Drug Discovery Workflow for PROTAC with a Focus on Eliminating Bottlenecks in Evaporation

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An emerging modality of therapeutics called PROteolysis TArgeting Chimeras (PROTACs) could enable the design of new drugs targeting "undruggable" proteins, which make up to 85% of the proteome. Their novel mode of action allows them to hijack a cells natural function and initiate degradation of the target protein for a particular disease. This development has led to great interest in the pharmaceutical and biotechnology industries. However, synthesis of PROTACs requires numerous evaporation steps, causing bottlenecks in their production and slowing the drug development process. This article introduces PROTACs and the benefits they can offer to the drug discovery field, before discussing how the issues with their synthesis can be overcome.

Traditionally, pharmaceutical drugs are small molecules that competitively inhibit the active site of an enzyme target. The success of these types of agents has led to established methods for the drug development process, and therapeutics that have this occupancy-driven mode of action (MOA) make up the majority of drugs on the market today. However, this MOA has two significant disadvantages; high drug doses are generally required to achieve the therapeutic effect and the majority of pharmacological protein targets to not have enzymatic activity.

A New Therapeutic Modality

In 2001, the Crews and Deshaies laboratories reported a new technology called PROteolysis TArgeting Chimeras (PROTACs); heterobifunctional molecules that hijack the body's own natural disposal system to initiate selective degradation of the protein of interest (POI). This MOA offers the potential to target the "undruggable" proteome, which comprises of about 85% of human proteins, including scaffolding proteins, transcription factors and regulatory proteins. In addition, much lower drug concentrations need to be administered in order to achieve the therapeutic effect, promising fewer adverse effects and reducing the possibility of drug resistance.

The first PROTAC induced the degradation of methionine aminopeptidase-2 (a key enzyme in blood vessel development of solid tumor cancers) and was peptide based, as were many other early PROTACs. However, these compounds are more difficult for the body to absorb so research moved towards the development of small molecule based PROTACs. In 2008, the first all small molecule based PROTAC was reported, which degraded the androgen receptor – a transcription factor that regulates the development and growth of the prostate. A few years later, in 2013, the first evidence of PROTACs function inhibiting tumor growth was demonstrated *in vivo*. Since then many more PROTACs have been developed, targeting

a range of POIs including BCR-ABL (found in types of cancer such as leukemia), Tau (associated with Alzheimer's diseases and Parkinson's disease) and human epidermal growth factor 2 (indicative of breast cancer). Of this range of potential therapeutics, two PROTACs have now reached phase I clinical trials, with the promise of more to follow.

The Event-Driven Mode of Action

Proteins in the human body are continually degraded and replaced by newly synthesized molecules. The rate of protein degradation within cells varies, from minutes to days, depending on the molecules function. For example, regulatory proteins such as transcription factors have a rapid turnover to allow a fast response to external stimuli. This recycling process also enables faulty or damaged proteins to be removed in order to eliminate any future issues.

The majority of intracellular proteins are degraded by the ubiquitin-proteasome system (UPS). In this process, a group of enzymes – E1 (ubiquitin-activating enzyme), E2 (ubiquitin-carrier) and E3 (ubiquitin-protein ligase) – identify the proteins for degradation and tag them by attaching multiple ubiquitin molecules to their surface. These polyubiquitinated proteins are then recognized by the proteasome, a large complex that then degrades the polyubiquitinated protein into small peptides.

PROTACs consist of three components: an E3 ligand, a ligand for the POI, and a linker which joins the E3 ligand and the POI ligand together. The E3 ligand and POI ligand bind to their respective targets, to form a ternary complex. Once bound, an E3 and POI are in close proximity, triggering the E3 to transfer multiple ubiquitin molecules to the POI. This polyubiquitinated POI is then recognized and degraded by the proteasome as part of the UPS. After the POI is degraded, the drug is released to continue its degradation mission.

