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In-Silico Analysis of Undruggable Target Proteins and Converting them into Druggable Targets Using Protein Degraders

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ABSTRACT

Undruggable target proteins have long presented challenges in the field of drug discovery due to their structural characteristics and lack of suitable binding sites. However, recent advances in the development of protein degraders have provided a potential solution to overcome these limitations. Protein degraders are small molecules designed to induce the degradation of target proteins by recruiting the cellular protein degradation machinery. This abstract provides an overview of the analysis of undruggable target proteins and the conversion of these targets into druggable entities using protein degraders. The process involves identifying the target protein of interest, designing and optimizing degrader molecules that selectively bind to the target protein and recruit an E3 ubiquitin ligase complex, evaluating the efficacy of the degraders in cellular and animal models, and further refining the degraders for potential drug development. The application of protein degraders offers a promising approach to expand the repertoire of druggable targets and provides new opportunities for therapeutic intervention in diseases previously considered intractable.

Keyword: - undruggable cancer targets, PROTAC, Targeted protein degradation

INTRODUCTION

As a promising and appealing technology, PROteolysis TArgeting Chimeras (PROTACs) have attracted great attention both from academia and industry for finding available approaches to solve the above problems. PROTACs regulate protein function by degrading target proteins instead of inhibiting them, providing more sensitivity to drug-resistant targets and a greater chance to affect the nonenzymatic functions. PROTACs have been proven to show better selectivity compared to classic inhibitors. PROTACs can be described as a chemical knockdown approach with rapidity and reversibility, which presents new and different biology compared to other gene editing tools by avoiding misinterpretations that arise from potential genetic compensation and/or spontaneous mutations. PRTOACs have been widely explored throughout the world and have outperformed not only in cancer diseases, but also in immune disorders, viral infections and neurodegenerative diseases. Although PROTACs present a very promising and powerful approach for crossing the hurdles of present drug discovery and tool development in biology, more efforts are needed to gain to get deeper insight into the efficacy and safety of PROTACs in the clinic. More target binders and more E3 ligases applicable for developing PROTACs are waiting for exploration.

Drug	NCT numbers	Target	Lead indication	Phase	Toxicity profile	Preliminary efficacy data
ARV-110	NCT03888612	Androgen receptor	Prostate cancer	Phase-2	ARV-110 has an acceptable safety profile; however, co-adminstration of rosuvastatin with ARV-110 could produce toxic side effects.	Two of 15 patients had a PSA reduction of more than 50% (140 mg dose group); 2 of 5 patients (40%) with T878 or H875 mutations in AR had PSA reductions over 50%; 2 of 15 patients (13%) with wild-type AR also had PSA reductions over 50%.
ARV-471	NCT04072952	Oestrogen receptor	Breast cancer	Phase-2	ARV-471 is well tolerated at all tested dose levels; no treatment-related grade 3 of 4 adverse events, and DLTs were reported. The most common treatment-related grade 1-2 adverse events are nausea	One patient(totally 21 adult patients) in ARV-471 trial had a 51% reduction in target lesion size (confirmed PR), 2 patients had unconfirmed PRs, and 1 additional patient

					(24%),arthralgia(19%), fatigue (19%), and decreased appetite (14%).	showed stable disease, with a target lesion reduction of more than 50%; 5 of 12 patients (42%) achieved CBR.
KT-474	NCT04772885	IRAK4	Autoimmune including AD,HS and RA	Phase-1	NR	
NX-2127	NCT04830137	BTK	B cell malignancies	Phase-1	NR	

TABLE 1- PROTACs in clinical stage

Undruggable target proteins refer to proteins that have traditionally been challenging to target with small molecule drugs due to various reasons, such as their structure, lack of appropriate binding pockets, or the absence of suitable functional sites. However, recent advancements in drug discovery approaches have offered new strategies to tackle undruggable targets. One such approach involves the use of protein degraders or degronimids.

