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BENZIMIDAZOLE SCAFFOLDS IN PROTAC DESIGN: BRIDGING E3 LIGASE RECRUITMENT AND TARGETED PROTEIN DEGRADATION – A REVIEW

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ABSTRACT

Benzimidazole scaffolds have gained renewed attention in the field of targeted protein degradation, particularly within the design of proteolysis-targeting chimeras (PROTACs). These heterobifunctional molecules operate by simultaneously engaging a protein of interest and an E3 ubiquitin ligase, ultimately leading to selective proteasomal degradation of the target protein. The structural features of benzimidazoles, including their planar heteroaromatic core, hydrogen-bonding potential, and synthetic flexibility, make them attractive for incorporation into both warhead and E3 ligase ligand domains of PROTACs. Recent advances have demonstrated that benzimidazole-based cereblon ligands can serve as alternatives to classical immunomodulatory imide drugs, offering opportunities to modulate binding profiles and reduce off-target degradation. Moreover, benzimidazole-containing inhibitors of kinases, bromodomains, and other therapeutic targets have been successfully adapted into PROTAC architectures. This review highlights current design strategies, structure-activity relationships, and emerging trends, underscoring the potential of benzimidazoles to expand the chemical diversity and therapeutic scope of PROTAC technology.

KEYWORDS: Benzimidazole, Chemical diversity, E3 ligase, Heterobifunctional, PROTACs.

INTRODUCTION

Proteolysis-targeting chimeras (PROTACs) have emerged as a promising modality for targeted protein degradation, offering a strategy to eliminate disease-associated proteins through the ubiquitin–proteasome system.[1] These hetero bifunctional molecules are composed of a ligand for a protein of interest (POI), a ligand for an E3 ubiquitin ligase, and a linker that connects these two functional domains.[2] The induced proximity between POI and the E3 ligase facilitates ubiquitination and subsequent proteasomal degradation of the target protein.[3] This catalytic mechanism enables PROTACs to function at sub-stoichiometric concentrations, potentially reducing the dosing frequency and overcoming the limitations of conventional occupancy-driven inhibitors.[4]

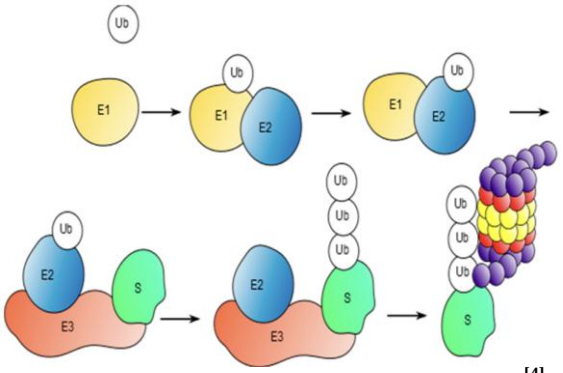

Figure 01: Overview of the ubiquitin system.[4]

Benzimidazole is a fused bicyclic heteroaromatic scaffold consisting of a benzene ring fused to an imidazole ring, known for its structural similarity to purine nucleobases.[5] Due to its rigid planar structure, hydrogen-bonding potential, and ease of chemical modification, benzimidazole has been extensively explored in medicinal chemistry.[6] It has been incorporated into inhibitors of kinases, bromodomains, tubulin, and other therapeutic targets.[7] More recently,

benzimidazole derivatives have been identified as novel cereblon (CRBN) ligands, enabling their use as E3 ligase recruiters in PROTAC design.[8]

This expansion of CRBN ligand chemistry beyond thalidomide-based immunomodulatory drugs (IMiDs) addresses issues such as off-target neo-substrate degradation and poor pharmacokinetic properties.[9]