This event-driven MOA means that PROTAC molecules can cycle through multiple rounds of activity, removing super-stoichiometric quantities of the POI, unlike the traditional occupancy-driven drugs that work on a stoichiometric basis. In addition, hijacking the cells natural protein degradation mechanism does not trigger overexpression of the target protein in order to compensate for the loss of protein function, an action observed with drugs that inhibit a proteins active site. For these reasons, lower concentrations of PROTAC can be given in order to produce a therapeutic response, enabling low doses to be administered to patients, potentially reducing adverse effects and decreasing the possibility of drug resistance.

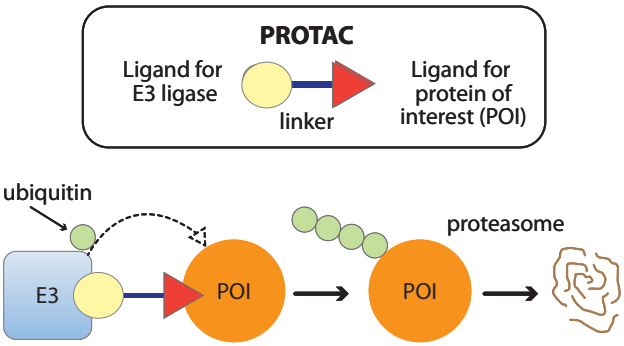


Figure 1: *The mechanism of action of a PROTAC. The heterobifunctional molecule comprises of an E3 ligase ligand (yellow oval), a chemical linker (blue bar) and ligand for the protein of interest (red triangle).*

PROTAC Synthesis

These heterobifunctional molecules emerged from university science labs, but the potential to target the "undruggable" proteome, coupled with the promising pre-clinical results, has led to all major pharmaceutical companies such as GSK, AZ, Pfizer and Bayer to invest in this emerging technology. However, developing the synthesis from small batches at the university to an industrialized, robust process that can produce high-quality products that meet the requirements for human administration is challenging. In addition, PROTAC molecules can be small molecule based or peptide based, so a multidisciplinary approach between chemistry and biology is required for their synthesis.

Evaporation Bottlenecks in PROTAC Synthesis

Production of both small molecule based and peptide based PROTACs require a number of evaporation steps; during synthesis, for removal of cleavage or deprotection groups; pre- and post-purification, for concentration of the crude mixture or the combined high performance liquid chromatography (HPLC) factions, respectively; and post-reformatting, for transforming the finished compound into the desired format for transportation. In the drug discovery process, thousands of potential compounds are initially synthesized for the first non-clinical in vitro testing steps before being whittled down to a promising few that enter the clinical testing phase. With the numerous evaporation steps required for PROTAC synthesis, these drying processes have been highlighted as a major bottleneck.

In addition, if these evaporation steps are performed poorly, the quality and integrity of the final product can be affected. There are several ways in which the compounds can be affected during evaporation. Firstly, many evaporators use heat lamps to speed up the evaporation process. This method directly heats the samples, but in an uncontrolled fashion, which can cause damage to the compounds. Secondly, different samples in the evaporators may dry at different rates which can lead to overheating of some compounds while others are still wet. If some samples have dried, but are still being heated because others are wet, these dried samples may sublime and end up being lost. Finally, samples that bump can result in solute and solvent showering the neighboring samples and causes sample loss or contamination of other samples.

SP-Genevac Evaporators can Eliminate Bottleneck Issues

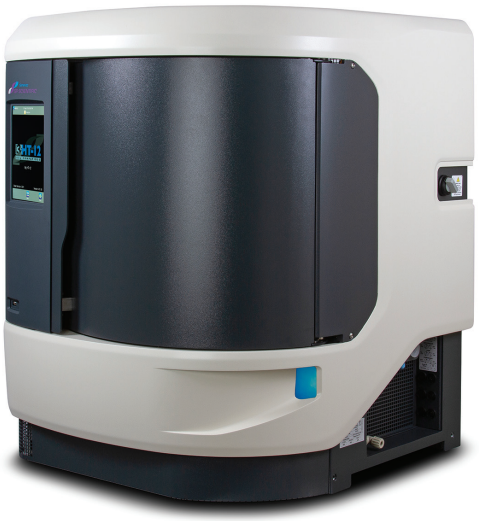
Drug development is already a lengthy and costly process without the issues of time-consuming evaporation steps, sample loss and cross-contamination. Therefore, it is essential that these problems can be overcome to make PROTACs a viable option for pharmaceutical and biotechnology companies to invest in. SP-Genevac is an industry leader in centrifugal evaporation and concentration processes and have developed two high throughput chemistry systems designed to eliminate evaporation issues; the HT Series 3i Evaporator (HTS3i) and the EZ-2 Personal Solvent Evaporator (EZ-2).