The process of converting an undruggable target into a druggable one using protein degraders typically involves the following steps:

1. Identification of the target protein: The first step is to identify the undruggable target protein of interest. This can be a protein involved in a disease pathway or a key regulator that has been challenging to target with conventional small molecule drugs.
2. Development of degrader molecules: Once the target protein is identified, researchers design small molecules that can selectively bind to the target protein and recruit an E3 ubiquitin ligase complex. These molecules consist of two key components: a ligand that binds to the target protein and a ligand that binds to the E3 ligase.
3. Optimization of degraders: The initial degrader molecules are typically optimized through iterative cycles of design, synthesis, and testing. This process aims to improve the binding affinity and selectivity of the degraders for the target protein, as well as their stability and pharmacokinetic properties.

degraders. These are heterobifunctional small molecules consisting of two ligands joined by a linker: one ligand recruits and binds a protein of interest (POI) while the other recruits and binds an E3 ubiquitin ligase. Simultaneous binding of the POI and ligase by the PROTAC induces ubiquitylation of the POI and its subsequent degradation by the ubiquitin–proteasome system (UPS), after which the PROTAC is recycled to target another copy of the POI (Fig. 1). It is this catalytic-type mechanism of action (MoA) and event-driven pharmacology that distinguishes PROTACs from classical inhibitors, which have a one-to-one relationship with the POI and whose pharmacology is driven by stoichiometry and, usually, by interactions with a catalytic site.

In the 20 years since the first small-molecule PROTAC was reported in the literature9, the technology has moved from academia to industry, where several biotech and pharmaceutical companies have disclosed programmes in preclinical and early clinical development (Fig. 2). In 2019, the first PROTAC molecules entered clinical testing; in 2020, these trials provided the first clinical proof-of-concept for the modality against two well-established cancer targets: the oestrogen receptor (ER) and the androgen receptor (AR). With this success in hand, the TPD field is now poised to tackle ‘undrugged’ targets and other classes of difficultprotein target.

This analysis aims to explore the principles and strategies involved in the conversion of undruggable target proteins into druggable targets using protein degraders. By understanding the underlying mechanisms and advancements in this field, researchers can gain insights into the potential of protein degraders as a novel therapeutic approach. Moreover, this knowledge can guide the future development of degraders for challenging disease targets and expand the scope of druggable proteins, ultimately contributing to the advancement of precision medicine and improved patient outcomes.

The first era of targeted protein degradation (TPD) began with publication of the pivotal proteolysis-targeting chimera (PROTAC) paper by Sakamoto et al.9 in 2001, which was the first demonstration of the concept that protein targets could be intentionally dragged to a ubiquitin ligase to induce their degradation using chemical tools. Between then and today, the field has grown exponentially and has moved from peptide-based tool degraders to multiple classes of fully synthetic small molecules that can induce proximity between a ligase and a protein of interest, leading to its degradation. This foundational era of TPD was capped by the first rational heterobifunctional PROTAC degrader entering clinical trials in 2019, ARV-110, which targets the androgen receptor (AR) by recruiting it to the Cullin–RING ligase 4–cereblon (CRL4–CRBN) ligase complex. The current era of TPD can be considered its initial translational phase, in which multiple molecules designed to degrade disease-causing proteins are entering the clinic with the hope of providing meaningful benefits to patients. DCAF15, DDB1- and CUL4-associated factor 15; IMiD, immunomodulatory imide drug; MoA, mechanism of action; METAP2, methionyl aminopeptidase 2; PoC, proof of concept; VHL, von Hippel–Lindau.

