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Chemical Strategies for Evaluating New Drug Targets

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1.1 Introduction

Discovering new drugs is difficult and expensive. Pharmaceutical companies typically spend at least $2.6 billion on average in research and development (R&D) for each drug before it reaches the market [1]. Interestingly, the high cost of R&D is not driven by the few programmes that succeed, but rather by the cost of pipeline projects that fail [2]. Only about 1 in 10 of drug candidates in phase I clinical trials actually makes it to become a new medicine [3, 4], and about half of the projects that fail in phase II clinical trials do so because of clinical efficacy [5, 6]. So why do so many drugs fail?

One answer is a lack of genetic evidence. An analysis of AstraZeneca's small molecule pipeline indicated that the success rate was over 70% for projects in phase II clinical trials with human genetic linkage of the target to the disease indication compared with 43% for projects without such a linkage [6]. Furthermore, another similar study concluded that selecting genetically supported targets can double the success rate in clinical development [7]. These observations have prompted some scientists to highlight the critical importance of the therapeutic hypothesis at the stage when a protein or gene is selected as a potential drug target [8]. However, it is often a long and difficult road between identifying a genetic link and understanding the underlying biological processes (see Chapter 6 for more details).

A major problem we are facing is that a large proportion of biomedical R&D focuses on only a small fraction of the genome despite the promised revolution in medicine following sequencing of the entire human genome [9]. Shortly after its announcement, scientists imagined that genome science would soon begin revealing the mysteries of hereditary factors in heart disease, cancer, diabetes, schizophrenia, and a host of other conditions and lead to new medicines [10]. Unfortunately, this has not happened. Indeed, more than 75% of protein research

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still focuses on the 10% of proteins that were known before the genome was mapped, even though many more have been genetically linked to disease [11]. A more recent analysis of drug targets highlights the continued dominance of a set of privileged target families across different disease areas, although there has also been a small growth of novel first-in-class mechanisms, particularly in oncology [12]. What can we do to help biomedical scientists worldwide to expand and prioritize the list of potential new drug targets?

One answer lies with high-quality chemical probes. We know that chemical tools can dramatically facilitate exploratory biomedical research. Let us take, for example, nuclear hormone receptors. When nuclear receptors were identified by sequence homology in the 1990s, all the family members were thought to have therapeutic potential. Scientists initially investigated those receptors that were found to have genetic links to disease or that had interesting knockout phenotypes. However, as time went on, research activity focused on a subset of eight of these receptors despite the fact that these eight were no more genetically interesting than the others. Indeed Edwards [11] postulated that the only connection among these eight receptors is that for each there exists a widely available, high-quality chemical probe that either enhances the receptor's activity or dampens it. In short, where high-quality tools are available, there is research activity; where there are no tools, there is none. What is a high-quality chemical probe and why are they so useful?

The Structural Genomics Consortium (SGC) (www.thesgc.org) is a large precompetitive public–private partnership between academia, private funders, and currently nine public pharmaceutical companies as well as patient advocacy and research organizations. The consortium has established a common set of principles for chemical probes, initially focused on epigenetic targets. A chemical probe is simply a small molecule that modulates the function of a protein in a specific and selective way. This allows a scientist to interrogate the biology and test hypotheses relating to the mechanism or role of the particular protein in a relevant cellular context [13]. The difference between specificity and selectivity is important to consider. Specificity is the capacity of a chemical probe to manifest only one kind of action. A chemical probe of perfect specificity of action might increase, or decrease, a specific function of a given cell type, but it would not do both, nor would it affect other receptors. In contrast, selectivity is the ability of a chemical probe to affect one cell population in preference to others, i.e. the ability of a chemical probe to affect one kind of cell, and produce effects, in doses lower than those required to affect other cells. This should not be confused with potency, i.e. the measure of the activity of a chemical probe, in terms of the concentration or amount required for producing a defined effect. Consequently, selectivity is actually a measure of the relative potency of a chemical probe in producing different effects.

The SGC has established a set of stringent criteria that a chemical tool compound must fulfil in order for its classification as a chemical probe (Figure 1.1). The compound must exhibit *in vitro* potency of less than 100 nmol/l for a single target or a small set (<5) of very similar targets and possess a minimum 30-fold selectivity relative to other sequence-related proteins of the same family. Furthermore, the probe must be profiled against a standard selection of other unrelated

pharmacologically relevant targets and large protein families of relevance to drug discovery (specificity) and, finally, have demonstrated on-target effects in cells at less than 1 μmol/l (cellular activity) [13]. These criteria were developed jointly by academia and industry to help guide scientists to choose the best chemical probe for their needs because, unfortunately, there are numerous examples of less well-characterized tools that may generate misleading results. Although the chemical probe criteria could be stricter (e.g. 10 nmol/l potency, selectivity for a single target >100-fold, cellular activity <100 nmol/l), the chemical probe criteria outlined here are a pragmatic compromise between the cost of creating such a chemical probe and the potential value it brings to science.

Seven pharmaceutical companies have since worked together in a precompetitive collaboration to make available a large number of innovative, high-quality chemical probes from previous terminated pharmaceutical projects [14]. These probes have all been subject to a rigorous and independent scientific review by the SGC, are accompanied by comprehensive data packages, and include an appropriate structurally related yet inactive control compound. All of this is made available to the wide scientific community free of intellectual property restrictions via the portal https://opensciencesprobes.sgc-frankfurt.de.

Regrettably, making a high-quality chemical probe entails a lot of effort especially for medicinal chemists. Indeed, the sort of efforts that are required to make such a tool are those that are only usually available for a full-fledged drug discovery project. How do we justify that level of investment if we do not know how important the target protein is *a priori*? Some scientists have likened it to a catch-22 situation. A scientist cannot justify the resources required for generating a chemical probe without knowing the importance of the target protein. On the other hand, the scientist cannot judge the importance of a protein without being able to interrogate its function with a chemical probe. But help is at hand. One way around this is to build chemogenomic sets of compounds for specific protein families known as chemogenomic libraries [15].