Ciulli et. al. (2019) in their research into iterative design and optimization of initially inactive PROTACs used the Genevac EZ-2 to dry fractions[11]

Both the HTS3i and the EZ-2 have the ability to dry many samples at once, so they are suitable for high throughput. These evaporators can also accommodate a wide range of sample formats including vials, tubes, flasks and plates, and the processes of drying, concentration and lyophilization can be performed in parallel. In addition, the systems can be pre-programmed and are able to detect when samples are dried or can be set to a timed run so unattended operation is possible. These features help reduce the time-consuming nature of PROTAC synthesis.

These evaporation systems also use SampleGuard technology, which monitors and directly controls sample temperature to avoid overheating, sample damage and compound sublimation. The DriPure®, anti-bumping technology eliminates bumping and therefore the possibility of sample loss and cross-contamination, ensuring the integrity and purity of the final product. Furthermore, both the HTS3i and the EZ-2 are compatible with all common organic solvents including corrosive acids such as hydrochloric acid (HCl) and trifluoracetic acid (TFA), explosive solvents such as diethyl ether, and high boiling point solvents such as dimethyl sulfoxide (DMSO) and N-Methyl-2-pyrrolidone (NMP). This ability enables a wide range of small molecule and peptide based PROTAC compounds to be synthesized using the same equipment.

SP-Genevac also offer additional components for the HTS3i and the EZ-2 evaporators, such as the SampleGenie™.



HTS3i

EZ-2

This accessory enables large volume samples to be dried or lyophilized directly into a small vial. This process typically requires four steps; (1) drying the large volume; (2) redissolving the sample in small volume of strong solvent (e.g. DMSO); (3) pipetting sample into the final vial; and (4) drying the sample in the final vial. The SampleGenie™ reduces this multi-step process into a single step, further reducing bottlenecks in PROTAC synthesis, and is particularly useful when combining HPLC fractions after sample purification. EXALT is another supplementary component that can be used with HT series evaporators. This option enables a wide range of solvents to be evaporated at the same time at slow rates to produce crystals for polymorph screening and structure characterization. This feature is highly beneficial to identify the stable and meta stable crystal forms of a small molecule at an early stage with only a few milligrams and the process can be automated so unattended operation is possible. It is compatible with a wide range of solvents with a boiling range from 40 °C to 165 °C and the evaporation time can be programmed to take 6 to 72 hours, or more if required. The EXALT uses baffles to control the solvent evaporation rate and modular toolkit allows researchers to create their own baffle configurations to achieve their desired evaporation profiles.

The Future of the PROTAC Therapeutic Modality

The development of PROTAC technology has grown dramatically over the last few decades, with many types of heterobifunctional molecules designed to target a wide range of POIs in cancer, neurodegenerative diseases and beyond. Arvinas are leading the way in the field and two of their PROTACS (ARV-110 for prostate cancer and ARV-471 for breast cancer) are currently undergoing phase I clinical trials. These trials are expected to complete mid-2020 and if they successfully reach the clinic, they have the potential to be the very first PROTAC drugs on the market.

However, there remain issues to be overcome before the compounds reach the market, in particular the evaporation steps during PROTAC synthesis. Centrifugal evaporators with the possibility for running multiple samples and technology to overcome sample loss and cross-contamination, such as the HTS3i and EZ-2 provided by SP Genevac, enable these problems to be eliminated.

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