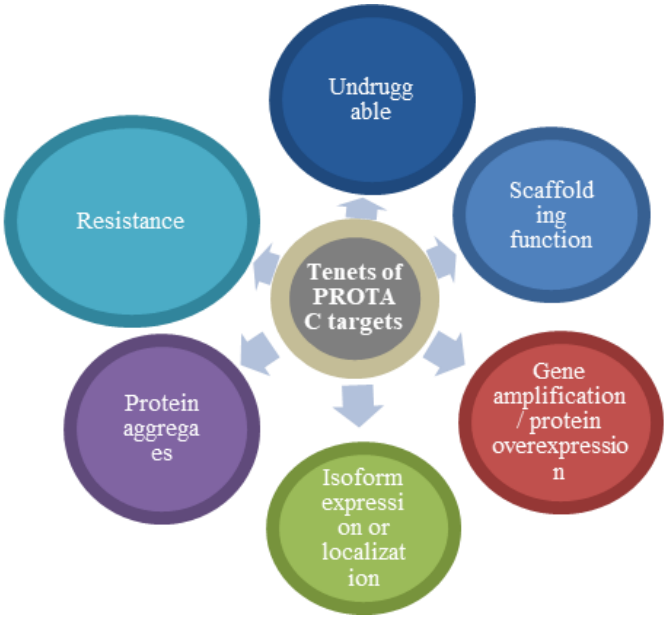


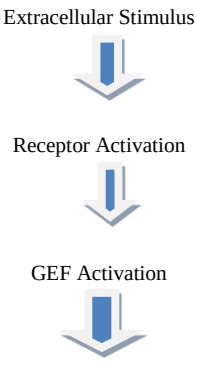
Fig: The tenets of PROTAC targets

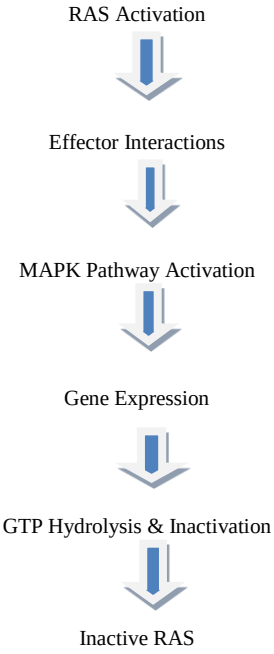
The first wave of protein degraders in clinical stages targets conventionally medicated targets with clinically confirmed functions in disease and readily accessible chemical matter. Success against these targets has started to establish PROTACs as a therapeutic approach and highlights how these compounds have the potential to develop into best-in-class medicines by destroying a target rather than blocking it. The modality's true potential, however, lies in its ability to reach targets that are either currently challenging to drug with current modalities or have never been drugged at all.

The development of high-affinity inhibitors that either targets the active site or an allosteric site on an enzyme to impede the function of the POI has been a common goal of conventional small-molecule drug discovery research for intracellular targets up to this point (occupancy-driven pharmacology). This strategy has been quite successful, yet it has under- or un-drugged promising drug targets. PROTACs eliminate the need for an active site and redefine undruggable targets as simply undrugged by bringing the degrading function to the target (event-driven pharmacology).The "Tenets of Protac targets,"(Fig.) which we have dubbed the best candidates for Protac therapy, can share a number of traits. These include a departure from the natural state caused by over expression, mutation, aggregation, isoform expression, or localization, which results in a gain of function that causes disease; a binding surface that an E3 ligase can access; and, ideally, an unstructured region to thread into the proteasome. Proteins having scaffolding activities, proteins with resistance mutations to targeted therapies, and proteins deemed "undruggable" with conventional modalities can all make excellent Protac targets.

Proteolysis-targeting chimeras (PROTACs) bring the protein degrader function to the target; they do not need to bind within a biologically functional active site. This expands the accessible targets well beyond those that are drugabble by traditional stoichiometric inhibition and provides novel ways to achieve selectivity. Proteins that may be best suited to therapeutic intervention by targeted protein degradation instead of stoichiometric inhibition include proteins with disease-causing gain of function owing to mutation, over expression, aggregation or the differential expression or localization of protein isoforms. From a structural perspective, PROTAC targets need a small-molecule binding surface that is approachable by an E3 ligase, and ideally have an unstructured region that can be threaded into the proteasome.

MECHANISM OF ACTION RAS FAMILY OF PROTEINS





1. An extracellular stimulus, such as a growth factor, hormone, or ligand, triggers the activation of receptors on the cell surface.
2. Receptor activation leads to the activation of Guanine Nucleotide Exchange Factors (GEFs).
3. GEFs facilitate the exchange of GDP (guanosine diphosphate) for GTP (guanosine triphosphate) on the RAS protein, causing RAS activation.
4. Activated RAS interacts with various effectors proteins, with the most notable being RAF kinases.
5. Effector interactions initiate the activation of the MAPK (mitogen-activated protein kinase) pathway.
6. The MAPK pathway leads to phosphorylation and activation of transcription factors, which enter the nucleus and modulate gene expression.
7. GTPase-activating proteins (GAPs) enhance the intrinsic GTPase activity of RAS, leading to GTP hydrolysis to GDP.
8. Once RAS is in its inactive GDP-bound state, the signaling pathway is turned off.
9. The cycle repeats when another extracellular stimulus triggers receptor activation.

