CatSci

EXCELLENCE IN MEDICINES DEVELOPMENT

CASE STUDY

Accelerating Targeted Protein Degradation (TPD) Programmes:

Building Platform Knowledge of Chemical Process Design and Development Prior to Candidate Selection

Introduction

To accelerate drug development programmes and expedite the advancement of new candidates into clinic, there is often a need to initiate process design studies prior to final selection of the NCE. With continuing pressure on time to launch, this will likely become a more regular demand. Despite not having a single target compound, a wealth of information can be gathered in advance from key experimental data designed to properly equip decision makers and accelerate subsequent development studies.

This whitepaper explores how CatSci's bespoke route scouting and process development strategies were harnessed on a time-sensitive proteolysis targeting chimera (PROTAC®) programme. This ensured the customer's timelines and milestones were achieved, despite late-stage selection of the lead candidate. Note that PROTAC® is a registered trademark of *Arvinas Operations, Inc*.

PROTACs

In recent years, the field of targeted protein degradation (TPD) has rapidly expanded through the power of these drugs to escape occupancy driven approaches and binding site limitations. PROTACs function through the labelling of specific disease-causing proteins, for recognition and destruction by the innate recycling systems within cells. Structurally, these PROTAC molecules consist of three components: a ligand for the protein of interest, a ligand for an E3 ubiquitin ligase for labelling, and a linker to join the two pieces that as a combination often leads to poor physical properties due to their size and flexibility. In turn, this leads to programmes requiring significant development effort. The return on this investment, however, is correspondingly significant as particularly apparent in the recent clinical trials of ARV-766, an Arvinas programme for the treatment of prostate cancer. Whereas small molecule antagonists, such as flutamide, function well against the wild-type cancer, mutations in the cancer can render such therapeutics less effective in binding or even to function as an agonist accelerating proliferation of the cancer. In contrast for PROTACs, such as ARV-776, the mode of action is agnostic to the nature of the binding (antagonist/ agonist), with ligation in any form seeking to label the protein for destruction and overall make it much harder for the cancer to evade treatment through receptor mutations and establish longer term resistance.[1]

Case Study

CatSci was tasked with route scouting and subsequent process development for a novel PROTAC ligand where at the project outset, the lead candidate had yet to be determined, with 12 possible targets still in play. Based on a similar core structure, all 12 targets had different substitution patterns, and the series included both enantiopure and racemic analogues.

While candidate selection was ongoing by the customer, CatSci designed and explored a myriad of routes towards the core structure. During the route scouting

[1] https://ir.arvinas.com/news-releases/news-release-details/arvinas-announces-interim-data-arv-766-phase-12-dose-escalation (8th June 2023)

process, the team focused on four key areas: (a) merits and feasibility of early or late introduction of the glutarimide ring, (b) understanding the impact of substituents and/or their tolerance to any key steps, (c) scalemic vs racemic routes and (d) protecting group requirements, including exploring orthogonal protecting group strategies (Figure 1).

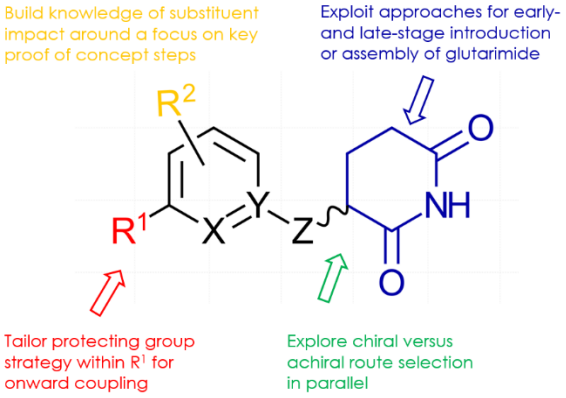

Figure 1 – Generic structure and areas of focus

1. Building Platform Knowledge

(a) Route design to core structure

When performing route ideation, CatSci sought to identify a range of synthetic approaches that could be applicable across the PROTAC candidate series. In line with our route design strategy, we utilised the wide experience across our scientific team, and harnessed our retrosynthesis software tools, to generate a multitude of ideas on paper. A route map was generated to visualise the synthetic strategies, highlight common intermediates, and identify the most direct routes to the target molecule (Figure 2).

