https://www.chemaxon.com>).

generation platinum-based drugs, such as Pt nanocluster (NC)-based nanodrugs, are also being investigated.²²²

Apart from platinum drugs, there are also natural product alkylating agents that covalently bind to the DNA minor groove, such as trabectedin, originally isolated from the marine tunicate *Ecteinascidia turbinata* and now produced synthetically. It is FDA-approved for the treatment of soft-tissue sarcoma, typically as a second-line therapy after doxorubicin failure. Trabectedin disrupts transcription by inhibiting transactivated TFs (such as Sp1), while also impairing replication and the NER pathway. Homologous recombination-deficient cells are particularly sensitive to trabectedin. In addition to sarcomas, it is under investigation for Ewing sarcoma, myxoid liposarcoma, and in various combination regimens, including immunotherapies.²²⁴

Another set of alkylating agents, called nitrogen mustards, also target DNA and are widely used in anticancer therapy.⁴⁷

However, their disruption of PDIs is generally less selective than that observed with platinum-based drugs, though evidence exists for specific effects, such as inhibition of Sp1 binding sites.²²⁵ Melphalan is a classical nitrogen mustard, consisting of an *l*-phenylalanine moiety linked to a bis(2-chloroethyl)amine group (the “mustard” group, which generates highly reactive aziridinium ions). It is highly reactive and has relatively low selectivity for DNA, as it also reacts with other nucleophiles in the cell such as proteins, leading to DNA–protein crosslinks (DPCs), drug resistance, and genotoxicity.^{226,227} In contrast, bendamustine is a non-classical nitrogen mustard, incorporating a benzimidazole ring that provides both alkylating activity (*via* the mustard group) and antimetabolite-like properties (mimicking purine bases).²²⁸ It is used in the treatment of non-Hodgkin’s lymphoma and multiple myeloma.²²⁹

Most small-molecule DNA-binding drugs preferentially target the minor groove, which is narrower and better suited to

their small sizes, allowing for strong van der Waals interactions. Major groove binders, mostly intercalators or alkylating agents, also exist, with very few non-covalent binders. Some metal-based small compounds, such as those of platinum, chromium, or rhodium, show this property. Non-covalent minor groove binders can prefer either AT- or GC-rich sites. The minor groove binding drugs include netropsin and distamycin.¹⁵⁵ They are pyrrole antibiotics that insert into the minor groove and form hydrogen bonds with donor atoms on adenine (A) or thymine (T) bases.²³⁰ These drugs are known to target TFs, such as E2F1/E2F4 and TBP (part of TFIID complex).⁴⁷ Though they themselves are not very good therapeutics because of binding, toxicity, or specificity issues, they have been the basis for designing many anti-cancer drugs as lead compounds.²³⁰

Dervan's polyamides are prime examples of improved sequence-selective minor groove binders based on these. Built from pyrrole-imidazole units, they adopt hairpin-like structures that allow them to recognise predetermined DNA sequences, following Dervan's pairing rules, where Im/Py recognises G-C, Py/Im recognises C-G, and Py/Py recognises both A-T and T-A base pairs.^{231,232} They have been used to disrupt transcription factor-DNA interactions, such as ER α binding and HIF-responsive elements; in principle, polyamide-peptide conjugates could function as artificial TFs capable of upregulating transcription.²³³ Although still in preclinical stages, they remain one of the most promising approaches for achieving programmable, sequence-specific targeting of DNA.²³⁴

Hoechst 33258 is another minor groove binder,^{47,155} which is used as a dye for staining DNA and used for fluorescent microscopy, flow cytometry, *etc.*²³⁵

In the case of FDA-approved drugs, mithramycin (also called plicamycin) is a natural product small-molecule inhibitor of TFs such as EWS-FLI1. Earlier, it was used for treating various types of cancers and Paget's disease. It has also been shown to prevent neural cell death in p53-mediated apoptosis by providing neuroprotection, but due to its hepatotoxicity, nephrotoxicity, and bone marrow toxicity, it was discontinued.²³⁶ However, it has now seen a resurgence in research and is being used in the therapy of Ewing sarcoma. Unlike most DNA-binding agents, mithramycin acts by directly targeting TFs. It binds in the minor groove of DNA as a Mg²⁺-coordinated dimer, recognising GC-rich sequences through a two-fold symmetry that complements the DNA site.²³⁷ In addition, it serves as a selective Sp1 inhibitor, where it competes for binding at GC-rich motifs, including those within *c-MYC* promoters.²³⁸ Studies have also explored mithramycin analogues (*e.g.*, mithplatin and EC-8042) for overcoming cisplatin resistance. A unique property of mithramycin is its ability to bind non-palindromic GC sequences, which distinguishes it from many other DNA-binding drugs.^{239,240}

Intercalation is a form of non-covalent binding where a flat, aromatic small-molecule inserts itself between the stacked base pairs of the DNA helix. The DNA becomes distorted as a result of this physical insertion, which unwinds the helix (by causing distortions in the sugar-phosphate backbone) and lengthens it

(as the drug inserts between bases and increases the spacing). Intercalation also sterically prevents proteins, such as TFs, from binding DNA, following the nearest-neighbour exclusion principle. Similar effects are seen in replication, and many intercalators poison topoisomerase enzymes, causing DNA breakage.²⁴¹ The unwinding effect is also not limited to that particular base pair, but the torsional strain can extend further along the DNA. The DNA-binding of intercalators is also strongly influenced by π - π stacking interactions between the drug's aromatic rings and the nucleotide base pairs.²⁴²

Actinomycin D (dactinomycin) is the first FDA-approved natural DNA intercalator drug, obtained from *Streptomyces*. Actinomycin D blocks the interaction of RNA polymerase with DNA, thus inhibiting transcription, particularly by halting ribosomal RNA synthesis. Unlike most intercalators, which are cationic (to neutralise DNA's negative charge and reduce repulsion), actinomycin D is largely neutral but possesses a dipole moment. It intercalates in GC-rich regions of the DNA minor groove and is mainly used to treat pediatric tumours, such as Wilms' tumour and rhabdomyosarcoma.²⁴³ It also inhibits TFs like Sp1 and Sox2, preventing their binding to DNA.²⁴⁴ In a different mechanism, it stabilises G4 DNA in oncogene promoters like *c-MYC*, blocking transcription, and reduces mRNA synthesis, effectively "turning off" the oncogene.²⁴⁵

Doxorubicin and daunorubicin are Topo II inhibitors of the anthracycline drug class. They intercalate into DNA and poison Topo II by trapping the enzyme-DNA cleavage complex, causing DSBs. These drugs also impact TFs; for example, they inhibit HIF-1, NF- κ B, Sp1, E2F1, and others by blocking their DNA-binding and suppressing gene expression. Doxorubicin is used in breast cancer, sarcoma, and lymphoma therapies, while daunorubicin is used in leukaemias. Their clinical use is limited by cardiotoxicity, among other toxicities. Most clinical intercalators are mono-intercalators (a single aromatic system inserts between bases). In contrast, bis-intercalators like echinomycin have two rings that intercalate at two distinct sites on the DNA. Echinomycin preferentially binds to CpG steps and inhibits transcription. It has been studied as a potential treatment for acute myeloid leukaemia (AML), but toxicity has limited its development.⁴⁷ For bis-intercalators, the nearest-neighbour exclusion principle does not apply, as they can occupy two sites at once.²⁴²

Nogalamycin is another anthracycline-related antibiotic. It uses a "threading intercalation" mechanism, interacting with both the major and minor grooves;²⁴⁶ however, due to severe toxicity, it never advanced beyond trials.²⁴⁷

Amsacrine, though not FDA-approved in the US, is approved in Europe for AML and Hodgkin's and non-Hodgkin's lymphomas. It stabilises the Topo II cleavage complex, causing DSBs.²⁴⁸ Combination therapy with cytarabine has proven effective in AML, especially for patients with cardiac problems, but recent advances focus on improving tolerability and efficacy.²⁴⁹

Voreloxin, a quinolone analogue, functions as a novel intercalator that stabilises G4 structures and selectively induces

damage in GC/GG sequences, demonstrating efficacy against AML and solid tumours. It remains in clinical trials, demonstrating sequence-selective cleavage and dual stabilisation of *c-MYC* and *BCL-2* G4s.^{250–252}

In contrast to other drugs, bleomycin chelates metal ions, primarily Fe(II); and when activated by oxygen, it releases free radicals that break DNA strands instead of merely intercalating.²⁵³ Cell cycle arrest and apoptosis result from the single- and double-strand breaks caused by this DNA cleavage.²⁵⁴ It has FDA approval for the treatment of squamous cell carcinomas, testicular cancer, and Hodgkin's lymphoma, among other conditions.²⁵⁵ Its efficacy can be enhanced when combined with Ku–DNA binding inhibitors of DNA-PK, which impair the NHEJ pathway essential for repair of bleomycin-induced DSBs.²⁵⁶ Therefore, while bleomycin does not directly interfere with protein–DNA binding, its DNA-cleaving action can lead to indirect effects on these interactions.

Beyond classical intercalators and groove binders, of DNA, non-canonical DNA structures like G4s are emerging targets for anticancer drugs. Camptothecin and indenoisoquinolines, though mainly topoisomerase inhibitors, can also interact with G4s. Camptothecin works synergistically with G4 stabilisers by blocking Topo I-mediated repair, whereas indenoisoquinolines can independently stabilise G4s, such as the *c-MYC* promoter G4, to suppress oncogene expression.^{257,258}

Several drugs are in development that directly target G4 structures, and CX-5461 (pidnarulex) is a prominent example. Initially characterised as an RNA polymerase I inhibitor, CX-5461 is now known to stabilise G4 DNA and trap Topo II. It exploits synthetic lethality in *BRCA1/2*-deficient cancers, including tumours resistant to PARP inhibition; its clinical development has been accelerated through FDA Fast Track designation, with early-phase trials in breast and ovarian cancers (see Table 4).²⁵⁹ Concerns regarding its safety arose from an *in vitro* study reporting extensive collateral mutagenesis in cell lines,²⁶⁰ however, subsequent analyses of clinical samples suggested that these effects may not translate to humans.²⁶¹ Another G4-targeting compound, QN-302, is in early clinical trials for advanced or metastatic solid tumours.²⁶²

Apart from G4s, other non-canonical DNA structures are also being explored as drug targets. Actinomycin D represents a classical model for hairpin-interactive compounds, preferentially binding GC sites within hairpin stems. Its binding affinity is influenced by loop composition and internal mismatches, leading to stabilisation of pathogenic trinucleotide-repeat hairpins.²⁶³ More recently, azacryptand ligands, such as tris-acridine and tris-naphthalene derivatives, have demonstrated the ability to bind imperfect DNA hairpins and other folded DNA junctions, supporting a multitargeting strategy that exploits structurally related non-B DNA conformations.²⁶⁴ In parallel, Z-DNA has emerged as an immunologically relevant target. Z-DNA-binding molecules can enhance anti-tumour immune responses and improve the efficacy of anti-PD-1 immunotherapy by unmasking ZBP1-mediated necroptotic signalling normally suppressed by ADAR1.²⁶⁵ In addition to targeting proteins or DNA separately, drugs can also act at the

interface between them, directly modulating protein–DNA complexes. This approach is discussed in the next section.

3.3 Targeting the protein–DNA complex

Drugs targeting DNA–protein complexes act at the bound interface between protein and DNA and are often called interfacial inhibitors.⁴⁵ This strategy addresses the problem of “flat”, otherwise undruggable protein surfaces, since the interface of two molecules creates a pocket for drug binding. The transient complex can be efficiently stabilised by drug binding, making the interaction thermodynamically favourable.²⁷⁵ The clinical applications and specific targets of these inhibitors are summarised in Table 5.

Prominent examples include etoposide and mitoxantrone (Topo II–DNA complex poisons), indenoisoquinolines, and camptothecin (Topo I–DNA complex poisons).⁴⁵ Other agents sometimes discussed in this context are amsacrine and dexrazoxane.