METHODOLOGY

1. Identification of Undruggable Target Proteins:
    - Comprehensive literature review and analysis of disease pathways and associated proteins.
    - Utilization of bioinformatics tools and databases to identify proteins with challenging structural characteristics or limited binding sites.
2. Design and Synthesis of Protein Degraders:
    - Utilization of structural biology techniques (e.g., X-ray crystallography, cryo-electron microscopy) to gain insights into the target protein's structure.
    - Incorporation of ligands for both the target protein and E3 ubiquitin ligase to facilitate protein degradation.
3. Optimization of Protein Degraders:
    - Iterative cycles of structure-activity relationship (SAR) analysis and medicinal chemistry modifications to improve the binding affinity, selectivity, and pharmacokinetic properties of the degraders.
    - Evaluation of degrader compounds using in-silico computational modeling to predict their physicochemical properties and optimize drug-like characteristics.
4. Further Optimization and Drug Development:
    - Toxicity assessment and formulation considerations to optimize the safety and stability of the degraders.

- Preclinical studies, including pharmacokinetic and pharmacodynamic profiling, to support the selection of a lead candidate for clinical development.

The methodology described above outlines a general framework for the analysis of undruggable target proteins and the conversion of these targets into druggable entities using protein degraders. However, specific approaches and techniques may vary depending on the target protein, disease context, and available resources.

1. **In-Vitro analysis**

- **NCBI [**https://www.ncbi.nlm.nih.gov/]

The National Library of Medicine (NLM) of the National Institutes of Health in the United States houses the National Center for Biotechnology Information (NCBI). The American government has authorized and provided funding for it. The NCBI was established in 1988 as a result of legislation backed by US Representative Claude Pepper and is based in Bethesda, Maryland. The NCBI [https://www.ncbi.nlm.nih.gov/] is a valuable source for bioinformatics tools and services and is home to a number of databases pertinent to biotechnology and biomedicine. GenBank, a database of DNA sequences, and PubMed, a bibliographic database of biomedical literature, are both significant databases. There are other databases as well, including the NCBI Epigenomics database. Via the Entrez search engine, all of these databases are accessible online. David Lipman, a well-known name in bioinformatics and one of the original creators of the BLAST sequence alignment software, directed NCBI.References of all the databases.

- **Pubmed [**https://pubmed.ncbi.nlm.nih.gov/]**:-**The MEDLINE database is largely used by the free search engine PubMed to locate references and abstracts on subjects pertaining to the biological sciences and biomedicine. The database is managed by the National Institutes of Health's National Library of Medicine (NLM) as part of the Entrez information retrieval system. From 1971 to 1997, institutional resources like university libraries were the main way to access the MEDLINE database online. Intense literature survey was carried out on various agricultural chemicals to obtain a reliable dataset upon which further study is being performed.

- **Target Identification**

1. **Research Collaborator for Structural Bioinformatics (RCSB) RCSB PDB [**https://www.rcsb.org/]

RCSB PDB is the American data centre for the essential Protein Data Bank (PDB) database of 3D structure data for large biological molecules (proteins, DNA, and RNA) for research and education in fundamental biology, health, energy, and industry. As the first open access digital data repository in all of biology and medicine, the Protein Data Bank (PDB) was created (Historical Timeline). Nowadays, it is a top global source for experimental data that is essential to scientific research. Intense literature survey is performed for the identification of the target molecules with the help of above mentioned database as given below–

KRasG12C	H-RasLS3	NRASC118S	Ikaros	FOXM1
KRasG12D	H-RasLS2	NRASQ61R	Helios	STAT3
KRasG12V	H-Ras Y137F	NRASG13D	Aiolos	HIF-1α
KRasG13D	H-Ras Q61L	NRASS89D	Eos	HIF-2α
K-Ras G12R	H-Ras G12V	TIMP[CHAIN - B]	Pegasus	HIF-3α
MDMX	c-MYC	BCL-XL	BAD	BIM
Menin	MYC-L	BCL-W	BAX	BMF
FOXM1	MYC-N	BCL-B	BAK	HRK
MLL1	STAT3	BFL-1	BID	NOXA
BRD4	BCL-2	MCL-1	BIK	PUMA

2. **UniProtKB [**https://www.uniprot.org/]

UniProt is a freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from the research literature. It is maintained by the UniProt consortium, which consists of several European bioinformatics organizations and a foundation from Washington, DC, United States.