Chemogenomics was coined as a term to describe the use of target family-directed chemical libraries in target or cell-based assays as a means of accessing new areas of biology and accelerating drug discovery research based on the assumption that similar receptors bind similar ligands [16–18]. Such sets, although containing compounds that individually do not fulfil the stringent criteria of a chemical probe, nevertheless, can be used to interrogate multiple members of protein families to help prioritize the most therapeutically relevant ones that could then form the basis of a chemical probe project (Figure 1.1). A chemogenomic approach involves a systematic screening of a set of small molecules (chemogenomic library) with well-annotated pharmacology against target families of functionally related proteins such as G-protein-coupled receptors (GPCRs), kinases, and ion channels. Usually target families that are homologous at the protein level have similar properties. Consequently, compounds synthesized for the purpose of one family member will, with a high probability, be active against other members of the same family. This strategy allows an economical use of chemical and biological knowledge for more efficient drug discovery. A recent example is the public chemogenomic set, known as the comprehensive kinase chemogenomic set (KCGS) (Published Kinase

Figure 1.1 Chemogenomic tools support the identification of potential target proteins, and chemical probes help interrogate biological systems selectively and specifically with the goal of ultimately developing safe and efficacious medicines.

Inhibitor Set [PKIS]), that is being developed for the entire family of human protein kinases [19]. This is a large collaborative endeavour that will be made available as a public resource of both data and physical compound samples to support basic research to all scientists that agree to be trustees of the set. We believe that researchers will be able to use publicly available chemogenomic libraries and chemical probes to interrogate exciting areas of biology that genetic studies have helped to identify. We use the terms target evaluation and target prioritization as part of the target validation process. However, we appreciate that we can only truly validate a drug target after the clinical effects have been demonstrated in pivotal human phase III clinical trials. This chapter will outline the chemical strategies used to evaluate and prioritize such protein targets and provide examples that illustrate the successful application of these strategies.

1.2 Use Cases and Case Studies for Chemogenomic Compounds and Chemical Probes

The inappropriate use of chemical tools can have serious deleterious consequences for the interpretation of experimental results for several different reasons. Here are some examples:

- Insufficient selectivity might lead to the conclusion that a target is relevant for an observed effect, which, in reality, is caused by an uncharacterized off-target effect.
- Low permeability might lead to the conclusion that a compound does not modulate the activity of its target in a cellular setting when, in fact, it does not even reach the target.
- General cytotoxicity caused by an inappropriate tool compound might be confused with a specific effect on a cancer target.
- Limited solubility might lead to the conclusion that a compound is inactive because it has precipitated.

These reasons illustrate the importance of defining high-quality and well-characterized tool compounds. Limited reproducibility of published work [20, 21] might, in part, be caused by the use of compounds that are not suitable for the experiments in which they have been used. Other authors have published requirements for high-quality compounds that should be applied when carrying out biological experiments as described above [13, 14, 22–24].

Table 1.1 provides an overview of the data necessary to assess the suitability of tool compounds for different experiments. For work with a purified recombinant protein, e.g. for developing biochemical or biophysical assays, only a limited number of parameters need to be assessed. For all work in a cellular context or *in vivo,* we recommend a significantly more comprehensive dataset.

1.2.1 Chemogenomic Libraries

There are a number of available chemogenomic libraries available either commercially from chemical suppliers or via collaboration (Table 1.2). While

Table 1.1 Relevance of data for the use of chemical tools.

Data needed to assess suitability for intended use	Assay development (biochemical)	Assay development (cellular)	Target validation *in vitro*[a)]	Target validation *in vivo*[a)]	Drug	SGC criteria (epigenetic probes)
Biochemical potency	Yes	Yes	Yes	Yes	Yes	<100 nmol/l
Cellular potency		Yes	Yes	Yes	Yes	<1 µmol/l
Solubility	Yes	Yes	Yes	Yes	Yes	Yes
Selectivity	Yes	Yes	Yes	Yes	Yes	>30-fold within family
No Pan-assay interference compounds (PAINS)	Yes	Yes	Yes	Yes	Yes	Yes
Cellular permissibility		Yes	Yes	Yes	Yes	Yes
Metabolic stability		Yes	Yes	Yes	Yes	Yes
No general cytotoxicity		Yes	Yes	Yes	Yes	Yes
Adequate pharmacokinetic properties for *in vivo* studies				Yes	Yes	
Inactive control	Yes	Yes	Yes	Yes		Yes

Data needed for the corresponding applications are marked by 'yes'. No values are provided when the requirements will vary considerably between different targets/target families.
a) For intracellular targets.

most chemogenomic libraries contain compounds that are not potent enough or characterized sufficiently to be true chemical probes based on the definition above, they are still valuable in that they often have a broader coverage of target space (Table 1.2, Broad Libraries). Libraries that are composed of high-quality chemical probes cover far fewer targets and have fewer compounds due to the high cost associated with discovering and characterizing chemical probes (Table 1.2, Chemical Probe Libraries) because of the inherent capacity constraints of generating such high-quality probes.

1.2.2 Inactive Control

In addition to interaction with the intended target, chemicals can also cause uncharacterized off-target or cytotoxic effects. To exclude misinterpretation of the observed results and to confirm that the activity is indeed caused by the biochemical inhibition of the annotated, intended target, we strongly recommend using an inactive control compound. An inactive control compound is defined as a compound that is chemically similar to the chemogenomic compound, but is inactive at the annotated target. Inactivity is often rationally designed using

Table 1.2 Examples of libraries of chemogenomic tools and chemical probes.

