Are We Ready to Formulate the Next Generation of Small Targeted Therapeutics?

The drug discovery pipeline is currently bursting with innovative small and medium sized modalities and targeted therapies. Progress in molecular biology and genome sequencing is fuelling the next wave of drug discovery advancements, re-inventing the space for small molecules through adding new mechanisms of action such as targeted protein degradation (TPD) previously only thought possible with larger protein and antibody therapeutics. Upon their discovery, monoclonal antibodies have revolutionised the biopharmaceutical market due to their enhanced pharmacokinetic profile and high selectivity and binding capability to extracellular targets (Pei *et al.*, 2019). With antibodies, though, it is difficult to target intracellular proteins/targets due to their large molecular size.

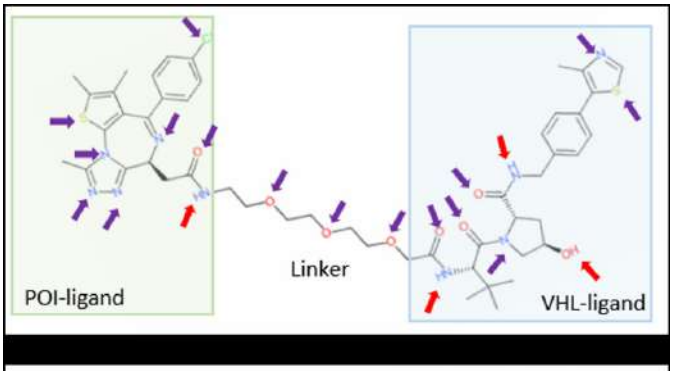
Shift towards druggability optimisation

Despite their phenomenal success, years of research have not yet unravelled the dilemma associated with druggability of many pathogenic targets and complete selectivity and safety of biologic treatment modalities. In fact, many compelling drug targets are yet to be drugged as 85% of the disease-causing proteins are believed to remain unexploited with the current large molecule approaches (Neklesa *et al.*, 2017). Therefore, a paradigm shift towards druggability optimisation has been noticed, in quest for molecules that serve as an extension of the oral druggable space. Ideally, these molecules possess the best of all preceding therapeutics, with drug-like characteristics of small molecules and selectivity of monoclonal antibodies and gene therapy (Neklesa *et al.*, 2017).

It is noteworthy that Lipinski's rule of five served as a rule-of-thumb for the selection of drug candidates with the potential of oral administration. However, the time has come to exploit molecules with drug-like properties far beyond these boundaries. PROTACs, or proteolysis targeting chimeras, come into play with their unique mode of action that distinguishes them from other therapeutic modalities. They induce the degradation of pathogenic proteins by utilising the body's natural protein disposal machinery known as ubiquitin-proteasome system (Pei *et al.*, 2019). PROTACs can unlock the undruggable space as they behave in a catalytic manner that is based on a transient interaction rather than constant, sustained binding. PROTACs tend to knockdown the target at lower doses compared to small molecule inhibitors, thus potentially minimising dose-related toxicity (Chen *et al.*, 2023).

Any Delivery or Formulation Challenges?

It seems that PROTACs intrinsically address the delivery challenges associated with the previously discussed therapeutic modalities as they seem to be more selective. However, our experience from a formulation perspective shows a number of challenges with these type of molecules despite their intricate molecular design. In fact, PROTACs possess challenging physiochemical properties as they fall within the chemical space of what is called the beyond-the-rule of five space (Bro-5 for short, Figure 1 shows an example of one of these molecules, MZ1) (Madan *et al.*, 2022). Because of the properties imposed by this chemical space such as their high molecular weight, polar surface area, and high lipophilicity, PROTACs come with their own set of challenges, namely poor cellular permeability, poor aqueous solubility, and subsequently low oral bioavailability (Chen *et al.*, 2023). They are also characterised with molecular flexibility, better described as chameleonicity, which adds to their complexity and may account for changes in their physicochemical properties depending on their conformation (Yokoo *et al.*, 2023). Yet, two Oral PROTACs, ARV 110 and ARV 471, managed to reach phase two clinical trials in 2022, reflecting the promising balance of efficacy and pharmacokinetic/CMC profile of these molecules (Yokoo *et al.*, 2023).