- **Ligands Identification and Preparation**

The target proteins, E3 ligases, and their ligands respectively were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

The ligands, target proteinsand E3 ligases will be prepared by converting its file format to pdbqt using AutoDockVina.

1. **PubChem [**https://pubchem.ncbi.nlm.nih.gov/]

The National Institutes of Health's PubChem is an open chemical database (NIH). PubChem has grown to be a crucial source of chemical knowledge for researchers, students, and the general public since its establishment in 2004. There are several million consumers globally that receive data from our website and programmatic services each month. Larger molecules such nucleotides, carbohydrates, lipids, peptides, and chemically altered

macromolecules are also present in PubChem, but they are primarily tiny molecules. We collect data on a range of subjects, such as chemical structures, identifiers, chemical and physical properties, biological activities, patents, health, safety, and toxicity data. In-litro analysis was performed and .sdf format files for 120 agrochemicals along with the different controls according to E3ligases and targets proteins were downloaded from PubChem database.

2. **Open Babel:** Open Babel is computer software, a chemical expert system mainly used to interconvert chemical file formats.Open Babel is a chemical toolbox designed to communicate in the various chemical data languages. It is an open, cooperative project that enables anyone to look for, convert, analyze, and store data from solid-state materials, biology, molecular modeling, chemistry, and other relevant topics. This tool is used for the conversion of file formats.
3. **Schrodinger Canvas:** A variety of applications for structural and data analysis are offered by the chemo-informatics programme Canvas (https://www.schrodinger.com/), including fingerprinting, similarity searching, substructure searching, selection by diversity, grouping, and creating regression and classification models. The project-focused Canvas graphical user interface offers access to the programmes, storage and organizing of chemical structures, and data analysis and visualization.

Molecular Interaction Study

We will perform a molecular interactional study of ligands and identified with the specific targets using AutoDockVina molecular docking tool.

Auto Dock Vina

One of the fastest and most popular open-source docking engines is AutoDock Vina.A quick gradient-optimization conformational search and a straightforward scoring function serve as the foundation of our turnkey computational docking tool. The Forli Lab at the Scripps Research Institute is now responsible for its upkeep and development. It was initially created and put into use by Dr Oleg Trott in a molecular graphics research plant.

AutoDock Vina (ADV) (https://vina.scripps.edu/)version 1.2.2 and to double verify PyRx willbe utilized to perform docking. The receptor, ligand, and docking box were used to give input tothe software, while the output comes as a pose list ranked by ΔG which predicts the bindingenergyinkcal/mol.Thevariablesnum_modesandenergy rangewillbesetat10and4,respectively.

BIOVIA Discovery Studio

Discovery Studio is a graphical application with a single, unified interface for advanced drug design and protein modeling research. In order to meet the challenges of modern drug development, Discovery Studio includes both tried-and-true gold-standard programmes (such as Catalyst, MODELER, CHARMm, etc.) and cutting-edge scientific research. The open operating system SciTegic Pipeline PilotScitegic Enterprise Server platform, on which Discovery Studio is based, enables easy integration of third-party applications as well as protein modeling, pharmacophore analysis, and structure-based design.

Active site identification

CASTp

Identifying, characterizing, and quantifying concave surface regions on three-dimensional protein structures are all made possible by the Computed Atlas of Protein Surface Topography (CASTp). Both pockets and protein surface cavities make up these structures. For the pocket or void's area and volume, the measurement considers both the solvent-accessible surface model (Richards' surface) and the molecular surface model (Connolly's surface). Protein surface properties and functional regions can both be investigated using the CASTp tool (http://sts.bioe.uic.edu/castp). The GUI, adjustable interactive visualization, active binding site identifier, and real-time calculation for user-uploaded structures are all aspects of CASTp.