Library	Number of compounds	Comments	Availability
Broad Libraries			
NCATS Pharmacologically Active Chemical Toolbox (NPACT)	>11 000	Collection from NCATS containing naturally occurring, nature-inspired, or synthetically created high-quality organism-agnostic and pharmacologically active compounds; diversity of physicochemical and pharmacological properties by a few best-in-class compounds with non-redundant chemotypes identified from the literature and worldwide patents https://ncats.nih.gov/preclinical/core/compound/npact	Available in collaboration with NCATS
NCATS Pharmaceutical Collection (NPC)	3 500	Collection from NCATS containing small molecular entities approved for clinical use by US, European Union, Japanese, and Canadian authorities; access to the collection through the Therapeutics for Rare and Neglected Diseases programme and the Toxicology in the 21st Century initiative https://ncats.nih.gov/expertise/preclinical/npc	Commercial and Collaborative
Sigma Library of Pharmacologically Active Compounds (LOPAC^{1280})	1 280	Commercially available collection containing approved drugs and pharmaceutical compounds annotated with biological activities dominated by GPCR targets; in literature most widely used chemogenomic library https://www.sigmaaldrich.com/catalog/product/sigma/lo3300?lang=de&region=DE	Commercial
Prestwick Chemical Library	1 280	A collection of 1280 off-patent drugs with high chemical and pharmacological diversity, as well as known bioavailability and safety in humans http://www.prestwickchemical.com/libraries-screening-lib-pcl.html	Commercial
Published Kinase Inhibitor Set (PKIS) (GlaxoSmithKline)	367	Collection containing adenosine triphosphate (ATP)-competitive kinase inhibitors published by GSK for screening by external users; set represents >20 different chemotypes covering a total of 58 kinases https://www.ebi.ac.uk/chembl/	Collaborative and commercial

(*continued*)

Table 1.2 (Continued)

Library	Number of compounds	Comments	Availability
Published Kinase Inhibitor Set (PKIS2)	654	Collection containing small molecule inhibitors published by academia and industry collaborators; set represents 86 diverse chemotypes [19]	Collaborative
Chemical Probe Libraries			
SGC Chemical Probe Library	44	Probes developed by SGC with academic and industry collaborators; compounds are available from the website until they are commercially available; then order by suppliers (Sigma, Tocris, and Cayman) https://www.thesgc.org/chemical-probes	Commercially available
Chemical Probes Portal	189	Web-based resource with accessible recommendations about chemical probes based on expert input from its Scientific Advisory Board, supporting data and guidance are available [13] www.chemicalprobes.org	Information repository about high-quality probes
opnMe Portal	25	Chemical probes designed by Boehringer Ingelheim; access to the molecules to order (M2O), only for some exceptions for collaborative researcher (molecules for collaboration [M4C]); associated data and negative control compounds are available https://opnme.com	M2O are freely available, M4C collaborative
SGC Donated Probes	54	Chemical probes developed by pharmaceutical companies (Takeda, Merck, Bayer, Boehringer Ingelheim, AbbVie, Pfizer, Janssen); all probes and negative controls are available from the website by the SGC; *in vitro* and *in vivo* data with recommendations on their use [14] www.sgc-ffm.uni-frankfurt.de	Commercially available

knowledge of the target's preferred pharmacophore and inactive compounds are often taken from stereo- or regio-isomers to maximize chemical similarity.

1.2.3 Use of Biological Target Panels and Profiling

The value of a tool compound depends on different factors:

- Fit to the agreed criteria in line with its intended use as described above.
- Accessibility (cost, time, and effort needed to obtain compound).

- Versatility of use (e.g. physicochemical properties enabling use over a broad concentration range, over a long period of time).
- Extent of annotation.

To phrase it differently, the value of every chemical probe or chemogenomic compound can be increased significantly by in-depth profiling with large assay panels as this will increase the confidence that the effects observed with compound treatment are specific and reproducible.

We recognize that selectivity needs to be closely monitored for large target families with conserved binding sites such as GPCRs or kinases. The most pragmatic way to assess the selectivity profile of a tool compound is to measure the effect on target family members with the highest sequence identity, the highest predicted similarity of the binding pocket (e.g. for kinases assessed via Kinase SARfari [www.ebi.ac.uk/chembl/sarfari/kinasesarfari]) and the expected relevance of the biological effect.

Innovative technologies such as Kinobeads are very useful to explore selectivity in a cellular setting (for details see Chapter 4). In addition, service providers such as Eurofins (www.eurofins.com) have established large assay panels for several relevant target families, e.g. roughly 500 different biochemical and cell-based kinase assays to cover the majority of all kinases. Efforts like the kinase chemogenomic library and other broad profiling [25] are of high value as they provide the scientific community not only with a unique library of kinase inhibitors but also with the associated data from the profiling in the Eurofins panel. Many published kinase inhibitors still lack selectivity data, and we would like to stress that such compounds should not really be used as tools for target evaluation.

Off-target effects are, however, not restricted to targets from the same target family. Previous work has recently shown that several inhibitors developed to target specific kinases also potently inhibit diverse bromodomains (BRD) [26]. When testing 628 kinase inhibitors on BRD-containing protein 4 (BRD4), 9 compounds were identified with a strong effect on BRD4. Examples include BI-2536, a clinical-phase polo-like kinase 1 (PLK1) inhibitor, and TG-101348, a clinical-phase Janus kinase 2 – Fms related tyrosine kinase 3 (JAK2-FLT3) inhibitor, which both have nanomolar activity on BRD4. This was unexpected as the primary sequences of BRD4 and the kinases are not related. While activity on more than one target might even be beneficial for a drug, it is clearly not desirable for a tool compound used for evaluating new targets. Here we want to understand if the effect induced by a tool compound is caused via the target it has been optimized and characterized for or by an unknown off-target effect. Experience shows that many drugs still exhibit effects on different target proteins. It is not at all unusual to identify hits in a high-throughput screen that originate from another project, either from the same target family or from unrelated targets.

To understand potential issues with specificity and selectivity, tool compounds should be profiled as broadly as possible. The donated chemical probes released by the SGC are a good example for broad annotation: after acceptance, all donated probes are profiled in target specific assays, in relevant selectivity panels, in a panel of over 500 kinases as well as in over 100 ion channels, GPCRs, and

proteases [14]. In this example, activity data related to more than 600 different targets are measured and made available. Nevertheless, these data relate mostly to well-known targets. To cover also underexplored targets, there is an urgent need to develop assays such as the thermal shift assay that are applicable to a broad range of targets with limited effort for specific assay development [27, 28].