Etoposide traps the Topo II–DNA cleavage complex and stabilises it by blocking DNA religation. Normally, Topo II introduces transient double-strand cleavages to relieve supercoiling. At therapeutic doses, it primarily produces SSBs as only one of the two catalytic sites within the Topo II dimer is trapped (Fig. 5).²⁷⁷ These SSBs remain covalently bound to the enzyme and act as obstacles on the DNA template. When replication or transcription machinery collides with these complexes, they are converted into DSBs or cause fork collapse, which drive cell death. Etoposide targets both Topo II α and Topo II β . Clinically, it is utilised in small-cell lung cancer (SCLC), testicular cancer, soft tissue sarcoma, and other solid tumours. Its main dose-limiting toxicity is bone marrow suppression.⁴⁵

Mitoxantrone acts as both a DNA intercalator and an interfacial inhibitor, stabilising the Topo II–DNA cleavage complex.^{45,258,278} Unlike anthracyclines such as doxorubicin, mitoxantrone undergoes less efficient redox cycling, generating fewer free radicals. Since anthracycline cardiotoxicity is strongly linked to free radical-mediated mitochondrial damage in the heart, mitoxantrone shows reduced cardiotoxicity in comparison. However, it can still cause dose-dependent cardiac toxicity, mainly through Topo II β poisoning in cardiomyocytes.^{48,258,278,279} Clinically, it is used in AML, acute promyelocytic leukaemia (APL), and multiple sclerosis.²⁷⁸

Camptothecin is a natural product inhibitor that traps the Topo I–DNA cleavage complex, preventing religation of single-strand breaks. This leads to replication fork collapse, S-phase toxicity, and apoptosis. Camptothecin is highly specific, as its only target is Topo I, and shows dose-dependent toxicity. It also halts DNA synthesis. Clinically utilised derivatives include irinotecan and topotecan. Resistance can arise from mutations in Topo I or altered drug efflux.²⁵⁸

Indenoisoquinolines are synthetic non-camptothecin Topo I inhibitors stabilising the Topo I–DNA cleavage complex. They show improved chemical stability and can overcome certain resistance mechanisms associated with camptothecins. Other interfacial inhibitors include raltegravir, elvitegravir, and

Table 5 Drugs binding to DNA–protein complexes

Drug	Target	Mechanism of action	Approval status	Drug class	Therapeutic area	Ref.
Etoposide	Topo II α -DNA complex	Stabilises cleavage complex, preventing religation, leading to persistent DSBs during replication	FDA approved	Podophylotoxin derivative, small-molecule	Leukaemia, lymphoma, testicular/lung cancer	45
Camptothecin	Topo I–DNA complex	Stabilises cleavage complex, prevents religation leading to replication fork collapse and DSBs	FDA approved	Plant alkaloid, small molecule	Colorectal, ovarian, lung cancers	45 and 258
Indeno-isoquinolines	Topo I–DNA complex	Stabilises the complex and inhibits the religation reaction of DNA	Phase I trials (NCT03030417)	Small-molecule	Solid tumours	45 and 283
Mitoxantrone	Topo II α -DNA complex (also intercalates DNA directly)	Stabilises the cleavage complex, blocking replication and repair	FDA approved	Anthracenedione, small molecule	AML, prostate cancer, breast cancer, multiple sclerosis	45 and 278

Abbreviations: Topo I, topoisomerase I; Topo II α , topoisomerase II alpha; DSB, double-strand break; AML, acute myeloid leukaemia

dolutegravir, which stabilise the HIV integrase–DNA complex, representing microbial applications of this principle.^{45,280}

Some drugs, such as doxorubicin and trabectedin, though primarily classified as DNA intercalators or groove binders, can also act partly as interfacial inhibitors.⁴⁵ However, as their dominant mechanism is DNA-binding, they are discussed in the preceding section.

Beyond classical small-molecule inhibitors, nanomaterials such as graphene quantum dots have been reported to act as interfacial inhibitors of DNA–protein complexes, including dual inhibition of Topo I and Topo II, with potential to overcome multidrug resistance,²⁸¹ as discussed in the Section 5.

Apart from the topoisomerase poisons, another type of inhibitor (Pol θ i) targeting the protein–DNA complex is being explored. It traps the Pol θ –Pol–DNA complex in a closed conformation by adding a nucleotide to the primer, thereby blocking the complex transition to the open conformation required for DNA repair. The inhibitor fits into the closed conformation pocket and is stabilised by hydrogen bonding, π -stacking, and hydrophobic interactions. Blocking the N (finger) and O subdomains prevents the polymerase from progressing and renders the complex temporarily resistant to endonuclease cleavage.²⁸²

4 Methodological overview: AI and computational approaches in protein–DNA modulator discovery

Rational drug design, supported by computer-aided drug design (CADD) and artificial intelligence (AI), has significantly advanced the discovery of modulators targeting PDIs.²⁸⁴ Molecules identified through AI approaches have reportedly shown higher success rates (up to 80–90%) in early clinical trials compared to conventional candidates.²⁸⁵ Traditional approaches such as structure-based drug design (SBDD) and fragment-based drug design (FBDD), when integrated with molecular modelling, binding site prediction, binding energy estimation and virtual screening, have accelerated the path to the identification of selective and potent inhibitors.^{284,286} Target identification and prediction represent the first step in drug discovery and have seen a wider application of AI. The integration of multi-omics data and network biology has facilitated the identification of therapeutic targets in complex diseases such as cancer.²⁸⁷ For example, structural modelling of TFs such as c-Myc, facilitated by AlphaFold and refined through molecular dynamics (MD) simulations revealed a druggable conformational switch within its transactivation domain.²⁸⁸ AI-based structural prediction models, including AlphaFold3,²⁸⁹ AlphaFold-Multimer,²⁹⁰ RoseTTAFoldNA,²⁹¹ and RoseTTAFold All-atom,²⁹² have enabled accurate prediction of novel DNA-binding proteins and protein–DNA complexes.

Successful targeting requires detailed information on the binding site. In this direction, both structure-based and sequence-based binding site predictors have been developed. Although the earlier methods were constrained by limited

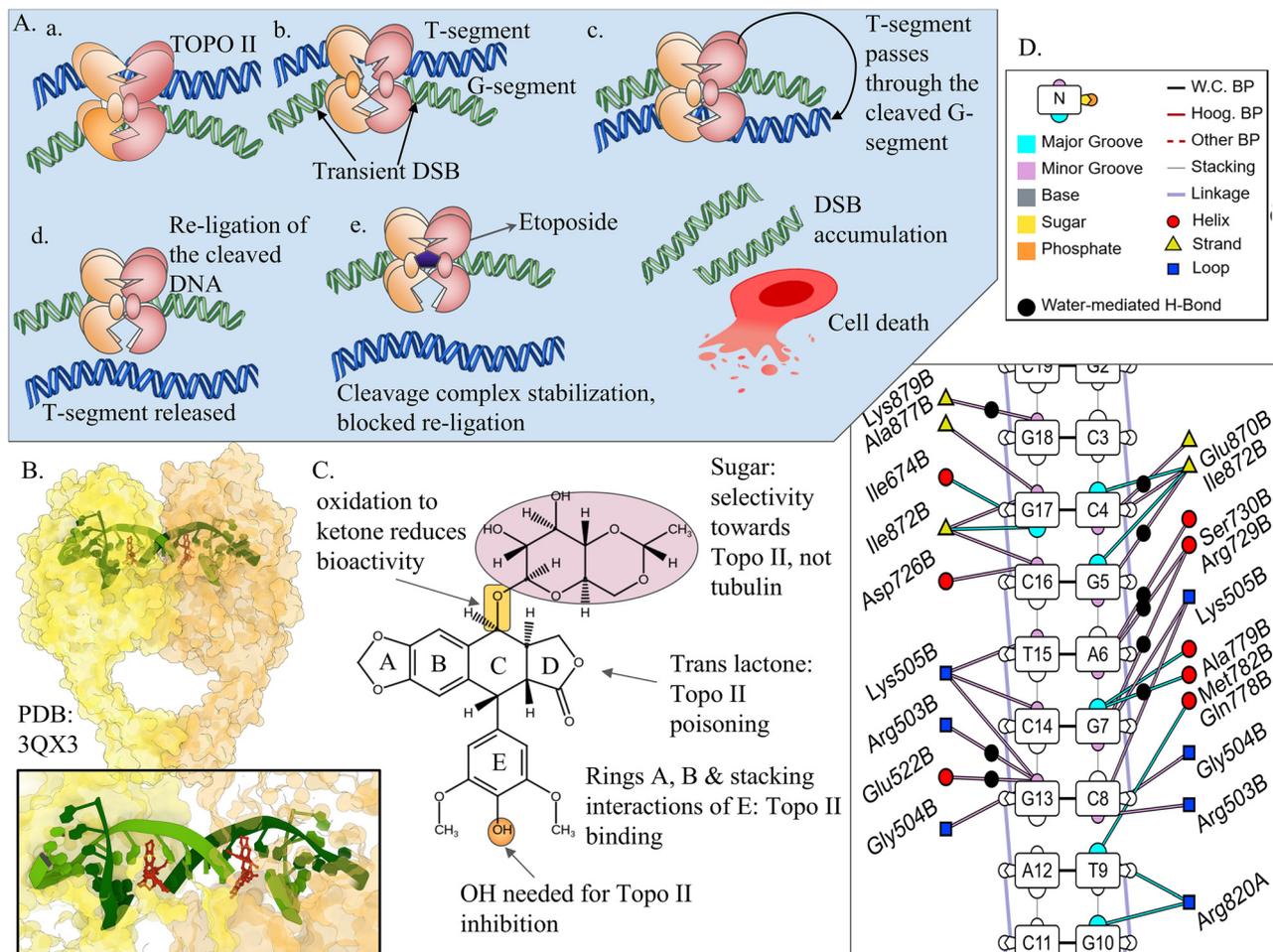


Fig. 5 Mechanistic and structural basis of topoisomerase II (Topo II) inhibition by etoposide. (A) Mechanism of action of etoposide showing stabilisation of the transient Topo II–DNA cleavage complex. During the catalytic cycle, Topo II introduces a double-strand break in the gate (G) segment DNA to permit passage of the transported (T) segment. Etoposide intercalates at the protein–DNA interface of the cleaved G segment, inhibits DNA religation, and traps the cleavage complex, leading to accumulation of DNA double-strand breaks, replication stress, and apoptosis. (B) Structure–activity relationship (SAR) of etoposide. (C) Crystal structure of the human Topo II β –DNA–etoposide complex (PDB: 3QX3) illustrating the overall surface geometry of the enzyme and the binding of two etoposide molecules at the protein–DNA interface within the cleavage complex.²⁷⁶ (D) NUCPLOTT representation of the Topo II β –DNA–etoposide complex highlighting representative protein–DNA and protein–drug interactions that stabilise the trapped cleavage intermediate. Visualisations generated via Mol*,⁶⁸ DNAProDB,⁹³ MarvinSketch for chemical structures, and Chemaxon (<https://www.chemaxon.com>).

structural data and biological complexity,^{293–296} recent AI advances have led to powerful binding site predictors such as ATMGBs, which combines protein language models and graph convolutional networks;²⁹⁷ DeepPBS, a geometric deep learning model predicting binding specificity from protein–DNA structures;¹⁴ and TransBind, an alignment-free deep learning framework that predicts DNA-binding proteins and residues directly from sequences.²⁹⁸ Tools and databases such as JASPAR,⁹¹ AIModules,²⁹⁹ MethMotif⁹⁰ and MEME Suite³⁰⁰ support motif identification, providing curated TF binding profiles and position weight matrices essential for motif discovery.

The use of curated chemical and structural databases, combined with machine learning (ML) and deep learning (DL) algorithms, enables efficient exploration of chemical space and prioritisation of candidate molecules. Molecular docking tools such as HADDOCK,³⁰¹ NPdock,³⁰² and MD simulations

further refine predicted protein–DNA complex structures, providing insights into binding stability and interaction mechanisms.³⁰³ These approaches have been applied to Gli1/DNA interactions as a druggable target for Hedgehog-dependent tumours,³⁰⁴ *in silico* modelling and screening of FOXO TF inhibitors,³⁰⁵ and STAT3 inhibition in cancer therapeutics.³ AI-enhanced MD simulations, incorporating ML force fields, improve the accuracy of studying dynamic PDIs.^{306,307} From the DNA side, Dictionary-based approaches (DNA shape and Dynaseq) hold great promise through their modelling of large-scale DNA shape and flexibility patterns, revealing conformational signatures crucial for drug binding.^{308,309} DL methods, such as Deep DNashape, predict DNA structure.³¹⁰ High-throughput screening (HTS)³¹¹ and DNA-encoded libraries (DELs)³¹² accelerate the identification of specific inhibitors, including for challenging targets like Myc

in cancer,¹⁴⁰ while AI-driven virtual screening platforms improve compound selection and druggability assessments.^{313,314} Chemical databases such as ChEMBL,³¹⁵ ZINC,³¹⁶ BindingDB,³¹⁷ and DrugBank³¹⁸ provide essential chemical and pharmacological data. Natural language processing (NLP) models extracting drug–target relationships from FDA drug labels further enhance regulatory assessment and drug development.³¹⁹ QSAR modelling, enhanced by ML, remains vital for predicting biological activity and optimising drug candidates, streamlining drug design effectively.³²⁰ Generative AI frameworks, including autoencoders, graph neural networks (GNNs), and reinforcement learning (RL) models, enable *de novo* molecule generation, opening new frontiers in drug discovery by designing novel compounds with optimised properties.^{321,322} Additionally, ALOPTAMER is an AI-driven pipeline that accelerates aptamer optimisation by integrating AI screening, structural modeling, deep learning scoring, and MD simulations, enhancing aptamer discovery.³²³ Beyond drug design, AI and ML-based frameworks have also advanced predictive oncology, enabling precise prediction of therapeutic response and treatment outcome.³²⁴ A recent pan-cancer, pan-treatment model further demonstrated that patient-derived xenograft-driven AI systems can accurately forecast drug responses across cancer types, accelerating preclinical testing and precision drug development.³²⁵

While various strategies to target protein–DNA interactions show promise, they face broader challenges related to selectivity, efficacy, and practical application, which are discussed in the next section.