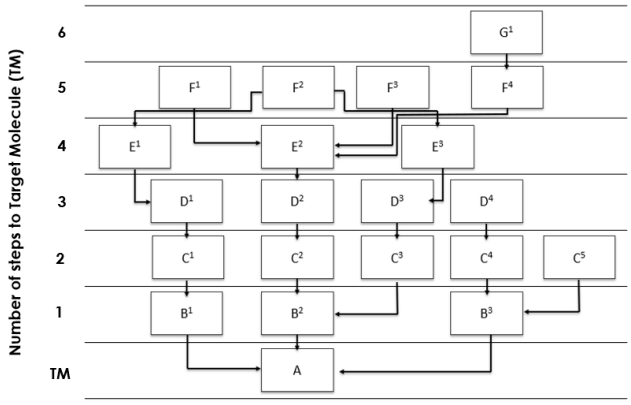

Figure 2 – Route map

In consultation with the customer, CatSci then generated a prioritised plan of routes to explore practically, by assessing factors such as cost, number of steps, and chance of technical success of each proposed route based on literature precedence and previous experience. Overall, CatSci shortlisted and explored 11 possible routes into the core structure, choosing to initially focus on routes that could be applicable to both racemic and enantiopure products. Further detail on our industry-leading route scouting and selection workflow can be found here.

(b) Running parallel screens for reagent selection

When investigating each synthetic route, CatSci ran parallel screens for the key steps to swiftly identify reaction conditions that would demonstrate proof-of-concept. Reaction parameters such as reagents, additives, solvents, and temperatures were widely screened to identify hit conditions, and confirm the feasibility of the route.

A significant secondary benefit to performing extensive screening during proof-of-concept studies was the additional information acquired about substrate reactivity and stability.

One proposed synthetic route on the project proceeded via an Ullmann cross-coupling reaction. Reaction screening focusing on ligands, bases and solvents, through which lead conditions were identified and proof-of-concept was achieved. Importantly, the screens also revealed that the starting material readily underwent undesired side reactions with some catalyst ligands (e.g. ethylene glycol), and that the desired Ullmann product was unstable under many of the reaction conditions, degrading faster when certain bases were used. The fragility of both the starting material and desired product under various reaction conditions was carefully noted by the team, and additional studies were performed to further increase reaction understanding and determine the feasibility of the route.

(c) Reaction profiling and impurity formation

To generate an accurate understanding of the reactivity and stability of the substrates under the different reaction conditions, key steps were profiled using automated sampling (MT EasySampler). Plotted graphs clearly depicted the trends of starting material consumption as well as product and side product formation (Figures 3a and 3b). The data enabled the CatSci team to focus their optimisation experiments on addressing the causes of the undesired side reactions at this early stage.

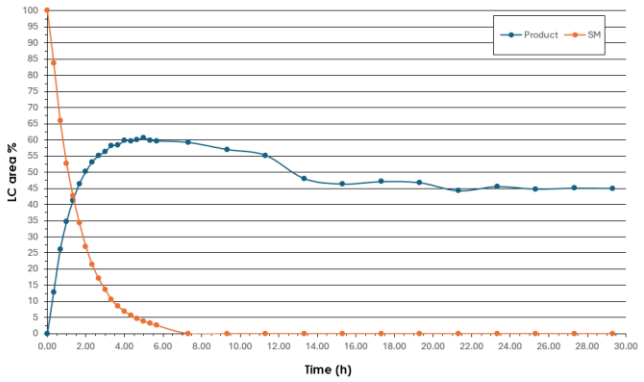

Figure 3a – Ullmann cross coupling reaction profile using automated sampling. Plotted LCAP values

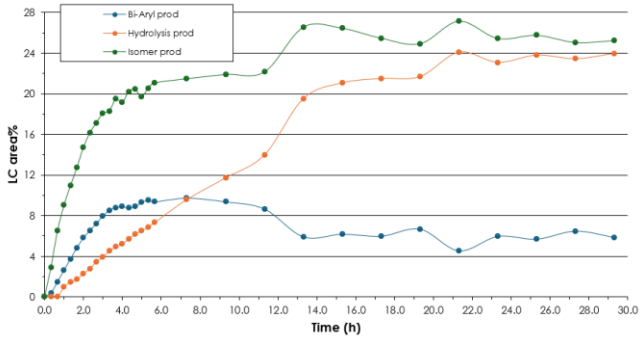

Figure 3b – Monitoring formation of Ullmann reaction side products

In the case of the Ullmann cross-coupling, the rate of regioisomer formation was fast and after 4h it existed in a 1:3 ratio with the desired product. The homo-coupled bi-aryl dimer was also observed, and the product was also seen to hydrolyse over time (24 LCAP). CatSci swiftly showed these latter impurities could be addressed by altering reaction conditions; dimerization was overcome through dilution of the reaction mixture, and hydrolysis was significantly reduced through performing water tolerance studies and setting specifications for water content. However, by noting poor regioselectivity with the initially proposed starting material, CatSci was able to highlight that an alternative would be required if the Ullmann-coupling route was to be a feasible option or outperform other routes.