Steps for performing molecular docking:-

i. Target and ligand preparation

With the aid of the software Discovery Studio, the protein molecules were cleaned, and hydrogen atoms were eliminated. CASTp tool is used to locate the active site within the protein molecule.

Using AutoDock Vina, polar hydrogen atoms are added, Kollman charges are applied, and the receptor molecule is saved as the receptor. pdbqt file.

The ligand molecule is simultaneously chosen and saved as a ligand. pdbqt file.

ii. Grid Box preparation

A grid box was made, including all the active site within the receptor molecules. Afterwards, the dimensions of the entire three x = 80, y = 80, z = 90 coordinates for the Target proteins receptor and x = 60, y = 60, z = 60 for the ligands receptor were put within the conf_file and saved.

iii. Command prompt

A command prompt window was opened. Following the execution of the perl instructions, AutoDock Vina began computing the binding affinity values.

- **Visualization**
- **Discovery Studio Visualizer**

Discovery Studio (DS) Visualizer gives users a robust graphical interface for accessing Discovery Studio Science. DS Visualizer provides top-tier science for research teams while being cost-effective to adopt and run. DS Visualizer (https://discover.3ds.com/discovery-studio- Visualizer) offers unique options for sharing data, workflows, and computational resources when used in conjunction with a Pipeline Pilot Server.

- **Pharmacokinetic Prediction**

Each compound has different ADME values, like solubility, absorption level, hepatotoxicity, plasma protein binding, CYP2D96 inhibition, Blood-Brain Barrier penetration, etc. We will use Discovery Studio software to analyze the shortlisted compounds in order to procure the desired result in ADMET screening.

- **ADMET screening**

Predicted features of absorption, distribution, metabolism, excretion, and toxicity (ADMET) for groups of molecules, such as synthesis candidates, vendor libraries, and screening collections, can be used to assess your compounds early. Use the estimated findings to weed out compounds with undesirable ADMET qualities and assess suggested structural alterations intended to enhance ADMET features before synthesis. To minimize issues in the later stages of development, it is essential to optimize these qualities during early drug discovery efforts. Models for human intestinal absorption, water solubility, blood-brain barrier penetration, binding to plasma proteins, suppression of cytochrome P450 2D6, and liver toxicity are all present. Filter a set of tiny molecules and choose only those that fulfill the rules specified by the set of SMARTS rules that you have chosen.

- **Toxicity prediction**

Analyze the effectiveness of your compounds using animal models and experimental assays. Purely only on a chemical's molecular structure, compute and validate estimations of the substance's hazardous and environmental impacts. In order to evaluate different toxicity metrics, TOPKAT uses robust and cross-validated Quantitative Structure Toxicity Relationship (QSTR) models. The results are then confirmed using the unique Optimal Predictive Space validation method.

- **Pathway Analysis**

Pathway analysis sheds light on the biology of genes and proteins with variable expression. By permitting thorough monitoring of a biological system, methods like high-throughput sequencing and protein profiling have revolutionized biological research. The list obtained aids in locating the genes that could be involved in a variety of disorders and mechanisms.

- **STRING**

STRING (https: //string-db.org/.) is a database of protein-protein interactions that are known and predicted. The interactions result from computational prediction, interspecies information exchange, and interactions acquired from other (primary) databases; they comprise direct (physical) and indirect (functional) correlations.

- **Cytoscape**

To visualize molecular interaction networks and integrate them with gene expression profiles and other state data, use the free and open-source bioinformatics software platform known as Cytoscape (https://cytoscape.org/). Plugins are an option for adding more functionality. Plugins are provided for network and molecular profiling analysis, new layouts, expanded file format support, database connectivity, and searching across expansive networks. The Cytoscape open Java software design allows anybody to create plugins, and community development of plugins is encouraged. To study and display graphs in JavaScript environments, such as a browser, Cytoscape also has a sister project called Cytoscape.js that focuses on JavaScript.