Parameter	Lipinski's Rule of 5	MZ1 Properties	Violations
Molecular weight	<500 Da	1002.64 Da	yes
Number of hydrogen bond donors	<5	4	No (represented by red arrow)
Number of hydrogen bond acceptors	<10	16	Yes (represented by purple arrow)
logP	<5	5.23	yes

Figure 1. Chemical structure of MZ1 with arrows highlighting the Bro-5 violating zones. The table shows how MZ1, as a model PROTAC, violates Lipinski's rule of five.

An ideal drug delivery system for these oral bioavailable molecules should be able to address the above challenging physiochemical properties to attain PROTACs with desirable pharmacokinetic profiles and enhance their performance.

What are the Available Formulation Options?

A number of technologies have been suggested to tackle the solubility, permeability, or site-specific accumulation of PROTACs. These efforts include formulating them into nano-sized carriers, prodrugs, and nucleic-acid based delivery systems (Chen *et al.*, 2022). For example, to address the solubility and stability of ARCC-4, a PROTAC targeting androgen receptor, Pöstges and colleagues (2023) embedded ARCC-4 into amorphous solid dispersions (ASD). They aimed to stabilise the amorphous PROTAC within the polymeric matrix by generating an aqueous supersaturated solution of the drug resulting in enhanced solubility.

In two other attempts, researchers have worked on addressing the delivery challenges of ARV-825, a PROTAC that degrades BRD4 which plays a role in tumour progression. Yang et al. (2022) incorporated the PROTAC in nanopolymeric micelles with the aim of overcoming its low water solubility and increasing its accumulation in the brain. Similarly, Saraswat *et al.* (2022) reported embedding ARV-825 in nanoliposomes anchored with galactose for enhanced targeting of hepatocellular carcinoma.

On the other hand, to improve the pharmacokinetic profile of a BRD4 targeting PROTAC, MZ1, another group of researchers reported encapsulating MZ1 in polymeric nanoparticles that were further conjugated with trastuzumab to treat HER2+ breast cancer (Cimas *et al.*, 2020). Compared to raw MZ1, the conjugated nanoparticles containing the PROTAC revealed stronger cytotoxic effect against MZ1 resistant cell lines. This could be attributed to improved internalisation and uptake by the cells that results in releasing most of drug molecules at the affected site upon conjugating the drug-loaded nanoparticles with trastuzumab.

At MESOX, we have formulated several complex molecules including the model PROTAC mentioned above, MZ1. Our preferred approach for this is a mesoporous carrier, called MesoPAC, engineered from an FDA-approved cellulose derivative (Figure 2). MZ1 dissolution was enhanced within this carrier by more than 2.5X in 30 min (from 19% to 49% release for raw and loaded drug, respectively), whilst stability of the molecule is ensured by a combination of surface anchoring (hydrophobic interactions) and spatial confinement (pore size 20 nm).

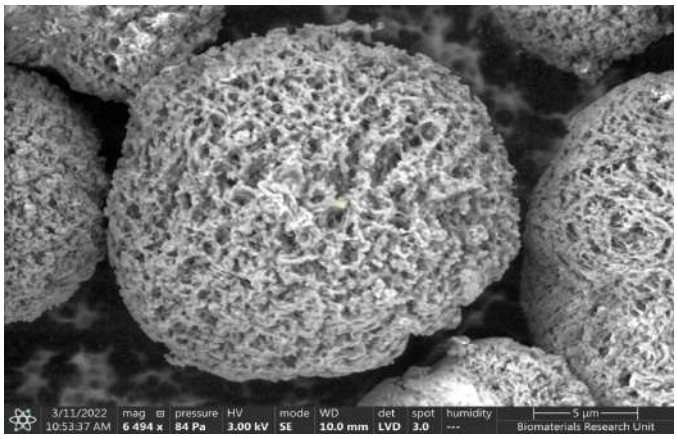


Figure 2: SEM image of the MesoPAC technology carrier.