Data supporting the selection of tool compounds for evaluating new drug targets are not always easy to find. Vendors usually provide some data and links to literature for the compounds they sell, but this is often limited to a few selected off-targets. Well established databases like ChEMBL are a very valuable source for activity data. In addition, new platforms like Probe Miner [29] and the Chemical Probes Portal [13] have recently been launched to help find information on tool compounds. Both platforms are user friendly and a valuable source of information when searching for the best tool compound for target validation.

1.3 Development of Chemical Probes

1.3.1 From BIX01294 to EPZ035544: Development and Improvement of G9a/GLP Inhibitors

The discovery of **EPZ035544** is an example of how a chemical probe was used to uncover a new biological link between epigenetic targets and haemoglobin expression and stimulate drug discovery for sickle cell anaemia.

The two closely related protein lysine methyltransferases (PKMTs) G9a (KMT1C/EHMT2) and G9a-like protein (GLP/KMT1D/EHMT1) are *S*-adenosyl methionine (SAM)-dependent enzymes responsible for the mono- and di-methylation of histone H3 lysine 9 (H3K9me1/H3K9me2) [30–33]. Dysregulation and overexpression of this post-translational epigenetic modification has been reported to be associated with a variety of human diseases, especially cancers [34, 35], including lung cancer [36–38], leukaemia [39–41], prostate carcinoma [39, 41, 42], breast carcinoma [43, 44], and hepatocellular carcinoma [45–47]. Over the past years, a number of selective G9a/GLP inhibitors have been used for the investigation of the cellular role of these PKMTs and developed as potential therapeutic drugs [35, 48].

The first selective and potent G9a/GLP inhibitor was discovered via high-throughput screening by Boehringer Ingelheim using a chemical library of 125 000 preselected compounds [49]. The only selective inhibition of G9a/GLP histone H3 lysine-9 di-methylation (H3K9me2) was observed for the diazepine-quinazoline-amine derivative **BIX01294** that competes with the peptide substrate and not with the methylation cofactor SAM (Figure 1.2). Although multiple studies of **BIX01294** have shown a successful application in cellular reprogramming [50, 51] and reactivation of latent HIV-1 [52], the use of the compound was limited due to the high cellular toxicity at concentrations (>4 μM) near the on-target cellular potency.

Further structure-based exploration of the **BIX01294** quinazoline scaffold [53, 54] led to the first potent, selective, and cell-active chemical probe **UNC0638** along with its inactive control **UNC0737** [55]. Additional optimization of

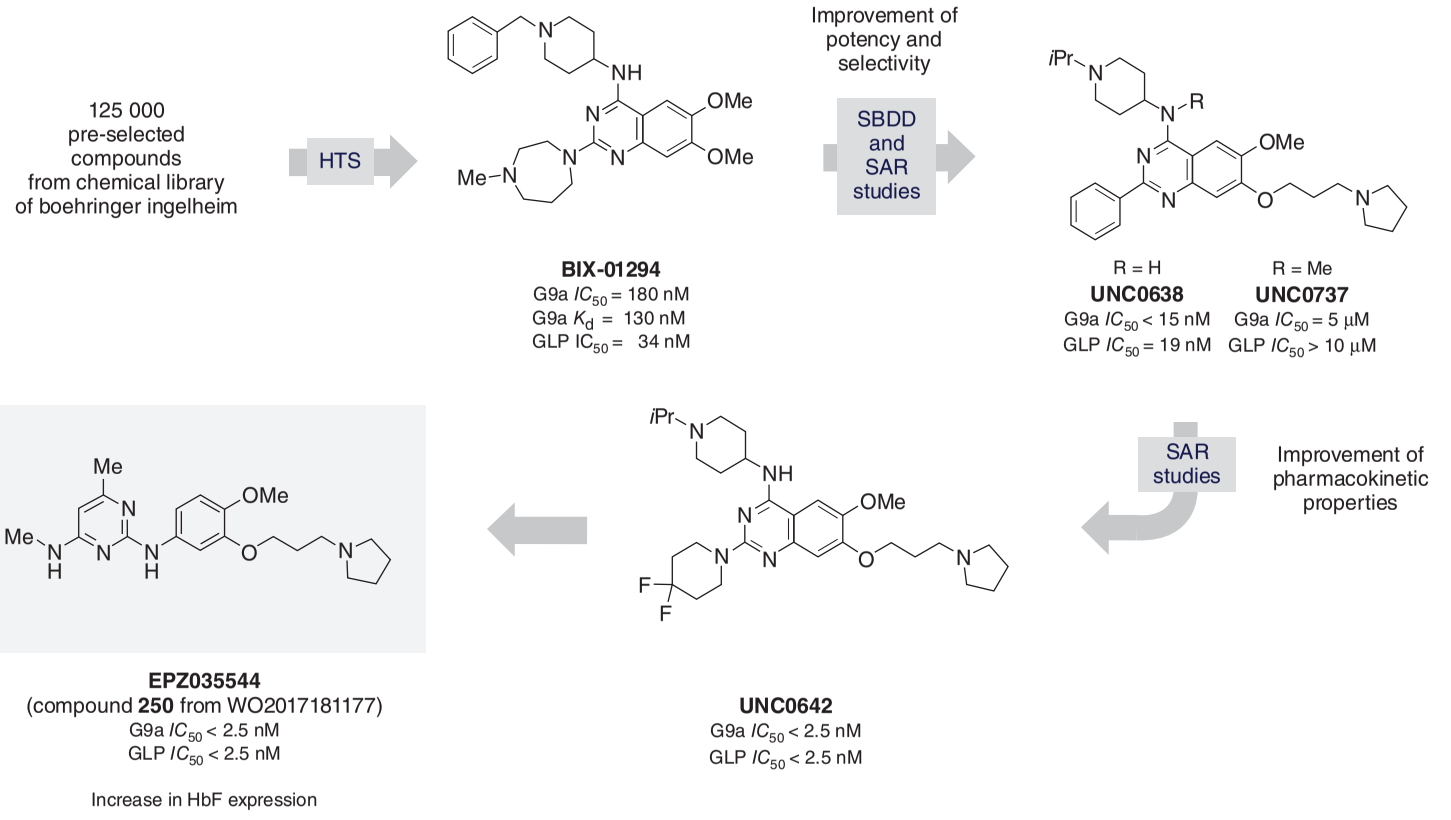

Figure 1.2 Development of G9a/GLP inhibitors. HTS, high-throughput screening.

pharmacokinetic properties produced **UNC0642**, a G9a/GLP chemical probe also suitable for *in vivo* use.