5 Challenges and future perspectives

Developing drugs for PDI targets is made complicated by two intersecting challenges: the biological complexity inherent to protein–DNA recognition and interaction surfaces, and technological limitations that restrict our understanding and experimental capacity.^{11,326,327} Many TFs, for instance, lack well-defined ligand-binding pockets and have historically been considered challenging to target.^{46,328} Many TFs are intrinsically disordered proteins (IDPs), such as c-Myc with its disordered TAD. As for IDPs, they are highly flexible and dynamic, lacking fixed 3D structures under normal physiological conditions and instead adopting multiple conformations. Their sequences are typically rich in polar residues and depleted of hydrophobic ones, leading to structural instability and a lack of rigid binding sites. Consequently, small-molecule inhibitors that can target them efficiently and specifically are scarce, since these compounds are known to typically bind to stable hydrophobic pockets; the lack of such pockets results in only low-affinity interactions. For example, the p53 binding site for MDM2 lies within a disordered region, making direct inhibition extremely challenging.³²⁶ There are attempts at targeting conserved genomic TAD regions belonging to specific targets of TF families, where specificity of binding remains the primary challenge. Even protein-based inhibitors designed to mimic or

disrupt these contacts often suffer from rapid degradation and limited stability *in vivo*.³²⁹

The intricate nature of PDIs also makes CADD difficult. The key computational technique of MD simulations, hugely successful in other systems, becomes challenging for TFs in PDIs due to their high flexibility and absence of stable structures. Classical force fields often fail to capture IDP behaviour accurately, prompting the use of coarse-grained models or enhanced sampling techniques to improve prediction. One would expect that some of these problems can be solved by using DNA sequences they recognise, instead of new small molecules. However, DNA fragments recognised by TFs are small and this approach may cause off-target effects due to a lack of precision and redundancy of similar DNA sites in the genome. Additionally, there remains a fundamental lack of understanding regarding the binding patterns and mechanisms of these proteins. Developing effective drugs depends critically on identifying their small functional sites and linear motifs. Technologies such as in-cell NMR and AI-driven structure prediction can ensure that compounds bind specifically to their intended targets while avoiding off-target effects.^{326,330}

Proteins involved in DNA repair and replication present their own set of therapeutic challenges. Many DNA repair proteins have broad, flat surfaces, which makes them hard to target. Many of these proteins, such as RAD51, RAD52, and Ku70/80, have large and relatively flat DNA-binding surfaces that are difficult to block selectively. PARP inhibitors, though successful, face resistance through restoration of HR or loss of PARP trapping efficiency.^{7,331} Synthetic lethality remains a powerful concept, yet maintaining its clinical effectiveness depends on identifying new pathway partners and rational drug combinations. High-throughput CRISPR–Cas9 screening has enabled the identification of novel synthetic lethal interactions outside the *BRCA*-mutant contexts, such as WRN inhibitors in MSI-H tumours or SMARCA2 degraders in *SMARCA4*-mutant cancers, providing avenues to circumvent PARP resistance and expand therapeutic options.³³² Similarly, inhibitors of DNA-PKcs face challenges related to kinase selectivity and toxicity, since the ATP-binding pocket is highly conserved across the PI3K family. The clinical failure of agents like AZD7648 highlights the unresolved balance between potency, specificity, and safety in targeting DNA repair enzymes.³³³

Drug resistance is another major phenomenon that complicates therapeutic targeting of PDIs. Defects in the MMR or NER pathways can alter responses to DNA-damaging agents such as cisplatin, temozolomide, and etoposide. Overexpression of repair proteins, such as ERCC1 and MGMT, or mutations in *MSH2* and *MLH1*, contributes to drug resistance by either enhancing lesion removal or impairing DNA damage signalling.^{334–336} The dynamic crosstalk between repair pathways means that blocking one mechanism often triggers compensatory mechanisms, highlighting the need for rationally designed drug combinations.³³⁷

Another inherent challenge in targeting PDIs for drug discovery arises from the limited chemical diversity of DNA itself. DNA is composed of only four bases, yet it encodes thousands

of genes, making it challenging to design drugs that selectively target a specific sequence without affecting others. While TFs typically recognise the major groove, small molecules often bind in the minor groove because it provides a snug, shape-complementary site for interaction. In contrast, the major groove is wide, relatively flat, and solvent-exposed, making selective binding of small molecules more difficult.^{155,234}

DNA intercalators remain potent but nonspecific. Bis-intercalators may increase DNA-binding strength but often at the cost of enhanced toxicity and off-target effects.⁴⁷ Similarly, interfacial inhibitors such as topoisomerase poisons still face limitations in selectivity. For example, lack of isoform-selective inhibitors, particularly those distinguishing Topo II α from Topo II β , results in unwanted side effects such as cardiotoxicity and bone marrow suppression.⁴⁸

Therefore, enhancing specificity, reducing toxicity, and addressing drug resistance remain key future directions that require substantial scientific effort.²⁰⁹ Solving these challenges depends on precise characterisation of how small molecules interact with DNA. High-resolution analytical platforms, such as single-molecule nanopore sensing, can detect and quantify subtle DNA structural differences at single-base-pair resolution, including canonical and non-canonical hydrogen-bonding states, as well as transient interactions between small molecules and DNA bases.^{338,339}

Combination therapy has shown progress in combating drug resistance. For example, co-inhibition of DOT1L and menin has demonstrated synergistic effects in *MLL*-rearranged leukaemias,²⁰⁹ while pairing topoisomerase inhibitors with TDP1 or TDP2 blockers may enhance the persistence of DNA damage in tumour cells.⁴⁸ Advanced drug delivery systems offer another solution. Nanoparticle-encapsulated inhibitors improve tumour targeting and reduce systemic side effects.⁴⁸ For example, graphene quantum dots (GQDs) enhance drug targeting, improve cellular uptake, and modulate PDIs through π - π stacking and electrostatic interactions. They can self-insert into the DNA major groove sites, enhancing interfacial inhibition of TFs and downregulating cancer stem cell genes, thereby overcoming multidrug resistance. However, optimisation of specificity and biocompatibility is still required.^{281,340} Liposomal delivery also improves drug safety, bioavailability, and efficacy, as exemplified by lipoplatin, but challenges remain in formulation, stability, and *in vitro*-*in vivo* translation. Multimodal liposomes and liposome-nanoparticle hybrids offer opportunities for the co-delivery of cytotoxic drugs and resistance pathway inhibitors, with potential for targeted and externally triggered release.³⁴¹

Drug repurposing offers a faster and less risky path to discovering PDI modulators. For example, eltrombopag, an FDA-approved drug for chronic immune thrombocytopenia, has recently been shown to inhibit transcription factor EB (TFEB), enhancing apoptosis inhibition and sensitising tumours to chemo- or radiotherapy.³⁴² Pentamidine, originally FDA-approved for protozoal infections (*e.g.*, PCP, trypanosomiasis, leishmaniasis),³⁴³ has been repurposed in cancer therapy.³⁴⁴ It binds to AT-rich regions in the DNA minor groove,

thereby interfering with TF-DNA interactions (*e.g.*, STAT3, SOX-2, and CDK4). Studies also show that pentamidine targets the suppression of HIF-1 α protein translation and inhibits the interaction between S100P and p53, through protein binding. While it shows activity in prostate and brain cancers and is being tested in combination therapies, challenges such as low bioavailability, delivery issues, and toxicity remain.³⁴⁴

Chemical biology tools, such as PROTACs, decoy PROTACs (D-PROTACs), molecular glues, and molecular glue degrader-antibody conjugates (MACs), offer indirect strategies to degrade, stabilise, or mislocalise PDIs, overcoming the limitations of classical inhibitors. While PROTACs induce targeted protein degradation *via* the ubiquitin-proteasome system,³⁴⁵ D-PROTACs further extend this strategy by fusing DNA decoys with E3 ligase ligands, enabling sequence-specific recruitment of DNA-binding proteins like STAT3 for targeted degradation and antitumour activity.³⁴⁶ Molecular glues complement these strategies by promoting proximity between E3 ligases and target proteins. Classical examples include thalidomide derivatives that recruit CRBN to degrade IKZF1 and IKZF3, while newer glues such as DKY709 and Helios CELMoD (targeting IKZF2) and WIZ modulators expand this approach to additional TFs.³⁴⁷ Furthermore, c-Myc-degrading MACs have recently demonstrated targeted degradation of the otherwise “undruggable” c-Myc *via* CRBN recruitment, achieving selective antitumour activity in PSMA-positive prostate cancer models.³⁴⁸ Though there are several limitations of traditional PROTACs, such as solubility, permeability and off-target issues, this has now been addressed using unconventional PROTACs.³⁴⁹

Recent advances in AI-driven structural biology have greatly enhanced our understanding of PDIs and their potential in drug design.^{290,291,350-352} Despite their promise, these AI-based tools have limitations: motif-specific recognition, non-canonical interactions, and highly dynamic regions can still be mispredicted. Integrating experimental techniques such as ChIP-seq, pull-down assays, or motif discovery algorithms like MEME is essential to refine predicted binding sites.³⁵² Data bias issues, limited representation of disordered/transient complexes and the “black box” of deep learning frameworks limit biological interpretability, suggesting a need for explainable AI (XAI).^{353,354}

Translating the computational findings into clinically viable drugs faces traditional hurdles, including poor stability, bioavailability and efficacy. Data integration across platforms is limited by heterogeneity, computational limitations, and biological interpretability. There is a need for a clear framework to facilitate the transition from *in silico* findings to *in vivo* applications, ultimately leading to the development of actual therapeutic drugs in real-life settings.³⁵⁵

Moving forward, a multidisciplinary approach combining structural biology, AI-driven modelling and predictions, chemical biology, nanotechnology, and multi-omics studies will be crucial for rationally designing drugs that precisely target PDIs. At the same time, careful evaluation of safety, specificity, and *in vivo* efficacy will be crucial for developing effective and clinically relevant therapies.

Conclusion

Transcription, replication, DNA repair, and chromatin regulation are key biological processes mediated by PDIs and provide important opportunities for drug targeting. PDIs have traditionally been considered “undruggable” due to the lack of conventional binding pockets; however a diverse range of targeting strategies have been developed to modulate these interactions. Success stories are evident in FDA-approved drugs, compounds currently under clinical investigation, and emerging modalities such as PROTACs. Targeting approaches span from small molecules that bind directly to DNA or proteins, to interfacial inhibitors that trap the protein–DNA interface in complex. In cases where direct inhibition is unfeasible, such as for histones or architectural proteins, indirect chemical biology strategies are increasingly being explored.

This review also highlights key challenges, including selectivity, off-target effects, and drug resistance, that need to be overcome to realise the full potential of PDI targeting. New technologies including AI-based structural predictions, advanced drug delivery systems (e.g., nanoparticles and liposomes), and combination therapies are helping to address these limitations. Rational drug design, guided by structural biology, chemical biology, and computational modelling, will be critical for developing precise, effective, and clinically viable outcomes in this direction.

Author contributions

This article was drafted by RM under the overall guidance of SA. Literature survey and data compilation were done by RM, whereas SA contributed to the overall structure, discussions and editing the draft versions. Both authors read and approved the final version.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Acknowledgements

This work was supported by DBT grants (BIC to JNU, NNP to IIITD, and NNP to CSIR-NEIST) and an ICMR grant on MTB drug-resistance to SA. This was also supported by a Junior Research fellowship to RM from UGC-India.