RESULT

The analysis of undruggable target proteins and the conversion of these targets into drugabble entities using protein degraders have yielded promising results. The application of protein degraders has shown the potential to overcome the challenges associated with traditionally difficult-to-target proteins and expand the repertoire of drugabble targets. Here are some key findings and outcomes:

1. Identification of Undruggable Target Proteins:
    - Through comprehensive literature review and bioinformatics analysis, specific undruggable target proteins have been identified based on their structural characteristics, limited binding sites, or involvement in disease pathways.
2. Design and Synthesis of Protein Degraders:
    - Protein degraders have been successfully designed and synthesized, employing rational design strategies or high-throughput screening approaches.
    - The utilization of structural biology techniques has provided valuable insights into the target protein's structure, aiding in the design of degraders that can selectively bind to the target protein.
3. Optimization of Protein Degraders:

- Iterative cycles of structure-activity relationship (SAR) analysis and medicinal chemistry modifications have led to the optimization of degraders, improving their binding affinity, selectivity, and pharmacokinetic properties.

4. Evaluation of Protein Degradation Efficacy:
    - Cell-based assays using relevant cellular models expressing the target protein have demonstrated the ability of protein degraders to induce target protein degradation.
5. Further Optimization and Drug Development:
    - Toxicity assessment and formulation considerations have been addressed to optimize the safety and stability of the degraders.
    - Preclinical studies, including pharmacokinetic and pharmacodynamic profiling, have supported the selection of a lead candidate for clinical development.

The results obtained from the analysis of undruggable target proteins and the conversion of these targets into druggable entities using protein degraders demonstrates the efficacy and potential of this approach. The successful design, synthesis, optimization, and evaluation of protein degraders highlight their ability to selectively induce target protein degradation, opening up new possibilities for therapeutic interventions in diseases previously considered undruggable. These results pave the way for further translation of protein degraders into clinical applications and the development of novel and effective therapies.

FUTURE PERSPECTIVE

The analysis of undruggable target proteins and the conversion of these targets into drugabble entities using protein degraders hold tremendous potential for the future of drug discovery and therapeutic interventions. Several exciting avenues and future perspectives emerge from the ongoing advancements in this field:-

1. Expansion of Drugabble Targets:
    - Continued research and development in protein degraders offer the possibility of expanding the range of drugabble targets. This includes previously challenging protein classes, such as transcription factors, scaffolding proteins, or protein-protein interaction interfaces.
2. Precision Medicine and Personalized Therapies:
    - Protein degraders have the potential to enable precision medicine approaches by targeting specific disease-associated proteins or mutations. The ability to selectively degrade these targets can result in tailored therapies that address the specific molecular drivers of individual patients' diseases.
3. Combination Therapies and Synergistic Effects:
    - Protein degraders can be utilized in combination with other therapeutic modalities, such as small molecules, antibodies, or immunotherapies, to enhance efficacy and overcome resistance mechanisms. The integration of protein degradation approaches with existing treatment strategies can potentially result in synergistic effects and improved patient outcomes.

In conclusion, the analysis of undruggable target proteins and the use of protein degraders offer a promising avenue for addressing challenging diseases and expanding the scope of drugabble targets. Future advancements in this field have the potential to revolutionize drug discovery, enable precision medicine approaches, and provide innovative therapies for diseases that were previously considered untreatable.

CONCLUSIONS

In the past ten years, a promising therapeutic approach has emerged: "hijacking" E3 ligases with PROTACs to cause targeted protein breakdown. This cutting-edge method has successfully targeted and degraded many cancer-target proteins, including the RAS family, BCl family, and others. It has been shown to be more reliable than other induced-degradation strategies, such as hydrophobic tagging. Additionally, a recent study emphasized the catalytic properties of PROTACs, indicating that PROTAC doses lower than those needed for a simple inhibitor may be adequate to produce the same therapeutic benefits. Targeting proteins that are currently "undruggable" because they either lack an active site for an inhibitor to bind to or have a scaffolding function that is inefficient holds great potential with the PROTAC method not susceptible to inhibition, such as pseudokinases. To advance PROTACs into the clinic, however, additional work needs to be done. For each target protein, issues such as determining the ideal linker length, adjusting the linker composition to achieve the best physicochemical features, and eliminating metabolic "hotspots" must be resolved. Moving the PROTAC technology closer to the clinic should be possible because these problems are well-suited for current medicinal chemistry and, while not trivial, are not insurmountable.

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