Although initially used in cancer studies, more recent work [56, 57] showed that **UNC0642** induced the expression of human foetal haemoglobin (HbF) in adult erythroid cells, despite its usual silencing after birth. Since the discovery of 5-azacytidine in 1982 [58], the reactivation of HbF synthesis has been widely explored for the treatment of blood disorders such as sickle cell anaemia and β-thalassaemia. Inspired by the results from **UNC0642**, the researchers at Epizyme developed additional G9a/GLP inhibitors as therapeutic agents to treat haemoglobin deficiency disorders [59]. Among the structures of a number of G9a/GLP inhibitors from an Epizyme patent application [60], **EPZ035544** (compound **205**), which shares some structural features to the previously disclosed chemical probes **UNC0638** and **UNC0642**, was identified as a potential candidate for the treatment of haemoglobin deficiencies such as sickle cell anaemia. The discovery of **UNC0642** and **EPZ035544** is an excellent example of using high-quality small molecule chemical probes to decipher new biology with the potential for great clinical benefit.

1.3.2 Development of BRD9 Inhibitors

The family of BRD are 'readers' of lysine acetylation (Kac) of proteins [61–64]. By reading histone acetylation, BRDs regulate gene transcription by serving as transcription factors themselves, thereby reorganizing the physical structure of chromatin (ATAD2) or recruiting transcription factors (BRD4) and chromatin remodellers (BRD7, BRD9). A dysfunction of BRD-containing proteins has been linked to diverse diseases such as cancer and inflammation.

For this reason, during the last decade, a number of single target and subfamily selective BRD chemical probes have been discovered [62–64]. All eight BRDs of the bromo- and extra-terminal (BET) subfamily were initially targeted, but chemical probes for most of the remaining BRD family have been described and allow near family-wide exploration of BRD effects on transcription and derived phenotypes. With the development of **LP99** from a fragment hit through structure-based drug design (SBDD), the first selective and potent BRD9 and BRD7 chemical probe was released [65]. Since the introduction of **LP99** by the SGC, there have been three additional structurally unrelated BRD9 and BRD7 inhibitors: I-BRD9 and **TP-472** [66] derived from BRD-focused libraries and **BI-9564** [67] also derived from a fragment hit (Figure 1.3). All of these BRD9 inhibitors are available to the scientific community to investigate the BRD9 biology, either by use of a single probe or a combination of several inhibitors. The availability of different BRD9 chemical probe chemotypes allows on-target effects to be confirmed (see Section 9.3 for further information on how cell biology techniques were used in combination to provide evidence that BRD9 target modulation is responsible for a cellular response).

I-BRD9, the first selective BRD9 chemical probe, has utility both in cellular assays and *in vivo* experiments [66]. A thienopyridone was identified as a hit by a cross-screening of GlaxoSmithKline (GSK) internal compounds to identify inhibitors of the BRD9 bromodomain. X-ray crystallography of analogues in