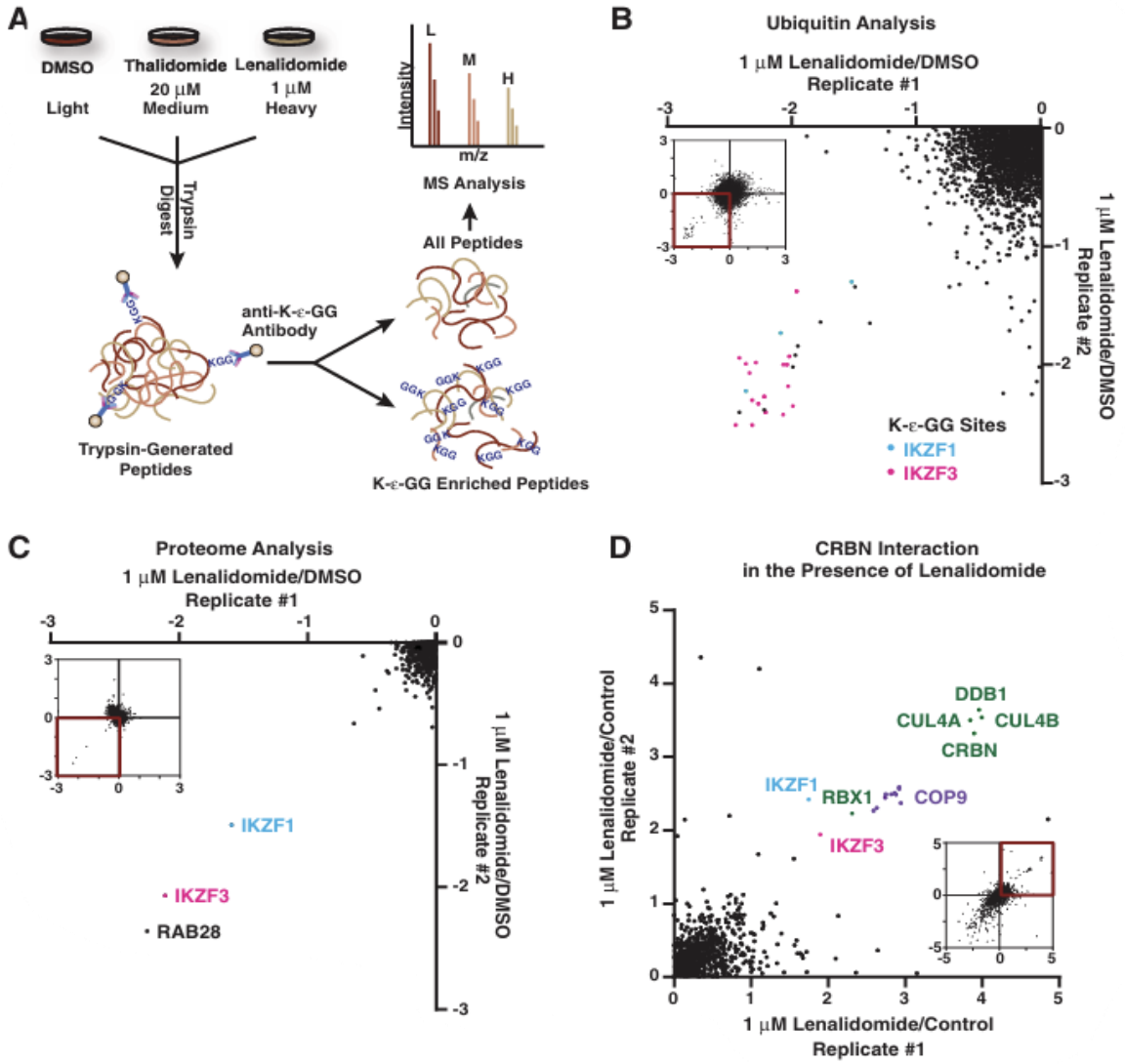

Figure 02: Proteomic analysis of lenalidomide-induced changes in ubiquitination, protein abundance, and CRBN interaction in MM1S cells.[9]

Benzimidazole as an E3 Ligase Ligand in PROTACs

Cereblon, a substrate receptor of the CRL4-CRBN E3 ubiquitin ligase complex, has been the most widely used ligase in PROTAC development due to the availability of high-affinity small-molecule ligands.[10] Traditional CRBN ligands such as thalidomide, lenalidomide, and pomalidomide are based on the glutarimide and phthalimide motifs.[11] However, these scaffolds induce degradation of endogenous neo-substrates such as Ikaros (IKZF1) and Aiolos (IKZF3), which can lead to undesirable immunomodulatory effects.[12] Benzimidazole-based CRBN ligands have emerged as alternative chemotypes that can retain cereblon binding affinity while modulating neo-substrate recruitment profiles.[13] In one study, phenyl-substituted benzimidazole derivatives were synthesized and shown to bind CRBN, leading to the development of BRD4-targeting PROTACs with potent degradation activity in cellular assays.[14] Structural modelling suggested that the benzimidazole core could occupy the CRBN binding pocket similarly to IMiDs, with potential for tuning interactions through substituent variation.[15]