References

- R. A. C. Ferraz, A. L. G. Lopes, J. A. F. da Silva, D. F. V. Moreira, M. J. N. Ferreira and S. V. de Almeida Coimbra, *Plant Methods*, 2021, **17**, 82.
- A. Alibes, A. D. Nadra, F. De Masi, M. L. Bulyk, L. Serrano and F. Stricher, *Nucleic Acids Res.*, 2010, **38**, 7422–7431.
- M. Radaeva, A.-T. Ton, M. Hsing, F. Ban and A. Cherkasov, *Drug Discovery Today*, 2021, **26**, 2660–2679.
- C. V. Dang, *Cell*, 2012, **149**, 22–35.
- Y. Shiroma, R.-u Takahashi, Y. Yamamoto and H. Tahara, *Cancer Sci.*, 2020, **111**, 1058–1064.
- M. W. Gonzalez and M. G. Kann, *PLoS Comput. Biol.*, 2012, **8**, 1–11.
- S. R. Hengel, M. A. Spies and M. Spies, *Cell Chem. Biol.*, 2017, **24**, 1101–1119.
- X. Li and W.-D. Heyer, *Cell Res.*, 2008, **18**, 99–113.
- C. Qu and H. Du, *Crop Design*, 2025, **4**, 100082.
- H. Mousavi and R. Emami, *Sci. Rep.*, 2025, **15**, 30565.
- A. A. Anashkina, *Biophys. Rev.*, 2023, **15**, 1007–1014.
- R. Nagarajan, S. Ahmad and M. Michael Gromiha, *Nucleic Acids Res.*, 2013, **41**, 7606–7614.
- Z. Liu, W.-R. Qiu, Y. Liu, H. Yan, W. Pei, Y.-H. Zhu and J. Qiu, *Anal. Biochem.*, 2025, 115862.
- R. Mitra, J. Li, J. M. Sagendorf, Y. Jiang, A. S. Cohen, T.-P. Chiu, C. J. Glasscock and R. Rohs, *Nat. Methods*, 2024, **21**, 1674–1683.
- L. R. Genz, S. Nair, A. Sweeney and M. Topf, *Curr. Opin. Struct. Biol.*, 2025, **95**, 103165.
- Sangeeta, S. K. Mishra and A. Bhattacharjee, *JACS Au*, 2024, **4**, 2640–2655.
- M. Brenowitz, D. F. Seneor, M. A. Shea and G. K. Ackers, *Methods in enzymology*, Elsevier, 1986, vol. 130, pp. 132–181.
- M. G. Fried, *Electrophoresis*, 1989, **10**, 366–376.
- D. S. Johnson, A. Mortazavi, R. M. Myers and B. Wold, *Science*, 2007, **316**, 1497–1502.
- P. Schuck and A. P. Minton, *Trends Biochem. Sci.*, 1996, **21**, 458–460.
- B. Gyrfas, F. Olasagasti, S. Benner, D. Garalde, K. R. Lieberman and M. Akeson, *ACS Nano*, 2009, **3**, 1457–1466.
- A. H. Squires, T. Gilboa, C. Torfstein, N. Varongchayakul and A. Meller, *Methods in enzymology*, Elsevier, 2017, vol. 582, pp. 353–385.
- J. Li, Y. Wang, L. Wang, Y. Wang, Z. Zhang, S. Liu and L. Wang, *ACS Mater. Lett.*, 2025, **7**, 2476–2481.
- Y.-L. Ying, Z.-L. Hu, S. Zhang, Y. Qing, A. Fragasso, G. Maglia, A. Meller, H. Bayley, C. Dekker and Y.-T. Long, *Nat. Nanotechnol.*, 2022, **17**, 1136–1146.
- S. Schmid and C. Dekker, *Essays Biochem.*, 2021, **65**, 93–107.
- V. Bijak, M. Szczygiel, J. Lenkiewicz, M. Gućwa, D. R. Cooper, K. Murzyn and W. Minor, *Expert Opin. Drug Discovery*, 2023, **18**, 1221–1230.
- S. Campagne, V. Gervais and A. Milon, *J. R. Soc., Interface*, 2011, **8**, 1065–1078.
- M. D. Wilson and A. Costa, *Biol. Crystallogr.*, 2017, **73**, 541–548.
- H. S. Kaya-Okur, D. H. Janssens, J. G. Henikoff, K. Ahmad and S. Henikoff, *Nat. Protoc.*, 2020, **15**, 3264–3283.
- A. Bartlett, R. C. O'Malley, S.-S. C. Huang, M. Galli, J. R. Nery, A. Gallavotti and J. R. Ecker, *Nat. Protoc.*, 2017, **12**, 1659–1672.
- J. Concepcion, K. Witte, C. Wartchow, S. Choo, D. Yao, H. Persson, J. Wei, P. Li, B. Heidecker and W. Ma, *et al.*, *Comb. Chem. High Throughput Screening*, 2009, **12**, 791–800.
- M. R. Corces, J. M. Granja, S. Shams, B. H. Louie, J. A. Seoane, W. Zhou, T. C. Silva, C. Groeneveld, C. K. Wong and S. W. Cho, *et al.*, *Science*, 2018, **362**, eaav1898.
- C. Sun and Y. Feng, *Protein J.*, 2024, **43**, 513–521.
- Y. Liu and B. Tian, *Briefings Bioinf.*, 2024, **25**, bbad488.
- A. Gress, V. Ramensky and O. V. Kalinina, *Oncogenesis*, 2017, **6**, e380–e380.
- H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, *Nucleic Acids Res.*, 2000, **28**, 235–242.
- H. M. Berman and S. K. Burley, *Q. Rev. Biophys.*, 2025, **58**, e9.
- G. O. Consortium, *Nucleic Acids Res.*, 2004, **32**, D258–D261.
- S. A. Aleksander, J. Balhoff, S. Carbon, J. M. Cherry, H. J. Drabkin, D. Ebert, M. Feuermann, P. Gaudet and N. L. Harris, *et al.*, *Genetics*, 2023, **224**, iyad031.

- 40 T. U. Consortium, *Nucleic Acids Res.*, 2024, **53**, D609–D617.
- 41 A. N. Koehler, *Curr. Opin. Chem. Biol.*, 2010, **14**, 331–340.
- 42 T. Helleday, E. Petermann, C. Lundin, B. Hodgson and R. A. Sharma, *Nat. Rev. Cancer*, 2008, **8**, 193–204.
- 43 H. A. Malone and C. W. Roberts, *Nat. Rev. Drug Discovery*, 2024, **23**, 661–681.
- 44 Y. S. Raouf, *Drug Discovery Today*, 2024, **29**, 104094.
- 45 Y. Pommier and C. Marchand, *Nat. Rev. Drug Discovery*, 2012, **11**, 25–36.
- 46 J. H. Bushweller, *Nat. Rev. Cancer*, 2019, **19**, 611–624.
- 47 J. Portugal, *Biochem. Pharmacol.*, 2018, **155**, 336–345.
- 48 Y. Pommier, *ACS Chem. Biol.*, 2013, **8**, 82–95.
- 49 M. R. Nathan and P. Schmid, *Oncol. Ther.*, 2017, **5**, 17–29.
- 50 T. M. Beer, A. J. Armstrong, D. E. Rathkopf, Y. Loriot, C. N. Sternberg, C. S. Higano, P. Iversen, S. Bhattacharya, J. Carles and S. Chowdhury, *et al.*, *N. Engl. J. Med.*, 2014, **371**, 424–433.
- 51 E. Garralda, M.-E. Beaulieu, V. Moreno, S. Casacuberta-Serra, S. Martinez-Martin, L. Foradada, G. Alonso, D. Masso-Valles, S. López-Estévez and T. Jauset, *et al.*, *Nat. Med.*, 2024, **30**, 762–771.
- 52 L. B. Snyder, T. K. Neklesa, R. R. Willard, D. A. Gordon, J. Pizzano, N. Vitale, K. Robling, M. A. Dorso, W. Moghrabi, S. Landrette, R. Gedrich, S. H. Lee, I. C. Taylor and J. G. Houston, *Mol. Cancer Ther.*, 2025, **24**, 511–522.
- 53 G. Kim, G. Ison, A. E. McKee, H. Zhang, S. Tang, T. Gwise, R. Sridhara, E. Lee, A. Tzou and R. Philip, *et al.*, *Clin. Cancer Res.*, 2015, **21**, 4257–4261.
- 54 S. M. Hoy, *Drugs*, 2018, **78**, 1939–1946.
- 55 S. Grant, C. Easley and P. Kirkpatrick, *Nat. Rev. Drug Discovery*, 2007, **6**, 21–22.
- 56 R. Straining and W. Eighmy, *J. Adv. Pract. Oncol.*, 2022, **13**, 158.
- 57 A. Bisht, D. Avinash, K. K. Sahu, P. Patel, G. Das Gupta and B. D. Kurmi, *Drug Delivery Transl. Res.*, 2025, **15**, 102–133.
- 58 D. Tsvetkova and S. Ivanova, *Molecules*, 2022, **27**, 2466.
- 59 T. K. Choueiri, T. M. Bauer, D. F. McDermott, E. Arrowsmith, A. Roy, R. F. Perini, D. Vickery and S. S. Tykodi, Phase 2 study of the oral hypoxia-inducible factor 2 α (HIF-2 α) inhibitor MK-6482 in combination with cabozantinib in patients with advanced clear cell renal cell carcinoma (ccRCC), 2021.
- 60 National Cancer Institute (NCI), *Olaparib in Combination with Vorinostat in Patients with Relapsed/Refractory and/or Metastatic Breast Cancer*, <https://clinicaltrials.gov/study/NCT03742245>, 2018, Accessed September 28, 2025.
- 61 S. M. Gough, J. J. Flanagan, J. Teh, M. Andreoli, E. Rousseau, M. Pannone, M. Bookbinder, R. Willard, K. Davenport, E. Bortolon, G. Cadelina, D. Gordon, J. Pizzano, J. Macaluso, L. Soto, J. Corradi, K. Diganantonio, I. Drulyte, A. Morgan, C. Quinn, M. Bekes, C. Ferraro, X. Chen, G. Wang, H. Dong, J. Wang, D. R. Langley, J. Houston, R. Gedrich and I. C. Taylor, *Clin. Cancer Res.*, 2024, **30**, 3549–3563.
- 62 K. K. Swinger, K. M. Lemberg, Y. Zhang and P. A. Rice, *EMBO J.*, 2003, **22**, 3749–3760.
- 63 U.-M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo and S. J. Lippard, *Nature*, 1999, **399**, 708–712.
- 64 D. B. Nikolov, H. Chen, E. D. Halay, A. Hoffman, R. G. Roeder and S. K. Burley, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 4862–4867.
- 65 M. Elrod-Erickson, M. A. Rould, L. Nekludova and C. O. Pabo, *Structure*, 1996, **4**, 1171–1180.
- 66 S. Dürr, BioIcons April 2024 Release, <https://bioicons.com>.
- 67 G. Tomasello, I. Armenia and G. Molla, *Bioinformatics*, 2020, **36**, 2909–2911.
- 68 D. Sehnal, S. Bittrich, M. Deshpande, R. Svobodova, K. Berka, V. Bazgier, S. Velankar, S. K. Burley, J. Koca and A. S. Rose, *Nucleic Acids Res.*, 2021, **49**, W431–W437.
- 69 L. Mariño-Ramrez, M. G. Kann, B. A. Shoemaker and D. Landsman, *Expert Rev. Proteomics*, 2005, **2**, 719–729.
- 70 M. Lin and J.-t. Guo, *Nucleic Acids Res.*, 2019, **47**, 11103–11113.
- 71 F. V. Murphy and M. E. Churchill, *Structure*, 2000, **8**, R83–R89.
- 72 Z. Zheng and Y. Wang, *Biomol. Concepts*, 2011, **2**, 293–303.
- 73 S. Ahmad, H. Kono, M. J. Arauzo-Bravo and A. Sarai, *Nucleic Acids Res.*, 2006, **34**, W124–W127.
- 74 M. J. Arauzo-Bravo and A. Sarai, *Nucleic Acids Res.*, 2008, **36**, 376–386.
- 75 A. B. Kolomeisky, *Phys. Chem. Chem. Phys.*, 2011, **13**, 2088–2095.
- 76 R. Murugan, *Biophys. J.*, 2010, **99**, 353–359.
- 77 W. K. Olson, A. A. Gorin, X.-J. Lu, L. M. Hock and V. B. Zhurkin, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 11163–11168.
- 78 N. M. Luscombe, R. A. Laskowski and J. M. Thornton, *Nucleic Acids Res.*, 2001, **29**, 2860–2874.
- 79 P. J. Sapienza, T. Niu, M. R. Kurpiewski, A. Grigorescu and L. Jen-Jacobson, *J. Mol. Biol.*, 2014, **426**, 84–104.
- 80 L.-A. Harris, L. D. Williams and G. B. Koudelka, *Nucleic Acids Res.*, 2014, **42**, 14053–14059.
- 81 S. Harteis and S. Schneider, *Int. J. Mol. Sci.*, 2014, **15**, 12335–12363.
- 82 R. Rohs, X. Jin, S. M. West, R. Joshi, B. Honig and R. S. Mann, *Annu. Rev. Biochem.*, 2010, **79**, 233–269.
- 83 R. Rohs, S. M. West, A. Sosinsky, P. Liu, R. S. Mann and B. Honig, *Nature*, 2009, **461**, 1248–1253.
- 84 K. B. Chhetri, Y. H. Jang, Y. Lansac and P. K. Maiti, *Phys. Chem. Chem. Phys.*, 2023, **25**, 31335–31345.
- 85 J. W. Schwabe, *Curr. Opin. Struct. Biol.*, 1997, **7**, 126–134.
- 86 R. Satange and M.-H. Hou, *RSC Chem. Biol.*, 2025, **6**, 699–720.
- 87 C. O. Pabo and R. T. Sauer, *Annu. Rev. Biochem.*, 1992, **61**, 1053–1095.
- 88 N. M. Luscombe, S. E. Austin, H. M. Berman and J. M. Thornton, *Genome Biol.*, 2000, **1**, 001-1.
- 89 V. Matys, E. Fricke, R. Geffers, E. Göföling, M. Haubrock, R. Hehl, K. Hornischer, D. Karas, A. E. Kel and O. V. Kel-Margoulis, *et al.*, *Nucleic Acids Res.*, 2003, **31**, 374–378.
- 90 M. Dyer, Q. X. X. Lin, S. Shapoval, D. Thieffry and T. Benoukraf, *Nucleic Acids Res.*, 2024, **52**, D222–D228.
- 91 I. Rauluseviciute, R. Riudavets-Puig, R. Blanc-Mathieu, J. A. Castro-Mondragon, K. Ferenc, V. Kumar, R. B. Lemma, J. Lucas, J. Chêneby and D. Baranasic, *et al.*, *Nucleic Acids Res.*, 2024, **52**, D174–D182.
- 92 L. Yang, T. Zhou, I. Dror, A. Mathelier, W. W. Wasserman, R. Gordán and R. Rohs, *Nucleic Acids Res.*, 2014, **42**, D148–D155.
- 93 R. Mitra, A. S. Cohen, J. M. Sagendorf, H. M. Berman and R. Rohs, *Nucleic Acids Res.*, 2025, **53**, D396–D402.
- 94 R. W. Tak Leung, X. Jiang, K. H. Chu and J. Qin, *Nucleic Acids Res.*, 2019, **47**, D322–D329.
- 95 Y. Cho, S. Gorina, P. D. Jeffrey and N. P. Pavletich, *Science*, 1994, **265**, 346–355.
- 96 A. Emamjomeh, D. Choobineh, B. Hajieghrari, N. MahdiNezhad and A. Khodavirdipour, *Mol. Biol. Rep.*, 2019, **46**, 3571–3596.
- 97 Z. Yan, F. Ge, Y. Zhang, H. Yan, Y. Liu, Y.-H. Zhu, J. Song and D.-J. Yu, *J. Chem. Inf. Model.*, 2025, **65**, 8335–8344.
- 98 D. Engelkamp and V. van Heyningen, *Curr. Opin. Genet. Dev.*, 1996, **6**, 334–342.
- 99 N. Chi and J. A. Epstein, *Trends Genet.*, 2002, **18**, 41–47.
- 100 J. Husby, A. K. Todd, S. M. Haider, G. Zinzalla, D. E. Thurston and S. Neidle, *J. Chem. Inf. Model.*, 2012, **52**, 1179–1192.
- 101 M. A. Samad, I. Ahmad, A. Hasan, M. H. Alhashmi, A. Ayub, F. A. Al-Abbasi, A. Kumer and S. Tabrez, *MedComm*, 2025, **6**, e70152.
- 102 P. Chen, B. Li and L. Ou-Yang, *Front. Endocrinol.*, 2022, **13**, 839005.
- 103 G. C. Shukla, A. R. Plaga, E. Shankar and S. Gupta, *Andrology*, 2016, **4**, 366–381.
- 104 M. Fischer and M. A. Sammons, *Cell Death Differ.*, 2024, **31**, 836–843.
- 105 D. W. Hagey, M. Bergsland and J. Muhr, *Development*, 2022, **149**, dev200547.
- 106 E. Shaulian and M. Karin, *Nat. Cell Biol.*, 2002, **4**, E131–E136.
- 107 S. Trop-Steinberg and Y. Azar, *Am. J. Med. Sci.*, 2017, **353**, 474–483.
- 108 P. B. Bhosale, H. H. Kim, A. Abusaliya, P. Vetrivel, S. E. Ha, M. Y. Park, H. J. Lee and G. S. Kim, *J. Evidence-Based Complementary Altern. Med.*, 2022, **2022**, 9797929.
- 109 M. Alcalay, N. Meani, V. Gelmetti, A. Fantozzi, M. Fagioli, A. Orleth, D. Riganelli, C. Sebastiani, E. Cappelli and C. Casciari, *et al.*, *J. Clin. Invest.*, 2003, **112**, 1751–1761.
- 110 X. Yuan, W. Ruan, B. Bobrow, P. Carmeliet and H. K. Eltzschig, *Nat. Rev. Drug Discovery*, 2024, **23**, 175–200.
- 111 D. P. Vageli, P. G. Doukas, K. Goupou, A. D. BENOS, K. Astará, K. Zacharouli, S. Sotiriou and M. Ioannou, *Oncol. Res.*, 2024, **32**, 1239.
- 112 G. Roviello, I. De Gennaro, I. Vascotto, G. Venturi, A. D'Angelo, C. Winchler, A. Guarino, S. Cacioppo, M. Modesti and M. M. Mela, *et al.*, *Genes*, 2024, **16**, 6.
- 113 J. W. Lee, J. Ko, C. Ju and H. K. Eltzschig, *Exp. Mol. Med.*, 2019, **51**, 1–13.