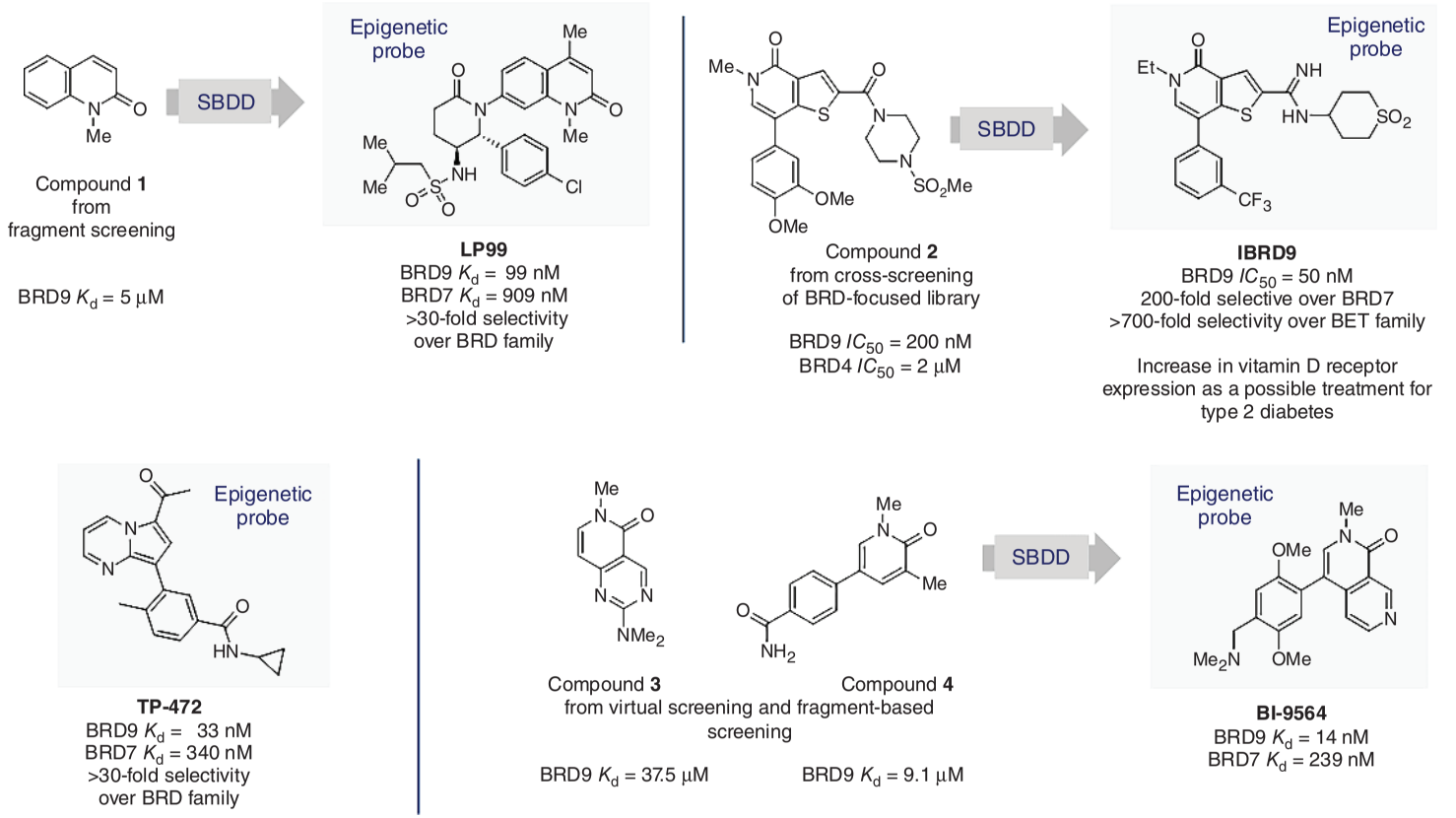

Figure 1.3 Development of BRD9 inhibitors with structure-based drug design (SBDD).

complex with BRD9 and BRD4 provided structural insight into substrate binding and selectivity for BRD9 over BRD4. Further optimization of the thienopyridone scaffold resulted in the synthesis of compounds with improved potency and selectivity for BRD9 culminating in the discovery of **I-BRD9** as a BRD9 chemical probe with nanomolar potency and a selectivity of greater than 700-fold over the BET family and greater than 200-fold over the highly homologous BRD7.

Besides the role of BRD9 in several cancer diseases, recent studies uncovered the utility of **I-BRD9** for treatment of progression of type 2 diabetes [68], which is caused by the dysfunction of pancreatic β-cells [69]. Previously, vitamin D receptors (VDRs) were identified as an important modulator of inflammation and pancreatic β-cell survival. Surprisingly, the use of **I-BRD9** [66] together with vitamin D [70] triggered an activation of VDRs and resulted in an enhanced survival of pancreatic β-cells and glucose homeostasis. Due to the restoration of pancreatic β-cell function by epigenetic regulation of VDR transcriptional response, BRD9 inhibitors consequently constitute a potential target for novel therapeutic approaches for the treatment of type 2 diabetes. The use of **I-BRD9** to link BRD9 to VDR expression and provide a new small molecule target for type 2 diabetes is another example of the utility of chemical probes in target discovery.

1.4 Compound-Based Target Evaluation with Patient-Derived Cells

Ideally, we need a cascade of assays from simple biochemical or physical tests all the way through to disease-relevant systems in order to discover new medicines (see Figure 1.4). In recent years, there has been an increased awareness of the limitations of conventional model systems (e.g. immortalized cell lines, rodent models). As a consequence, the interest to source and build translational assays and test systems using cells and tissue derived from patients has increased significantly in both industry and academia, since such systems hold the promise to recapitulate the disease state more accurately (also see Chapter 7 for more details) [71–73].

1.4.1 Compound-Based Target Evaluation

Translational medical research is to a large extent dependent upon high-quality antibody and chemical-based modulators of protein function for discovery and confirmation of target–disease associations. This can be carried out either as hypothesis-driven or as unbiased approaches. In hypothesis-driven studies, underlying data from genetic studies provide initial evidence for a specific protein target that is subsequently confirmed by the use of a functional antibody or chemical compound. An example of the unbiased and target agnostic approach is phenotypic cell-based screens with biomarker read-outs. Such screens will provide hit compounds in the absence of an initially identified target. Both approaches have their merits and drawbacks, but are complementary and can also be combined, as outlined below [74].