(d) Exploring alternative starting materials

At project outset, the client recommended key starting materials, based on their in-house research. As part of route design, CatSci explored alternative raw materials, which were cheaper and more widely available. Accordingly, given the potential benefits, different starting materials were heavily studied when assessing how substituents on the aryl ring impacted the key proof of concept steps. For example, a nitro-containing heterocycle was proposed as a core building block. This was screened alongside numerous other possible substrates and CatSci was able to quickly identify the reactivity of the nitro-aryl was inferior to alternatives that had not previously been considered. Each substrate would slightly alter the downstream chemistry and as such the most promising substrates were taken forwards to the next steps for process evaluation. After establishing the baseline route conditions from each of the shortlisted starting materials, CatSci was able to share extensive experimental data with the customer to justify the selection of the optimal starting material for process development.

(e) Protecting group requirements

For all explored routes, CatSci tested the limits of protecting group requirements. Eager to cut down on steps where possible, CatSci performed chemistry on the unprotected substrates, and retained markers for side products when formed, to support future process development. Where it was proven that protecting groups were required, CatSci tested whether protecting groups could be cleaved in one-pot, to avoid extra steps. And finally, with such complex structures, the CatSci team took the time to seek and identify orthogonal protecting group strategies that could simplify or otherwise help with downstream steps.

(f) Retention of enantiopurity

During route scouting, fit-for-purpose chiral LC and GC methods were developed in-house and beneficially deployed, enabling the chiral purity of reaction intermediates to be studied. This allowed the quick evaluation of each route to discern those which resulted in high enantiomeric purity and which, if any, steps were problematic for chiral erosion.

2. Expediting Process Development

Through these route scouting activities, CatSci collected invaluable information about the reactivity of the candidate series, enabling putatively attractive manufacturing routes to be sensibly discounted, due to poor yields, competing side reactions or other synthetic challenges that would be difficult to overcome on scale.

Understanding the protecting group requirements of the substrates resulted in a higher degree of confidence in the step count for each route and therefore enabled better comparison between the route options. At the point of candidate selection, CatSci had acquired substantial experimental information and was able to rank the synthetic routes in order of preference by comparing each against weighed criteria using a Pugh Matrix (Figure 4). CatSci was thus able to highlight and clearly advocate for a lead route in front of the customer, demonstrating the value created by a genuine innovation partnership.

Criteria	Weighing	Route A	Route B	Route C	Route D
Multiple SM suppliers	3	0	-	+	+
Number of steps (inc. PGs)	4	+	-	-	+
Number of solid intermediates	4	+	+	-	+
Isolation by crystallisation	4	+	+	+	-
Access to chiral API	1	+	0	0	-
Telescope opportunities	2	0	+	+	+
Cost	4	+	-	+	+
Weighted Score	-	17	-1	5	12

0	reference concept
+	design is better than reference
-	design is worse than reference

Figure 4 – Pugh Matrix comparing potential routes against customer criteria

With a wealth of knowledge in hand and the lead route established, process development was able to hit the ground running. Once candidate selection had been confirmed by the customer, CatSci developed the final manufacturing process within months, providing the requisite process descriptions, tech transfer documents and analytical methods to ensure accommodation into the pilot plant in a timely manner. Working in a three-way collaboration with the customer and CMO, CatSci ensured the transfer of the novel process for development manufacture for clinical supply, troubleshooting and solving accommodation issues in the initial demonstration batches arising from the plant location. This collaborative approach was rewarded with the first campaign performing on a 100 kg input scale.

About CatSci

CatSci Ltd is an award-winning innovation partner, dedicated to developing economically and environmentally sustainable pharmaceutical manufacturing processes. We proudly serve customers across the globe with projects, meeting their needs from candidate selection to product launch and beyond.

Our tailored services include route scouting and selection, initial scale-up and risk management for early development. For later development, we provide process design, assessment and optimisation, scale-up for clinical and commercial manufacture, tech transfer and post-approval improvements. We possess specialist facilities in Process R&D, catalysis, high pressure reactions, crystallisation, preformulation, analytical development, HPAPI development, and non-GMP supply, and recently launched our oligonucleotides capability. Through our partnership with AGC Pharma Chemicals, we offer scalable small molecule API manufacturing, from grams to tonnes, with complete accountability of tech transfer.

Recent recognition includes the highly esteemed Queen's Award for Enterprise: International Trade 2022, Wales STEM Awards 2022: STEM Company of the Year and the UK Fast Growth 50 Index 2023: Innovative Growth.

Contact us to learn more about how CatSci can support your project: enquiries@catsci.com