- 114 S. Jaiswal, X. Han and H. P. Lu, *J. Phys. Chem. B*, 2022, **126**, 997–1003.
- 115 R. Dueva and G. Iliakis, *NAR Cancer*, 2020, **2**, zcaa022.
- 116 C. Laguri, I. Duband-Goulet, N. Friedrich, M. Axt, P. Belin, I. Callebaut, B. Gilquin, S. Zinn-Justin and J. Couprie, *Biochemistry*, 2008, **47**, 6199–6207.
- 117 D. Jaworski, B. Brzozczyk and L. Szyberg, *Cells*, 2023, **12**, 1375.
- 118 D. M. Wilson III and V. A. Bohr, *DNA Repair*, 2007, **6**, 544–559.
- 119 A. Piscone, F. Gorini, S. Ambrosio, A. Noviello, G. Scala, B. Majello and S. Amente, *Cells*, 2025, **14**, 112.
- 120 D. C. George, F. E. Bertrand and G. Sigounas, *Adv. Biol. Regul.*, 2024, **91**, 101013.
- 121 A. Smogorzewska, Chapter 1: The Fanconi Anemia DNA Repair Pathway, <https://www.fanconi.org/images/uploads/other/Chapter1.pdf>, n.d., Fanconi Anemia Research Fund; Accessed: 2025-09-26.
- 122 M. F. Sharp, V. J. Murphy, S. V. Twest, W. Tan, J. Lui, K. J. Simpson, A. J. Deans and W. Crismani, *Sci. Rep.*, 2020, **10**, 7959.
- 123 B. Cheng, W. Pan, Y. Xing, Y. Xiao, J. Chen and Z. Xu, *Eur. J. Med. Chem.*, 2022, **230**, 114109.
- 124 M. Crewe and R. Madabhushi, *Front. Aging Neurosci.*, 2021, **13**, 751742.
- 125 J. L. Nitiiss, *Nat. Rev. Cancer*, 2009, **9**, 327–337.
- 126 Y. Pommier and N. Osheroff, *DNA Topoisomerases and Cancer*, Springer, 2011, pp. 145–154.
- 127 M. Li and Y. Liu, *Genomics, Proteomics Bioinf.*, 2016, **14**, 166–171.
- 128 L. Broderick, S. Yost, D. Li, M. D. McGeough, L. M. Booshehri, M. Guaderrama, S. D. Brydges, K. Kucharova, N. C. Patel and M. Harr, *et al.*, *Sat. Commun.*, 2019, **10**, 3644.
- 129 H. Zhang, S.-J. Lee, B. Zhu, N. Q. Tran, S. Tabor and C. C. Richardson, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 9372–9377.
- 130 R. Bythell-Douglas and A. J. Deans, *Structure*, 2021, **29**, 99–113.
- 131 G. Wang and K. M. Vasquez, *Nat. Rev. Genet.*, 2023, **24**, 211–234.
- 132 A. Bansal, S. Kaushik and S. Kukreti, *Front. Genetics*, 2022, **13**, 959258.
- 133 F. Pan, Y. Zhang, P. Xu, V. H. Man, C. Roland, K. Weninger and C. Sagui, *Comput. Struct. Biotechnol. J.*, 2021, **19**, 2819–2832.
- 134 G. Matos-Rodrigues, J. A. Hisey, A. Nussenzweig and S. M. Mirkin, *Mol. Cell*, 2023, **83**, 3622–3641.
- 135 P. Thumapati, S. N. Rai, C. Prajapati, K. Ramakrishna and S. K. Singh, *Sci. Pharm.*, 2025, **93**, 6.
- 136 S.-L. Zhang, H. Fu, Y. Ma, Q. Lin, Y. Xu, Q. Yang, P. He and Z. Wei, *Org. Biomol. Chem.*, 2025, **23**, 1112–1119.
- 137 A. Sengupta, S. S. Roy and S. Chowdhury, *Acc. Chem. Res.*, 2020, **54**, 46–56.
- 138 Z.-Y. Sun, X.-N. Wang, S.-Q. Cheng, X.-X. Su and T.-M. Ou, *Molecules*, 2019, **24**, 396.
- 139 I. Frasson, V. Pirota, S. N. Richter and F. Doria, *Int. J. Biol. Macromol.*, 2022, **204**, 89–102.
- 140 J. R. Whitfield and L. Soucek, *Nat. Rev. Drug Discovery*, 2025, 1–13.
- 141 M. Al Ojaimi, B. J. Animortada, A. Alragheb, R. S. Hajir, C. Alves, D. Walid, A. Raza and A. W. El-Hattab, *Hum. Genomics*, 2025, **19**, 47.
- 142 S.-H. Song and T.-Y. Kim, *Genomics Bioinf.*, 2017, **15**, 114.
- 143 M. Bustin and R. Reeves, *Prog. Nucleic Acid Res. Mol. Biol.*, 1996, **54**, 35–100b.
- 144 S. Date and L. K. Bhatt, *Inflammopharmacology*, 2025, **33**, 767–784.
- 145 S. Wu, Y. Yu, Z. Zheng and Q. Cheng, *Eur. J. Med. Res.*, 2023, **28**, 430.
- 146 B. Wen, Y.-t. Wei and K. Zhao, *Mol. Cell. Biochem.*, 2021, **476**, 1729–1739.
- 147 S. G. Swygert and C. L. Peterson, *Biochim. Biophys. Acta, Gene Regul. Mech.*, 2014, **1839**, 728–736.
- 148 T. Kouzarides, *Cell*, 2007, **128**, 693–705.
- 149 R. Parveen, D. Harihar and B. P. Chatterji, *Cancer*, 2023, **129**, 3372–3380.
- 150 Z. Yao, Y. Chen, W. Cao and N. Shyh-Chang, *Cell Proliferation*, 2020, **53**, e12898.
- 151 G. A. Holdgate, C. Bardelle, A. Lanne, J. Read, D. H. O'Donovan, J. M. Smith, N. Selmi and R. Sheppard, *Drug Discovery Today*, 2022, **27**, 1088–1098.
- 152 A. Jain, S. Mittal, L. P. Tripathi, R. Nussinov and S. Ahmad, *Comput. Struct. Biotechnol. J.*, 2022, **20**, 4415–4436.
- 153 D. Wu, N. Potluri, J. Lu, Y. Kim and F. Rastinejad, *Nature*, 2015, **524**, 303–308.
- 154 X. Ren, X. Diao, J. Zhuang and D. Wu, *Mol. Pharmacol.*, 2022, **102**, 240–247.
- 155 M. Lambert, S. Jambon, S. Depauw and M.-H. David-Cordonnier, *Molecules*, 2018, **23**, 1479.
- 156 J.-j. Zhuang, Q. Liu, D.-l. Wu and L. Tie, *Acta Pharmacol. Sin.*, 2022, **43**, 2474–2481.
- 157 D. A. Sallman, *Haematologica*, 2020, **105**, 1470.
- 158 G. Garcia-Manero, *Lancet Haematol.*, 2023, **10**, e71–e78.
- 159 J. Schust, B. Sperl, A. Hollis, T. U. Mayer and T. Berg, *Chem. Biol.*, 2006, **13**, 1235–1242.
- 160 C.-L. Li, H.-Y. Chen, J.-C. Yen, S.-J. Yu, T.-Y. Chou, S.-W. Yeh, H.-Y. Chuang and F.-L. Huang, *Mol. Med. Rep.*, 2025, **31**, 1–11.
- 161 M. M. Kasembeli, P. Singhmar, J. Ma, J. Edralin, Y. Tang, C. Adams, C. J. Heijnen, A. Kavelaars and D. J. Tweardy, *Biochem. Pharmacol.*, 2021, **192**, 114688.
- 162 S. Moverare-Skrtic, A. E. Borjesson, H. H. Farman, K. Sjogren, S. H. Windahl, M. K. Lagerquist, A. Andersson, A. Stubelius, H. Carlsten, J. Ake Gustafsson and C. Ohlsson, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 1180–1185.
- 163 J. Hoffman-Censits and W. K. Kelly, *Clin. Cancer Res.*, 2013, **19**, 1335–1339.
- 164 X. Wu, D. Lazris, R. Wong and S. S. Tykodi, *Ther. Adv. Med. Oncol.*, 2025, **17**, 17588359251317846.
- 165 R. Srinivasan, O. Iliopoulos, K. E. Beckermann, V. Narayan, B. L. Maughan, S. Oudard, T. Else, J. K. Maranchie, A. B. Iversen and J. Cornell, *et al.*, *Lancet Oncol.*, 2025, **26**, 571–582.
- 166 R. J. Motzer, Y. Liu, R. F. Perini, Y. Zhang and D. Y. C. Heng, Phase III study evaluating efficacy and safety of MK-6482+ lenvatinib versus cabozantinib for second-or third-line therapy in patients with advanced renal cell carcinoma (RCC) who progressed after prior anti-PD-1/L1 therapy, 2021.
- 167 T. K. Choueiri, J. Bedke, J. A. Karam, R. R. McKay, R. J. Motzer, S. M. Pal, C. Suárez, R. Uzzo, H. Liu, J. E. Burgents, *et al.*, Phase 3 LITESPARK-022: Pembrolizumab (pembro) plus hypoxia-inducible factor 2 α (HIF-2 α) inhibitor belzutifan as adjuvant treatment for clear cell renal cell carcinoma (ccRCC), 2023.
- 168 S. Goel, M. J. DeCristo, S. S. McAllister and J. J. Zhao, *Trends Cell Biol.*, 2018, **28**, 911–925.
- 169 A. Andrikopoulou, S. Chatzinikolaou, E. Panourgias, M. Kaparelou, M. Liontos, M.-A. Dimopoulos and F. Zagouri, *Breast*, 2022, **63**, 157–167.
- 170 Y. Xu, Q. Yu, P. Wang, Z. Wu, L. Zhang, S. Wu, M. Li, B. Wu, H. Li and H. Zhuang, *et al.*, *Adv. Sci.*, 2022, **9**, 2104344.
- 171 C. Calvis, A. Beier, M. Feichtinger, T. Höfurtherner, M. Moreno, R. Messeguer, R. Konrat, S. Esteban and L. Nevola, *Cancer Res.*, 2021, **81**, 2471.
- 172 A. Starodub, A. Gollerkeri, C. De Savi, J. Dey, S. Agarwal, S. Donohue, R. Perea, C. Klaus and J. Gollob, *J. Clin. Oncol.*, 2022, **40**, TPS3171.
- 173 J. Fallah, M. H. Brave, C. Weinstock, G. U. Mehta, D. Bradford, H. Gittleman, E. W. Bloomquist, R. Charlab, S. S. Hamed and C. P. Miller, *et al.*, *Clin. Cancer Res.*, 2022, **28**, 4843–4848.
- 174 M.-F. Langelier, L. Zandarashvili, P. M. Aguiar, B. E. Black and J. M. Pascal, *Nat. Commun.*, 2018, **9**, 844.
- 175 K. Ryan, B. Bolanos, M. Smith, P. B. Palde, P. D. Cuenca, T. L. VanArsdale, S. Niessen, L. Zhang, D. Behenna and M. A. Ornelas, *et al.*, *J. Biol. Chem.*, 2021, **296**, 100251.
- 176 C. J. Lord and A. Ashworth, *Science*, 2017, **355**, 1152–1158.
- 177 A. P. Wiegman, M. Miranda, S. W. Wen, F. Al-Ejeh and A. Möller, *Oncotarget*, 2016, **7**, 60087.
- 178 A. Ward, K. K. Khanna and A. P. Wiegman, *Cancer Treat. Rev.*, 2015, **41**, 35–45.
- 179 Y. Pan, Q. Zhang, C. Xu, Y. Sun, Q. Zheng, S. Yang and S. Lv, *Comput. Biol. Med.*, 2025, **191**, 110105.
- 180 P. S. VanderVere-Carozza, N. S. Gavande, S. I. Jalal, K. E. Pollok, E. Ekinici, J. Heyza, S. M. Patrick, A. Masters, J. J. Turchi and K. S. Pawelczak, *Front. Oncol.*, 2022, **12**, 826655.
- 181 M. T. Roth and S. Das, *Expert Rev. Anticancer Ther.*, 2021, **21**, 229–238.
- 182 M. T. van Bussel, A. Awada, M. J. de Jonge, M. Mau-Sørensen, D. Nielsen, P. Schöffski, H. M. Verheul, B. Sarholz, K. Berghoff and S. El Bawab, *et al.*, *Br. J. Cancer*, 2021, **124**, 728–735.
- 183 J. M. Kelm, A. Samarbaksh, A. Pillai, P. S. VanderVere-Carozza, H. Aruri, D. S. Pandey, K. S. Pawelczak, J. J. Turchi and N. S. Gavande, *Front. Oncol.*, 2022, **12**, 850883.