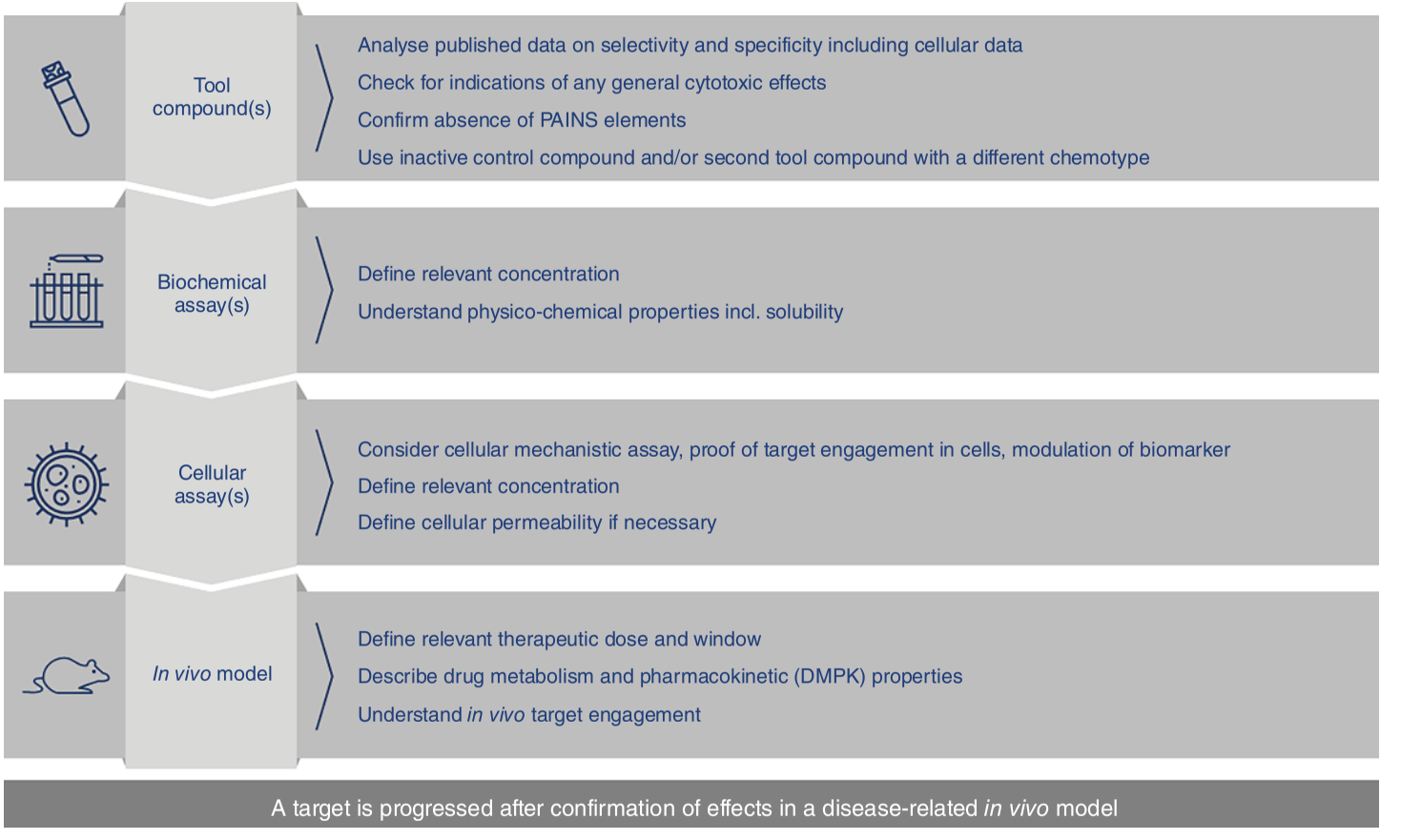

Figure 1.4 Workflow for target validation using tools compounds. Best practices are indicated for the individual steps.

1.4.2 Patient-Derived Cell Assays

Preclinical studies with conventional models of disease often fail to translate into clinical reality. This holds true for most therapeutic areas, ranging from infectious diseases to oncology [75, 76]. In addition, the path from discovery to clinical trials and ultimate market approval is long. For example, a recent analysis of the history of first-in-class kinase inhibitors revealed that often more than a decade transpired between the first published conclusive target–disease association and the initiation of clinical studies [77]. This delay occurred despite the fact that clinical success rates for kinase inhibitors and other targeted therapies are higher compared to classical small molecule medicines [78, 79]. One of the reasons for this is likely that multiple sources of data from different research groups need to, over time, provide converging and supporting disease-link evidence before the pharmaceutical or biotech industry became convinced of embarking on costly drug discovery and development programmes. However, there are also examples where the use of disease models based on patient cells has provided the necessary and supporting validation data. For example, therapeutic antibodies against tumour necrosis factor alpha for rheumatoid arthritis was developed with significant support from data using synovial cell samples from patients, enabled by the development and optimization of new more advanced cell culture conditions [80]. Thus, applying specific and high-quality probes as small molecules or antibodies to patient-derived test systems holds an enormous potential for target discovery and interrogation.

However, clinical samples are not always easily obtainable. The procurement involves direct collaboration with hospitals and clinicians and ethical approval of sample collection and analytical procedures and most importantly requires the consent of the patients themselves. Usually samples are of limited size or volume, remain in a near-native state for a short time, and often do not constitute the foundation for the generation of a renewable resource such as stem cells and organoids. The inherent variability between donors and patients, especially if combined with a relatively low number of samples available, results in observed trends rather than rigid statistical significance of effect. Hence, regular access to samples from multiple patients, along with stringent clinical as well as experimental inclusion and exclusion criteria, is required to allow sufficient amounts of data to be generated and analysed in a meaningful way.

1.4.3 Target Evaluation Approach

The approach that we recommend combines targeted and unbiased approaches: (i) targeted since the compounds used have well-defined protein specificities and if a disease modifying response is observed, it is likely driven by a defined modulation of that protein function, and (ii) unbiased since we apply all chemical probes to all assays, thereby allowing potential new discoveries in the absence of previously established disease-link evidence. Importantly, the same chemical probes are used in all assays from all disease areas, thereby allowing with time direct comparative studies (data integration and analysis) across all diseases studied.

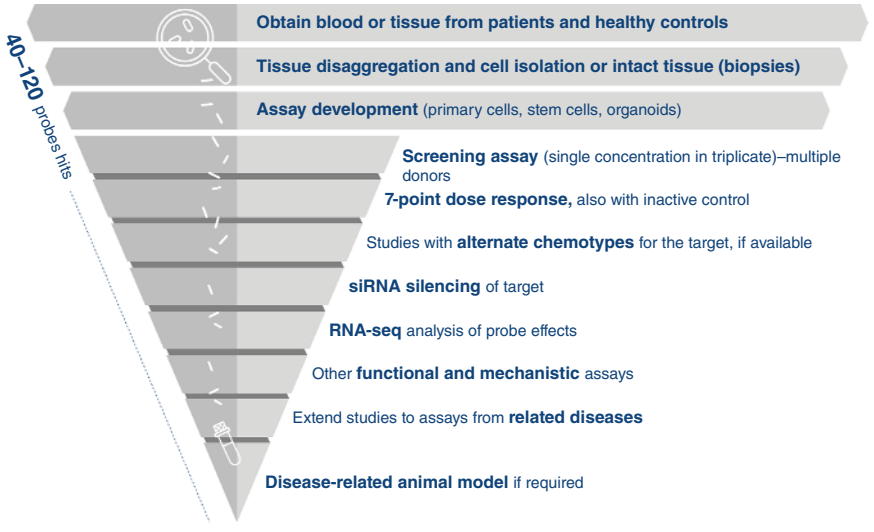

Figure 1.5 A generalized overview of the target validation process carried out at the SGC Tissue Platforms, from obtaining patient samples to functional studies. Source: Adapted from original by Fiona McCann, University of Oxford.