Unlike traditional ASDs and other nanocarriers for oral delivery, mesoporous carriers such as MesoPAC offer complete release and tuneable release profiles which can be used to design the desired pharmacokinetic profile and adjust the dose given to patients. Furthermore, it is compactable at high drug loads into tablets (being a plastically deforming material) and ensures a smooth translation to high speed tableting machines. Therefore, mesoporous carriers emerge as contender drug delivery system that could accelerate the development of these new therapeutic modalities.

The formulation challenges of the new generation of small/medium sized molecules are not to be underestimated in the future. This is an area where little formulation experience exists in the field due to non-conformance to existing small molecules 'rules'. Selecting the right enabling technology is a critical decision for any biotechnology or pharma company developing these novel modalities.

REFERENCES

1. Chen, Q., Liu, C., Wang, W., Meng, X., Cheng, X., Li, X., Cai, L., Luo, L., He, X., Qu, H., Luo, J., Wei, H., Gao, S., Liu, G., Wan, J., Israel, D. I., Li, J. & Dou, D. (2022) Optimization of PROTAC ternary complex using DNA encoded library approach. ACS Chemical Biology, 18(1): 25–33
2. Chen, Y., Tandon, I., Heelan, W., Wang, Y., Tang, W. & Hu, Q. (2022) Proteolysis-targeting chimera (PROTAC) delivery system: advancing protein degraders towards clinical translation. Chemical Society Reviews, 51(13): 5330–5350
3. Cimas, F., Niza, E., Juan, A., Noblejas-López, M., Bravo, I., Lara-Sanchez, A., Alonso-Moreno, C. & Ocaña, A. (2020) Controlled delivery of BET-PROTACS: *in vitro* evaluation of MZ1-loaded polymeric antibody conjugated nanoparticles in breast cancer. Pharmaceutics, 12(10): 986
4. He, Y. et al. (2017) Mesoporous silica nanoparticles as potential carriers for enhanced drug solubility of paclitaxel. Materials Science and Engineering C, 78: 12–17
5. Madan, J., Ahuja, V., Dua, K., Samajdar, S., Ramchandra, M. & Giri, S. (2022) PROTACS: current trends in protein degradation by proteolysis-targeting chimeras. BioDrugs, 36: 609–623
6. Neklesa, T., Winkler, J., & Crews, C. (2017) Targeted protein degradation by PROTACs. Pharmacology & Therapeutics, 174: 138–144
7. Pei, H., Peng, Y., Zhao, Q. & Chen, Y. (2019) Small molecule PROTACs: an emerging technology for targeted therapy in drug discovery. RSC Advances, 9(30): 16967– 16976
8. Pöstges, F., Kayser, K., Appelhaus, J., Monschke, M., Gütschow, M., Steinebach, C. & Wagner, K. G. (2023) Solubility enhanced formulation approaches to overcome oral delivery obstacles of PROTACS. Pharmaceutics, 15(1), 156
9. Roots analysis (2023) Targeted Protein Degradation Market: Focus on Technology Platforms and Therapeutics (2nd Edition) [online]. Available from: https://www.rootsanalysis.com/reports/view_document/protein-degradation-market/289.html.
10. Saraswat, A., Vemana, H., Dukhande, V. & Patel, K. (2022). Galactose-decorated liver tumor-specific nanoliposomes incorporating selective BRD4-targeted PROTAC for hepatocellular carcinoma therapy. Heliyon, 8(1): e08702
11. Yang, T., Hu, Y., Miao, J., Chen, J., Liu, J., Cheng, Y. & Gao, X. (2022) A BRD4 PROTAC nanodrug for glioma therapy via the intervention of tumor cells proliferation, apoptosis and M2 macrophages polarization. Acta Pharmaceutica Sinica. B, 12(6): 2658–2671
12. Yokoo, H., Naito, M. & Demizu, Y. (2023) Investigating the cell permeability of proteolysis-targeting chimeras (PROTACs). Expert Opinion on Drug Discovery

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