- 184 J. Zhu, L. Zhou, G. Wu, H. Konig, X. Lin, G. Li, X.-L. Qiu, C.-F. Chen, C.-M. Hu and E. Goldblatt, *et al.*, *EMBO Mol. Med.*, 2013, **5**, 353–365.
- 185 F. Huang, O. M. Mazina, I. J. Zentner, S. Cocklin and A. V. Mazin, *J. Med. Chem.*, 2012, **55**, 3011–3020.
- 186 F. Huang and A. V. Mazin, *PLoS One*, 2014, **9**, e100993.
- 187 F. Huang, N. A. Motlekar, C. M. Burgwin, A. D. Napper, S. L. Diamond and A. V. Mazin, *ACS Chem. Biol.*, 2011, **6**, 628–635.
- 188 P. C. Fong, D. S. Boss, T. A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, P. Mortimer, H. Swaisland, A. Lau and M. J. O'Connor, *et al.*, *N. Engl. J. Med.*, 2009, **361**, 123–134.
- 189 F. T. Zenke, A. Zimmermann, C. Sirrenberg, H. Dahmen, V. Kirkin, U. Pehl, T. Grombacher, C. Wilm, T. Fuchss and C. Amendt, *et al.*, *Mol. Cancer Ther.*, 2020, **19**, 1091–1101.
- 190 M. Elias, S. Gani, Y. Lerner, K. Yamin, C. Tor, A. Patel, A. Matityahu, M. Dessau, N. Qvit and I. Onn, *iScience*, 2023, **26**, 107498.
- 191 J. Antony, C. V. Chin and J. A. Horsfield, *Int. J. Mol. Sci.*, 2021, **22**, 6788.
- 192 A. B. Alharbi, U. Schmitz, C. G. Bailey and J. E. Rasko, *Nucleic Acids Res.*, 2021, **49**, 7825–7838.
- 193 Y. Kuroki, K. Yamagata, Y. Aikawa, Y. Shima, H. Shioneri and I. Kitabayashi, *Cancer Sci.*, 2025, 3016–3026.
- 194 Y. Liu, P. Liu, S. Duan, J. Lin, W. Qi, Z. Yu, X. Gao, X. Sun, J. Liu and J. Lin, *et al.*, *Cell Death Differ.*, 2025, **32**, 745–762.
- 195 Y. Han, W. Sheng, X. Liu, H. Liu, X. Jia, H. Li, C. Wang, B. Wang, T. Hu and Y. Ma, *Sci. Rep.*, 2024, **14**, 24948.
- 196 G. Vergoten and C. Bailly, *Med. Drug Discovery*, 2020, **7**, 100058.
- 197 E. San José-Enériz, N. Gimenez-Camino, X. Agirre and F. Prosper, *Cancers*, 2019, **11**, 1794.
- 198 T. N. Teknos, J. Grecula, A. Agrawal, M. Old, E. Ozer, R. Carrau, S. Kang, J. Rocco, D. Blakaj and V. Diavolitsis, *et al.*, *Invest. New Drugs*, 2019, **37**, 702–710.
- 199 T. H. Luu, R. J. Morgan, L. Leong, D. Lim, M. McNamara, J. Portnow, P. Frankel, D. D. Smith, J. H. Doroshow and D. R. Gandara, *et al.*, *Clin. Cancer Res.*, 2008, **14**, 7138–7142.
- 200 P. Smolewski and T. Robak, *Expert Opin. Drug Discovery*, 2017, **12**, 859–873.
- 201 C. Sequera, M. Grattarola, F. Cannet, A. Dobric, P. Michea Veloso, M. Methia, S. Richelme, A. El Kaoutari, P. Kousteridou and D. Debayle, *et al.*, *Nat. Commun.*, 2025, **16**, 7919.
- 202 S. M. Horwitz, A. J. Nirmal, J. Rahman, R. Xu, E. Drill, N. Galasso, N. Ganesan, T. Davey, H. Hancock and L. Perez, *et al.*, *Nat. Med.*, 2024, **30**, 2517–2527.
- 203 J. P. Laubach, P. Moreau, J. F. San-Miguel and P. G. Richardson, *Clin. Cancer Res.*, 2015, **21**, 4767–4773.
- 204 M. J. Homan, A. Franson, K. Ravi, H. Roberts, M. P. Pai, C. Liu, M. He, A. Matvekas, C. Koschmann and B. L. Marini, *Cancer Chemother. Pharmacol.*, 2021, **88**, 555–562.
- 205 M. Monje, T. Cooney, J. Glod, J. Huang, C. J. Peer, D. Faury, P. Baxter, K. Kramer, A. Lenzen and N. J. Robison, *et al.*, *Neuro-Oncology*, 2023, **25**, 2262–2272.
- 206 A. Aartsma-Rus, *Front. Cell Dev. Biol.*, 2025, **12**, 1514898.
- 207 F. Perner, T. Berg, D. Sasca, S.-L. Mersiowsky, J. Y. Gadrey, J. Thomas, M. W. Kühn and M. Lübbert, *Int. J. Cancer*, 2025.
- 208 E. M. Stein, G. Garcia-Manero, D. A. Rizzieri, R. Tibes, J. G. Berdeja, M. R. Savona, M. Jongen-Lavrenic, J. K. Altman, B. Thomson and S. J. Blakemore, *et al.*, *Blood*, 2018, **131**, 2661–2669.
- 209 A. Schurer, S. G. Glushakow-Smith and K. Gritsman, *Stem Cells Transl. Med.*, 2024, **14**, szae089.
- 210 S. Zehatabcheg, H. Soleimani Samarkhazan, M. Asadi, M. Zabihi, S. Parkhideh and M. H. Mohammadi, *Biomarker Res.*, 2025, **13**, 73.
- 211 L. L. Marzochi, C. I. Cuzziol, C. H. V. Do Nascimento Filho, J. A. Dos Santos, M. M. U. Castanhole-Nunes, É. C. Pavarino, E. N. S. Guerra and E. M. Goloni-Bertollo, *Eur. J. Pharmacol.*, 2023, **944**, 175590.
- 212 H. Dhulkifle, M. I. Diab, M. Algonaiyah, H. M. Korashy and Z. H. Maayah, *ACS Pharmacol. Transl. Sci.*, 2024, **7**, 546–559.
- 213 Y. N. Lamb, *Drugs*, 2024, **84**, 849–856.
- 214 S. M. Hoy, *Drugs*, 2020, **80**, 513–521.
- 215 T. Maes, C. Mascaráo, I. Tirapu, A. Estiarte, F. Ciceri, S. Lunardi, N. Guibourt, A. Perdones, M. M. Lufino and T. C. Somerville, *et al.*, *Cancer Cell*, 2018, **33**, 495–511.
- 216 K. K. Ray, S. J. Nicholls, K. A. Buhr, H. N. Ginsberg, J. O. Johansson, K. Kalantar-Zadeh, E. Kulikowski, P. P. Toth, N. Wong and M. Sweeney, *et al.*, *JAMA*, 2020, **323**, 1565–1573.
- 217 A. M. J. Fichtinger-Schepman, J. L. Van der Veer, J. H. Den Hartog, P. H. Lohman and J. Reedijk, *Biochemistry*, 1985, **24**, 707–713.
- 218 D. Sahoo, P. Deb, T. Basu, S. Bardhan, S. Patra and P. K. Sukul, *Bioorg. Med. Chem.*, 2024, **112**, 117894.
- 219 M.-H. Baik, R. A. Friesner and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 14082–14092.
- 220 R. D. Wood, *Biochimie*, 1999, **81**, 39–44.
- 221 A. Yimit, O. Adebali, A. Sancar and Y. Jiang, *Nat. Commun.*, 2019, **10**, 309.
- 222 C. Zhang, C. Xu, X. Gao and Q. Yao, *Theranostics*, 2022, **12**, 2115.
- 223 S. Li, W. K. Olson and X.-J. Lu, *Nucleic Acids Res.*, 2019, **47**, W26–W34.
- 224 A. K. Larsen, C. M. Galmarini and M. D'Incalci, *Cancer Chemother. Pharmacol.*, 2016, **77**, 663–671.
- 225 X.-M. Chen, C. Cullinane, P. J. Gray and D. R. Phillips, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 1999, **445**, 45–54.
- 226 M. Shang, M. Ren and C. Zhou, *Chem. Res. Toxicol.*, 2019, **32**, 2517–2525.
- 227 V. L. Souliotis, M. A. Dimopoulos and P. P. Sfrikakis, *Clin. Cancer Res.*, 2003, **9**, 4465–4474.
- 228 L. M. Leoni, B. Bailey, J. Reifert, H. H. Bendall, R. W. Zeller, J. Corbeil, G. Elliott and C. C. Niemeyer, *Clin. Cancer Res.*, 2008, **14**, 309–317.
- 229 T. W. Dennie and J. M. Kolesar, *Clin. Ther.*, 2009, **31**, 2290–2311.
- 230 G. S. Khan, A. Shah and D. Barker, *et al.*, *J. Photochem. Photobiol., B*, 2012, **115**, 105–118.
- 231 P. B. Dervan, *Bioorg. Med. Chem.*, 2001, **9**, 2215–2235.
- 232 L. Pett, J. A. Hartley and K. Kiakos, *Curr. Top. Med. Chem.*, 2015, **15**, 1293–1322.
- 233 P. B. Dervan and B. S. Edelson, *Curr. Opin. Struct. Biol.*, 2003, **13**, 284–299.
- 234 J. L. Yap, J. Chauhan, K.-Y. Jung, L. Chen, E. V. Prochownik and S. Fletcher, *MedChemComm*, 2012, **3**, 541–551.
- 235 L. H. Fornander, L. Wu, M. Billeter, P. Lincoln and B. Nordén, *J. Phys. Chem. B*, 2013, **117**, 5820–5830.
- 236 J. Kormanec, R. Novakova, D. Csolleiova, L. Feckova, B. Rezuchova, B. Sevcikova and D. Homerova, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 7701–7721.
- 237 C. Hou, S. Weidenbach, K. E. Cano, Z. Wang, P. Mitra, D. N. Ivanov, J. Rohr and O. V. Tsodikov, *Nucleic Acids Res.*, 2016, **44**, 8990–9004.
- 238 S. F. Sleiman, B. C. Langley, M. Basso, J. Berlin, L. Xia, J. B. Payappilly, M. K. Kharel, H. Guo, J. L. Marsh and L. M. Thompson, *et al.*, *J. Neurosci.*, 2011, **31**, 6858–6870.
- 239 J. Portugal, *Pharmacol. Ther.*, 2024, **260**, 108672.
- 240 S. S. Bhosale, A. Mandal, C. Hou, J. R. McCorkle, D. Schweer, K. S. Hill, V. Subramanian, J. M. Kolesar, O. V. Tsodikov and J. Rohr, *ChemMedChem*, 2023, **18**, e202200368.
- 241 S. Venugopal, V. Sharma, A. Mehra, I. Singh and G. Singh, *Chem. Biol. Drug Des.*, 2022, **100**, 580–598.
- 242 F. Gago, *Methods*, 1998, **14**, 277–292.
- 243 C. Avendano and J. C. Menendez, *Medicinal Chemistry of Anticancer Drugs*, Elsevier, Amsterdam, 2008, pp. 199–228.
- 244 J. T. Taylor, S. Ellison, A. Pandeley, S. Wood, E. Nathan, G. Forte, H. Parker, E. Zindy, M. Elvin and A. Dickson, *et al.*, *Neuro-Oncology*, 2020, **22**, 1289–1301.
- 245 H.-J. Kang and H.-J. Park, *Biochemistry*, 2009, **48**, 7392–7398.
- 246 T. Banerjee, S. Banerjee, S. Sett, S. Ghosh, T. Rakshit and R. Mukhopadhyay, *PLoS One*, 2016, **11**, e0154666.
- 247 B. K. Bhuyan and C. G. Smith, in *Nogalamycin*, ed. A. C. Sartorelli and D. G. Johns, Springer Berlin Heidelberg, Berlin, Heidelberg, 1975, pp. 623–632.
- 248 A. C. Ketron, W. A. Denny, D. E. Graves and N. Osheroff, *Biochemistry*, 2012, **51**, 1730–1739.
- 249 D. Kuron, A. Pohlmann, L. Angenendt, T. Kessler, R. Mesters, W. E. Berdel, M. Stelljes, G. Lenz, C. Schliemann and J.-H. Mikesch, *Ann. Hematol.*, 2023, **102**, 755–760.
- 250 R. E. Hawtin, D. E. Stockett, J. A. W. Byl, R. S. McDowell, N. Tan, M. R. Arkin, A. Conroy, W. Yang, N. Osheroff and J. A. Fox, *PLoS One*, 2010, **5**, e10186.
- 251 Y. Singh, N. Bhatia, A. Biharee, S. Kulkarni, S. Thareja and V. Monga, *Expert Opin. Drug Discovery*, 2023, **18**, 1151–1167.
- 252 J. Yin, P. Jia, X. Qu, Z. Han, L. Yao, S. Wang and J. Gao, *Chem. Biol. Drug Des.*, 2024, **104**, e70034.
- 253 E. A. Sausville, J. Peisach and S. B. Horwitz, *Biochemistry*, 1978, **17**, 2740–2746.

