28. PROTAC: Proteolysis-Targeting Chimera

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Pharmacology, together with medicinal-pharmaceutical chemistry, is one of the pillars of the drug discovery process (Drews, 2000), which is highly dynamic. Thus, we must remain attentive to new strategies that arise, especially when they represent a paradigm shift, as is the case with PROTACs. PROTAC is an acronym for PROteolysis TArgeting Chimera, which designates a heterobifunctional small molecule composed of two active domains (hence the term *chimera*) linked by a spacer, capable of activating protein degradation through proteolysis via the proteasome. One active domain binds to the target protein, while the other recognizes the E3 ligase responsible for initiating the ubiquitination process. In simplified terms, PROTACs act by bringing the ubiquitination machinery into close proximity with the target protein, catalyzing the onset of the degradation cascade. Owing to this mechanism, the PROTAC strategy, based on protein degradation, has several advantages over the classical inhibition of protein activity, as pointed out by Churcher (2018):

1. High (cellular) potency due to the catalytic mode of action. Indeed, unlike the high level of receptor/target occupancy generally required to inhibit a protein, in the case of PROTACs, low occupancy may be sufficient to maintain a degradation rate that exceeds the rate of protein production, rapidly leading to significant depletion of the target protein and thus to the desired pharmacological effect. Consequently, high drug concentrations are unnecessary, since the cellular EC_{50} value is lower than the K_d. It should be noted that, in these studies, the parameter typically used is DC_{50}, not EC_{50}, to define the observed concentration that produces 50% of maximal **degradation**.
2. High selectivity, which can be achieved in different ways (Smith et al., 2019).
3. Broad applicability in terms of cellular targets and *in vivo* systems, since the ubiquitin ligase system is highly conserved.
4. Possibility of extending the duration of the pharmacological effect if a PROTAC can rapidly deplete a population of proteins whose synthesis is slow.

Given the mechanism of PROTACs, certain characteristics are advantageous while others are not. One advantage is the lack of a requirement for a well-defined binding site to which an inhibitor must attach to block protein activity; the “tagging” for disposal can occur anywhere on the protein. On the other hand, the presence of bell-shaped (inverted U) concentration-effect curves makes it difficult to choose an appropriate dose to administer (Bondeson et al., 2015).

For those interested in historical aspects, the term PROTAC first appeared in the literature in 2001 (Sakamoto et al., 2001). The second major milestone was the description of the first non-peptidic PROTAC, a nutlin-derived compound capable of binding to Mdm2, a ubiquitin ligase, and thereby inducing the proteasome-mediated degradation of the androgen receptor (Schneekloth et al., 2008). Given the progress in the field, the first spinout company (Arvinas) was founded in 2013 following licensing of PROTAC technology by Yale University, marking the possibility that this strategy could become something more than an experimental tool. Proof of concept for the real clinical applicability of PROTACs came in 2015 with the publication of four articles, one of which demonstrated the complete catalytic inhibition of a protein *in vivo* (Bondeson et al., 2015). In this context, the recent review on the topic, twenty years after the theoretical concept of PROTACs was proposed, is worth reading (Békés et al., 2022).

In practical terms, what are the prospects of having a PROTAC drug in clinical use soon? We still do not know, but several clinical trials are already underway, with the two most advanced being conducted by the pioneering company mentioned above (Arvinas). These trials already have positive preliminary results regarded as clinical proof of concept for the therapeutic use of PROTACs (Békés et al., 2022):

- a phase 1/2 clinical trial with an orally bioavailable PROTAC (ARV-110) that degrades the androgen receptor in patients with metastatic castration-resistant prostate cancer; and
- a phase 1/2 clinical trial with the PROTAC ARV-471, alone and in combination with palbociclib, in patients with locally advanced or metastatic ER+/HER2- breast cancer (mBC).

By the end of 2021, ten additional PROTACs had entered phase I clinical trials, and many more are expected to emerge soon (Békés et al., 2022). Furthermore, there are now small specialized companies, such as BOC Sciences, offering services such as designing, synthesizing, and optimizing PROTAC molecules, as well as establishing analytical methods and performing biological evaluation of such compounds.

Finally, it is worth highlighting the recent initiative by Schneider et al. (2021), who used a systematic approach, based on genome-wide target discovery projects, to support decision-making regarding whether a specific target may or may not be amenable to modulation using a PROTAC. These authors estimate that 1,336 proteins may be viable PROTAC targets.

We conclude that PROTACs may represent a powerful tool to extend the chemical space into new types of targets previously considered undruggable or for which rational drug design would not have been feasible (Békés et al., 2022).

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