Benzimidazole as a POI Warhead in PROTACs

Beyond E3 ligase binding, benzimidazole scaffolds have been widely used as POI ligands in PROTACs targeting various proteins. Kinase inhibitors incorporating benzimidazole cores, such as those targeting BCR-ABL, VEGFR, and EGFR, have been adapted into PROTAC architectures.[16] For example, benzimidazole-derived

kinase inhibitors with solvent-exposed substituents have been functionalized with linkers to connect to CRBN or VHL ligands, resulting in degradation of kinase targets *in-vitro*.[17] Similarly, benzimidazole-containing bromodomain inhibitors have been converted into PROTACs that degrade BRD2, BRD3, and BRD4.[18] The synthetic approach typically involves identifying a solvent-accessible position on the benzimidazole ring system to attach the linker without compromising POI binding affinity.[19]

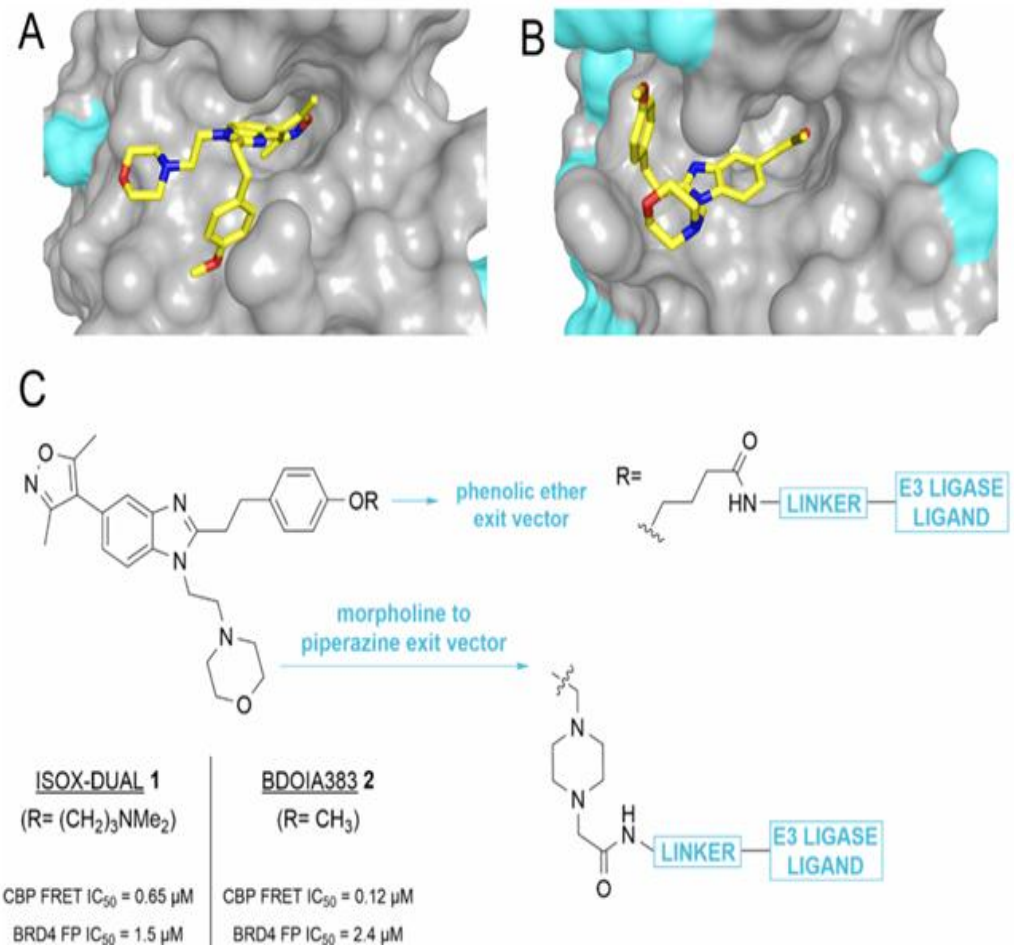

Figure 03. Choice of the starting scaffold and exit vectors. (A) Crystal structure of the CBP bromodomain with bound inhibitor 2 (5CGP). (B) Structure of the first bromodomain of BRD4 (5CFW) with the same inhibitor, showing ether (OMe) and morpholine solvent-exposed prospective exit vectors. Surface lysines are shown in cyan. (C) Structure of ISOX-DUAL 1 and 2 with representative structural design of the proposed phenolic ether and piperazine exit vectors.[33]