- 254 O. Tounekti, A. Kenani, N. A. Foray, S. Orlowski and L. M. Mir, *Br. J. Cancer*, 2001, **84**, 1272–1279.
- 255 J. M. Bennett and S. D. Reich, *Ann. Intern. Med.*, 1979, **90**, 945–948.
- 256 P. L. Mendoza-Munoz, N. S. Gavande, P. S. VanderVere-Carozza, K. Pawelczak, J. Dynlacht, J. Garrett and J. Turchi, *NAR Cancer*, 2023, **5**, zcad003.
- 257 K.-B. Wang, M. S. A. Elsayed, G. Wu, N. Deng, M. Cushman and D. Yang, *J. Am. Chem. Soc.*, 2019, **141**, 11059–11070.
- 258 A. Ali and S. Bhattacharya, *Bioorg. Med. Chem.*, 2014, **22**, 4506–4521.
- 259 H.-X. Li, Y.-M. He, J. Fei, M. Guo, C. Zeng, P.-J. Yan, Y. Xu, G. Qin and F.-Y. Teng, *J. Transl. Med.*, 2025, **23**, 457.
- 260 G. C. C. Koh, S. Boushaki, S. J. Zhao, A. M. Pregnall, F. Sadiyah, C. Badja, Y. Memari, I. Georgakopoulos-Soares and S. Nik-Zainal, *Nat. Genet.*, 2024, **56**, 23–26.
- 261 S. Kanwal, N. Brajanovski, J. Zhan, J. Chan, R. Rebello, O. Hofmann, S. Grimmond, L. Furic, R. D. Hannan and G. McArthur, *et al.*, *MedRxiv*, 2025, 2025.09.30.25336525, DOI: [10.1101/2025.09.30.25336525](https://doi.org/10.1101/2025.09.30.25336525).
- 262 A. A. Ahmed, W. Greenhalf, D. H. Palmer, N. Williams, J. Worthington, T. Arshad, S. Haider, E. Alexandrou, D. Guneri and Z. A. Waller, *et al.*, *Molecules*, 2023, **28**, 2452.
- 263 R. M. Wadkins, *Curr. Med. Chem.*, 2000, **7**, 1–15.
- 264 J. Zell, K. Duskova, L. Chouh, M. Bossaert, N. Chéron, A. Granzhan, S. Britton and D. Monchaud, *Nucleic Acids Res.*, 2021, **49**, 10275–10288.
- 265 T. Zhang, C. Yin, A. Fedorov, L. Qiao, H. Bao, N. Beknazarov, S. Wang, A. Gautam, R. M. Williams and J. C. Crawford, *et al.*, *Nature*, 2022, **606**, 594–602.
- 266 E. A. Elmorsy, S. Saber, R. S. Hamad, M. A. Abdel-Reheim, A. F. El-Kott, M. A. AlShehri, K. Morsy, S. A. Salama and M. E. Youssef, *Eur. J. Pharm. Sci.*, 2024, **203**, 106939.
- 267 R. C. Todd and S. J. Lippard, *Metallomics*, 2009, **1**, 280–291.
- 268 P. D. O'Dowd, D. F. Sutcliffe and D. M. Griffith, *Coord. Chem. Rev.*, 2023, **497**, 215439.
- 269 A. Le Cesne, J.-Y. Blay, D. Cupissol, A. Italiano, C. Delcambre, N. Penel, N. Isambert, C. Chevreau, E. Bompas and F. Bertucci, *et al.*, *Ann. Oncol.*, 2021, **32**, 1034–1044.
- 270 G. D. Demetri, M. Von Mehren, R. L. Jones, M. L. Hensley, S. M. Schuetze, A. Staddon, M. Milhem, A. Elias, K. Ganjoo and H. Tawbi, *et al.*, *J. Clin. Oncol.*, 2016, **34**, 786–793.
- 271 M. van Kan, K. E. Burns and N. A. Helsby, *Cancer Chemother. Pharmacol.*, 2021, **88**, 755–769.
- 272 2w?>U.S. Food and Drug Administration, Cosmegen® (dactinomycin for injection) Prescribing Information, 2018, https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/050682s0331bl.pdf, Accessed: 2025-09-26.
- 273 M. Kciuk, A. Gielecinska, S. Mujwar, D. Kolat, Z. Kaluzinska-Kolat, I. Celik and R. Kontek, *Cells*, 2023, **12**, 659.
- 274 A. Banerjee, S. Sanyal, K. K. Kulkarni, K. Jana, S. Roy, C. Das and D. Dasgupta, *FEBS Open Bio*, 2014, **4**, 987–995.
- 275 Y. Pommier and J. Cherfilis, *Trends Pharmacol. Sci.*, 2005, **26**, 138–145.
- 276 C.-C. Wu, T.-K. Li, L. Farh, L.-Y. Lin, T.-S. Lin, Y.-J. Yu, T.-J. Yen, C.-W. Chiang and N.-L. Chan, *Science*, 2011, **333**, 459–462.
- 277 V. Singh, P. Johansson, E. Ekedahl, Y.-L. Lin, O. Hammarsten and F. Westerlund, *Biochem. Biophys. Res. Commun.*, 2022, **594**, 57–62.
- 278 B. J. Evison, B. E. Sleebs, K. G. Watson, D. R. Phillips and S. M. Cutts, *Med. Res. Rev.*, 2016, **36**, 248–299.
- 279 D. Cardinale, F. Iacopo and C. M. Cipolla, *Front. Cardiovasc. Med.*, 2020, **7**, 26.
- 280 G. N. Maertens, A. N. Engelman and P. Cherepanov, *Nat. Rev. Microbiol.*, 2022, **20**, 20–34.
- 281 B. Geng, J. Hu, P. Li, D. Pan and L. Shen, *Carbon*, 2022, **187**, 365–374.
- 282 W. Fried, M. Tyagi, L. Minakhin, G. Chandramouly, T. Tredinnick, M. Ramanjulu, W. Auerbacher, M. Calbert, T. Rusanov and T. Hoang, *et al.*, *Nat. Commun.*, 2024, **15**, 2862.
- 283 B. Ko, A. P. Chen, S. Kummar, M. Hogu, L. V. Rubinstein, N. Takebe, R. Piekarz, E. Sharon, J. H. Beumer, D. Wilsker, D. Ketchum, K. V. Ferry-Galow, R. E. Parchment, J. L. Holleran, J. J. Deppas, J. M. Collins, B. C. Johnson, S. P. Ivy, J. H. Doroshov and G. H. O'Sullivan Coyne, *J. Clin. Oncol.*, 2023, **41**, 3022.
- 284 H. Gamal, E. M. Shoeib, A. Hajjaj, H. E. A. Abdullah, E. H. Elramy, D. A. Abd Ellah, S. M. El-Sayed and M. F. Khder, *RSC Pharm.*, 2025, 1019–1033.
- 285 M. K. Jayatunga, M. Ayers, L. Bruens, D. Jayanth and C. Meier, *Drug Discovery Today*, 2024, **29**, 104009.
- 286 H. Wei and J. A. McCammon, *npj Drug Discovery*, 2024, **1**, 1.
- 287 Y. You, X. Lai, Y. Pan, H. Zheng, J. Vera, S. Liu, S. Deng and L. Zhang, *Signal Transduction Targeted Ther.*, 2022, **7**, 156.
- 288 D. Lama, T. Vosselman, C. Sahin, J. Liaño-Pons, C. P. Cerrato, L. Nilsson, K. Teilum, D. P. Lane, M. Landreh and M. Arsenian Henriksson, *Nat. Commun.*, 2024, **15**, 1865.
- 289 J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A. J. Ballard and J. Babrick, *et al.*, *Nature*, 2024, **630**, 493–500.
- 290 A. Omid, M. H. Möller, N. Malhis, J. M. Bui and J. Gsponer, *Proc. Natl. Acad. Sci. U. S. A.*, 2024, **121**, e2406407121.
- 291 M. Baek, R. McHugh, I. Anishchenko, H. Jiang, D. Baker and F. DiMaio, *Nat. Methods*, 2024, **21**, 117–121.
- 292 R. Krishna, J. Wang, W. Ahern, P. Sturmfels, P. Venkatesh, I. Kalvet, G. R. Lee, F. S. Morey-Burrows, I. Anishchenko and I. R. Humphreys, *et al.*, *Science*, 2024, **384**, ead12528.
- 293 M. Andrabi, K. Mizuguchi, A. Sarai and S. Ahmad, *BMC Struct. Biol.*, 2009, **9**, 30.
- 294 S. Chauhan and S. Ahmad, *Proteins: Struct., Funct., Bioinf.*, 2020, **88**, 15–30.
- 295 D. M. Varghese, R. Nussinov and S. Ahmad, *NAR: Genomics Bioinf.*, 2022, **4**, lqac091.
- 296 S. Mirza, P. Pandey, S. Hasnain and S. Ahmad, Emerging Topics in Protein–DNA Interactions, in *Reference Module in Life Sciences*, Elsevier, 2025, pp. 373–393.
- 297 X. Li, W. Peng and X. Zhu, *Briefings Bioinf.*, 2025, **26**, bbaf457.
- 298 M. T. Tahmid, A. M. Hasan and M. S. Bayzid, *Commun. Biol.*, 2025, **8**, 568.
- 299 M. Aydinli, C. Liang and T. Dandekar, *Sci. Rep.*, 2022, **12**, 17588.
- 300 S. L. Nystrom and D. J. McKay, *PLoS Comput. Biol.*, 2021, **17**, e1008991.
- 301 R. V. Honorato, M. E. Trellet, B. Jiménez-Garca, J. J. Schaarschmidt, M. Giulini, V. Reys, P. I. Koukos, J. P. Rodrigues, E. Karaca and G. C. van Zundert, *et al.*, *Nat. Protoc.*, 2024, **19**, 3219–3241.
- 302 I. Tuszyńska, M. Magnus, K. Jonak, W. Dawson and J. M. Bujnicki, *Nucleic Acids Res.*, 2015, **43**, W425–W430.
- 303 M. M. Gromiha and K. Harini, *Curr. Opin. Struct. Biol.*, 2025, **90**, 102955.
- 304 P. Infante, M. Mori, R. Alfonsi, F. Ghirga, F. Aiello, S. Toscano, C. Ingallina, M. Siler, D. Cucchi and A. Po, *et al.*, *EMBO J.*, 2015, **34**, 200–217.
- 305 G. Calissi, E. W.-F. Lam and W. Link, *Nat. Rev. Drug Discovery*, 2021, **20**, 21–38.
- 306 Y. Wang, J. M. L. Ribeiro and P. Tiwary, *Curr. Opin. Struct. Biol.*, 2020, **61**, 139–145.
- 307 O. T. Unke, S. Chmiela, H. E. Sauceda, M. Gastegger, I. Poltavsky, K. T. Schutt, A. Tkatchenko and K.-R. Müller, *Chem. Rev.*, 2021, **121**, 10142–10186.
- 308 M. Andrabi, A. P. Hutchins, D. Miranda-Saavedra, H. Kono, R. Nussinov, K. Mizuguchi and S. Ahmad, *Sci. Rep.*, 2017, **7**, 4071.
- 309 M. Kalsan, A. Jabeen and S. Ahmad, *Transcriptome Data Analysis*, Springer, 2024, pp. 317–343.
- 310 J. Li, T.-P. Chiu and R. Rohs, *Nat. Commun.*, 2024, **15**, 1243.
- 311 M. J. Wildey, A. Haunso, M. Tudor, M. Webb and J. H. Connick, *Annu. Rep. Med. Chem.*, 2017, **50**, 149–195.
- 312 A. A. Peterson and D. R. Liu, *Nat. Rev. Drug Discovery*, 2023, **22**, 699–722.
- 313 T. A. A. Program, *Sci. Rep.*, 2024, **14**, 7526.
- 314 R. Qureshi, M. Irfan, T. M. Gondal, S. Khan, J. Wu, M. U. Hadi, J. Heymach, X. Le, H. Yan and T. Alam, *Heliyon*, 2023, **9**, e17575.
- 315 B. Zdrzil, E. Felix, F. Hunter, E. J. Manners, J. Blackshaw, S. Corbett, M. De Veij, H. Ioannidis, D. M. Lopez and J. F. Mosquera, *et al.*, *Nucleic Acids Res.*, 2024, **52**, D1180–D1192.
- 316 J. J. Irwin and B. K. Shoichet, *J. Chem. Inf. Model.*, 2005, **45**, 177–182.
- 317 T. Liu, L. Hwang, S. K. Burley, C. I. Nitsche, C. Southan, W. P. Walters and M. K. Gilson, *Nucleic Acids Res.*, 2025, **53**, D1633–D1644.
- 318 C. Knox, M. Wilson, C. M. Klinger, M. Franklin, E. Oler, A. Wilson, A. Pon, J. Cox, N. E. Chin and S. A. Strawbridge, *et al.*, *Nucleic Acids Res.*, 2024, **52**, D1265–D1275.
- 319 Y. Shi, P. Ren, Y. Zhang, X. Gong, M. Hu and H. Liang, *Front. Res. Metrics Anal.*, 2021, **6**, 670006.