The general experimental approach at the platforms is as follows (Figure 1.5):

1. The initial assay is run at one single compound concentration, in triplicate. Depending on the compound properties, this normally ranges from 0.1 to 1 µmol/l concentration (proven non-toxic concentration). Typically, we include the SGC's chemical probes and compounds within the pharma donated probes collection [23, 24], as well as other compounds from academic and industrial collaborators. For compounds providing a positive response (hit compounds), the experimental steps 2–5 are usually carried out. Data from each step needs to be supportive and conclusive for it to pass on further in the validation cascade.
2. A dose–response curve is generated, typically with seven concentrations (0.1–10 µmol/l). The hit compound is used alongside an inactive control compound (using the aforementioned definition). The hit compound needs to exhibit reproducible dose–response effects, whereas the inactive control should not affect the assay read-outs.
3. If available, a compound with an alternate chemical or chiral structure and specificity profile, but hitting the same primary target, is used. If similar data are generated, this strengthens the case significantly.
4. Moreover, if the chemical probe screen identifies a promising target–disease association, an orthogonal genetic method is applied. Typically, siRNA is used for this purpose, as it is fast and relatively straightforward and, in our experience, mimics compound effects well in the patient-derived test systems.
5. Finally, and depending on the exact purpose of the study, other assays and technologies are applied. These can include gene expression profiling as a consequence of compound treatment, proteomics, and other functional or mechanistic studies, as well as collaborative studies in other related disease areas.

1.4.4 Case Story: Inflammatory Bowel Disease (IBD) Tissue Platform

In the Western world, inflammatory bowel disease (IBD) has been increasing over the last decade, and the prevalence now exceeds 0.3% in North America and Europe [81]. The primary clinical manifestations are severe diarrhoea, abdominal pain, and weight loss resulting from gut chronic inflammation, in particular the colon. Treatment options are few and suboptimal, and the molecular drivers of the disease are poorly understood. Thus, the identification of new intervention points for improved IBD therapies is greatly needed.

The IBD Tissue Platform at Karolinska Institutet and University Hospital was initiated in mid-2017. The aim of the study is to define and validate new intervention points for future therapies. The study is initially planned for three years, during which up to 300 consenting patients can be included. Patients will provide samples of blood and colon biopsies for the generation of patient-based assays.

To date, two assay formats have been generated, one directly on biopsies of colon mucosa, which are maintained under specific culture conditions for 24 hours, and thereafter spontaneous cytokine release is measured. Biopsies retain their inflammatory phenotype during culture, and a clear distinction is observed between non-inflamed and inflamed tissue. The biopsies also respond as expected to drugs in clinical use, such as prednisolone (Figure 1.6).

Since a limited number of biopsies per patient can be obtained and used to study only very few prototype drugs, we also needed to develop blood-based screening assays. Here, the first is a whole blood assay in which we induce inflammation by stimulating nucleotide-binding oligomerization domain-containing protein 2 (NOD2) signalling with muramyl dipeptide (MDP), and thereafter cytokine release is measured. Around 70 open-source chemical probes have been tested to date, reconfirming the effect of previously published kinase inhibitors (such as mitogen-activated protein kinase (MAPK) and receptor-interacting serine/threonine-protein kinase 2 (RIPK2) inhibitors), but also implicating other novel targets; verification studies are currently underway. The study also includes the generation of novel screening platforms based on colon organoids as well as a deep *-omics* patient characterization study of approximately 60

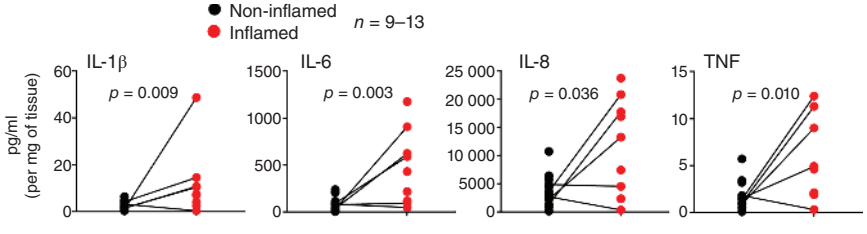

Figure 1.6 Preliminary results (Mann–Whitney test) on spontaneous pro-inflammatory cytokine release in colon tissue culture supernatant, comparing inflamed (red) and non-inflamed (black) mucosa from 17 IBD patients with heterogeneous inflammation and medication profiles. Donor-matched inflamed and non-inflamed tissues were available for five patients (connecting lines). IL, interleukin; TNF, tumour necrosis factor.

patients, including whole genome sequencing (blood and colon mucosa) and gene expression profiling studies.

In conclusion, patient-derived cell and tissue assays can be a powerful tool and complement in preclinical research as described above. However, the establishment of such test systems is complex and resource intense as compared to conventional disease-related models with animals. Hence, it is of utmost importance that the research tools and reagents used, such as chemical probes, are of the highest quality to ensure high-quality assay read-outs and best use of precious patient samples.

1.5 Summary and Outlook

The evaluation of potential new drug targets can be performed in a variety of different ways. Genetic approaches such as gene editing [82] are routinely used to study the function of a target of interest by suppressing its expression (see Chapter 9 for more details). As genetic methods usually remove or suppress the entire protein, they cannot easily reveal the function of an individual domain. Furthermore, the effects are not reversible, which limits their value as a stand-alone method of prioritizing a target for small molecule drug discovery. In contrast, we can use chemical probes and chemogenomic compounds to interrogate any particular function of a targeted protein or protein domain in a dose- and time-dependent manner, and such chemical tools should be used whenever possible to complement other approaches for evaluating and prioritizing new potential targets.

In-depth understanding of the potential and limits of each tool compound is needed to correctly interpret the results. Indeed, there are several examples where tools of poor quality and compounds being used incorrectly have generated misleading results [13]. Nevertheless, user-friendly tools are available to support scientists' search for an appropriate tool compound for target evaluation. Even with the very broad annotation, we must expect effects from uncharacterized off-targets and therefore highly recommend using several tool compounds with different chemotypes whenever possible, as well as applying orthogonal approaches to strengthen the validation case.

The different steps in the path towards evaluating a new target using chemicals tools are depicted in Figure 1.4 along with best practices that we suggest for the individual steps. Biochemical and cellular assays performed with carefully selected tools compounds provide valuable information on the relevance of a novel drug target, but we wish to stress that a target is only fully validated after successful pivotal phase III clinical studies in humans. The number of open-access chemical probes and chemogenomic compounds has significantly increased over the last few years, supported by both academia and pharma companies, such as the recent donated probes initiative [14]. Still, there is an urgent need to identify high-quality chemical tools for a large area of the underexplored druggable genome, as well as de-orphanize new druggable target families. A concerted worldwide effort is needed to fill this gap.

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