Linker Design Considerations

The linker is critical for PROTAC activity, influencing ternary complex formation, degradation efficiency, and selectivity.[20] When using benzimidazole-based ligands, linker attachment points are often chosen at the 2-, 5-, or 6-position to avoid disrupting key hydrogen bonds with the target.[21] Polyethylene glycol (PEG) linkers are commonly used to impart flexibility and solubility[22], while alkyl or rigid aromatic linkers can be used to restrict conformational freedom and improve ternary complex stability.[23] Structure-based design and linker scanning remain essential for optimizing benzimidazole PROTACs.[24]

CASE STUDIES OF BENZIMIDAZOLE PROTACS

Case 1 - BRD4 degradation with benzimidazole CRBN ligands: A phenyl-substituted benzimidazole CRBN ligand linked to a JQ1-based bromodomain inhibitor resulted in potent BRD4 degradation with DC_50 values in the low nanomolar range.[14]

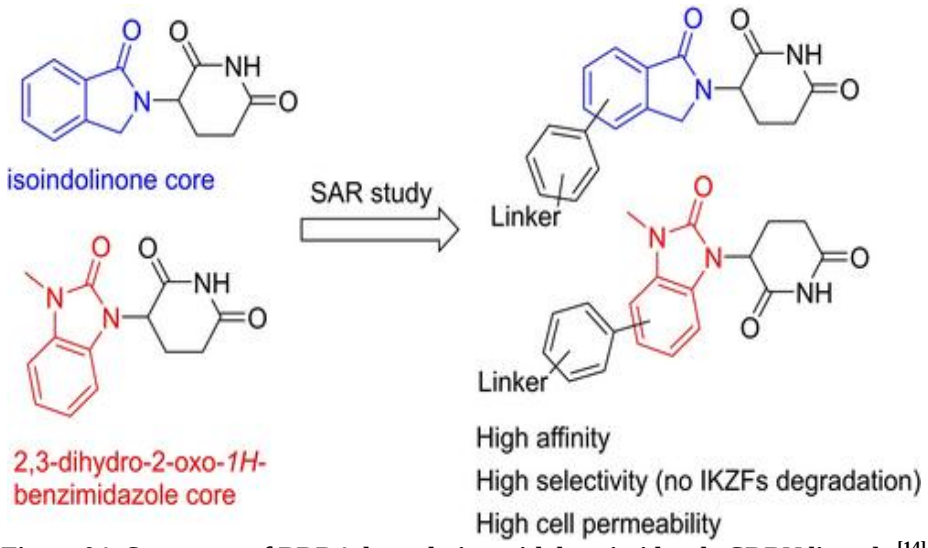

Figure 04: Structures of BRD4 degradation with benzimidazole CRBN ligands.[14]

Case 2 - Kinase-targeting benzimidazole PROTACs VEGFR2 inhibitors containing a benzimidazole scaffold were modified at the solvent-exposed end to attach a PEG-based linker and pomalidomide ligand, achieving selective VEGFR2 degradation in endothelial cells.[17]

Case 3 - HDAC6 degradation: A benzimidazole-containing hydroxamate was used as the HDAC6-binding warhead, linked to a CRBN ligand, leading to selective HDAC6 degradation without affecting HDAC1 or HDAC2.[25]

CHALLENGES AND LIMITATIONS

Despite their promise, benzimidazole PROTACs face certain challenges. The relatively rigid aromatic core may limit linker attachment options without loss of binding affinity.[26] Moreover, benzimidazole derivatives often require fine-tuning of lipophilicity to ensure adequate cell permeability for large PROTAC molecules.[27] Off-target effects due to CRBN neo-substrate degradation, although potentially reduced with benzimidazole ligands, still need comprehensive profiling.[28]

FUTURE DIRECTIONS

Further optimization of benzimidazole CRBN ligands is expected to produce scaffolds with improved selectivity and pharmacokinetic profiles.[29] Structural biology, particularly crystallography of benzimidazole-CRBN complexes, will guide rational ligand design.[30] Artificial intelligence and computational modelling are likely to accelerate linker and ligand optimization.[31] Expansion of benzimidazole use to other E3 ligases beyond CRBN could diversify degradation profiles and broaden therapeutic applicability.[32]

CONCLUSION

Benzimidazoles have proven to be a valuable scaffold in PROTAC development, offering strong target-binding potential, structural flexibility, and favourable drug-like properties. Their integration as warheads or linker components has led to degraders with improved potency, selectivity, and stability. Ongoing advances in structure-based design and linker optimization are expected to expand their therapeutic applications, positioning benzimidazole-based PROTACs as promising candidates for next-generation targeted protein degradation strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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