- 320 A. Cherkasov, E. N. Muratov, D. Fourches, A. Varnek, I. I. Baskin, M. Cronin, J. Dearden, P. Gramatica, Y. C. Martin and R. Todeschini, *et al.*, *J. Med. Chem.*, 2014, **57**, 4977–5010.
- 321 A. Gangwal, A. Ansari, I. Ahmad, A. K. Azad, V. Kumarasamy, V. Subramaniam and L. S. Wong, *Front. Pharmacol.*, 2024, **15**, 1331062.
- 322 D. Crucitti, C. Pérez Miguez, J. A. Daz Arias, D. B. Fernandez Prada and A. Mosquera Orgueira, *Front. Hematol.*, 2024, **3**, 1305741.
- 323 T. Gupta, P. Sharma, S. Malik and P. Pant, *Mol. Pharm.*, 2025, 4076–4090.
- 324 T.-G. Chang, S. Park, A. A. Schäffer, P. Jiang and E. Ruppin, *Nat. Cancer*, 2025, 1–15.
- 325 S. Gupta, V. K. Mohani, G. Ghislat, P. J. Ballester and S. Ahmad, *NAR: Genomics Bioinf.*, 2025, **7**, lqaf111.
- 326 T. Lazar, A. Connor, C. F. DeLisle, V. Burger and P. Tompa, *Nat. Rev. Drug Discovery*, 2025, 1–21.
- 327 A. van der Vaart, *Biochim. Biophys. Acta, Gen. Subj.*, 2015, **1850**, 1091–1098.
- 328 J. E. Darnell Jr, *Nat. Rev. Cancer*, 2002, **2**, 740–749.
- 329 R. Edaibis, R. Akel and J. A. Shin, *Transcription*, 2025, **16**, 67–85.
- 330 Q. Sun, H. Wang, J. Xie, L. Wang, J. Mu, J. Li, Y. Ren and L. Lai, *Chem. Rev.*, 2025, **125**(13), 6309–6365.
- 331 N. S. Gavande, P. S. VanderVere-Carozza, K. S. Pawelczak, P. Mendoza-Munoz, T. L. Vernon, L. A. Hanakahi, M. Summerlin, J. R. Dynlacht, A. H. Farmer and C. R. Sears, *et al.*, *Nucleic Acids Res.*, 2020, **48**, 11536–11550.
- 332 N. Y. Ngoi, D. Gallo, C. Torrado, M. Nardo, D. Durocher and T. A. Yap, *Nat. Rev. Clin. Oncol.*, 2025, **22**, 46–64.
- 333 T. A. Yap, P. LoRusso, R. E. Miller, R. Kristeleit, A. G. Paulovich, S. McMorn, L. Oplustil O'Connor, B. Lombardi, P. Marco-Casanova and E. T. Gangl, *et al.*, *Br. J. Cancer*, 2025, 1–10.
- 334 S.-H. Chen and J.-Y. Chang, *Int. J. Mol. Sci.*, 2019, **20**, 4136.
- 335 L. Dong, H. Jiang, Z. Kang and M. Guan, *Clin. Chim. Acta*, 2023, **544**, 117338.
- 336 N. J. Curtin, L.-Z. Wang, A. Yiakouvaki, S. Kyle, C. A. Arris, S. Canan-Koch, S. E. Webber, B. W. Durkacz, H. A. Calvert and Z. Hostomsky, *et al.*, *Clin. Cancer Res.*, 2004, **10**, 881–889.
- 337 R. C. van Waardenburg and E. S. Yang, *Cancer Drug Res.*, 2021, **4**, 837.
- 338 S. Liu, J. Li, Y. Wang, Z. Zhang, Y. Wang, L. Wang and L. Wang, *Small Methods*, 2025, **9**, e01445.
- 339 W. Li, J. Li, L. Wang, Y. Wang, Z. Zhang, S. Liu, B. Gong, Y. Wang and L. Wang, *Nano Lett.*, 2025, **25**, 1706–1714.
- 340 Y. Su, K. Ye, J. Hu, Z. Zhang, Y. Wang, B. Geng, D. Pan and L. Shen, *Adv. Healthcare Mater.*, 2024, **13**, 2304648.
- 341 M. Stepie, J. Zajda, B. K. Keppler, A. R. Timerbaev and M. Matczuk, *Talanta*, 2025, 128331.
- 342 Y. Lin, Q. Shi, G. Yang, F. Shi, Y. Zhou, T. Wang, P. Xu, P. Li, Z. Liu and H. Sun, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**, e2213670120.
- 343 M. Sands, M. A. Kron and R. B. Brown, *Rev. Infect. Dis.*, 1985, **7**, 625–6344.
- 344 N. Rastegar-Pouyani, M. A. Farzin, J. Zafari, M. Haji Abdolvahab and S. Hassani, *J. Transl. Med.*, 2025, **23**, 258.
- 345 H. Jung and Y. Lee, *Cancers*, 2025, **17**, 1871.
- 346 S. Li, X. Wang, J. Huang, X. Cao, Y. Liu, S. Bai, T. Zeng, Q. Chen, C. Li and C. Lu, *et al.*, *Cell Death Dis.*, 2025, **16**, 197.
- 347 C. Kang and W. Xu, *J. Med. Chem.*, 2025, **68**, 2048–2051.
- 348 Y. Tao, Y. Lu, B. Yu and Y. Wang, *Trends Pharmacol. Sci.*, 2025, 520–534.
- 349 X. Zhang, Z. Song, X. Zhang, J. Zou, G. Yu, X. Chen and S. Wang, *Angew. Chem., Int. Ed.*, 2025, **64**, e202507702.
- 350 X. Yang, H. Zhu, L. Shi, T. Song, W. Gong, S. He, S. Shan, C. Xu and Z. Zhou, *Nucleic Acids Res.*, 2025, **53**, gkaf735.
- 351 L. Gerasimavicius, S. C. Biddie and J. A. Marsh, *Nucleic Acids Res.*, 2026, **54**(1), gkaf1417.
- 352 P.-Y. Lin, S.-C. Huang, K.-L. Chen, Y.-C. Huang, C.-Y. Liao, G.-J. Lin, H. Lee and P.-Y. Chen, *Botanical Studies*, 2025, **66**, 14.
- 353 M. R. Karim, T. Islam, M. Shajalal, O. Beyan, C. Lange, M. Cochez, D. Rebholz-Schuhmann and S. Decker, *Briefings Bioinf.*, 2023, **24**, bbad236.
- 354 A. Blanco-Gonzalez, A. Cabezon, A. Seco-Gonzalez, D. Conde-Torres, P. Antelo-Riveiro, A. Pineiro and R. Garcia-Fandino, *Pharmaceuticals*, 2023, **16**, 891.
- 355 F. J. Ferreira and A. S. Carneiro, *ACS Omega*, 2025, **10**(23), 23889–23903.