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Seeding Collaborations to Advance Kinase Science with the GSK Published Kinase Inhibitor Set (PKIS)

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Abstract: To catalyze research on historically untargeted protein kinases, we created the PKIS, an annotated set of 367 small molecule kinase inhibitors. The set has been widely distributed to academic collaborators as an open access tool. It has been used to identify chemical starting points for development of chemical probes for orphan kinases and to investigate kinase signaling in high content phenotypic assays. Access to the set comes with few restrictions other than the requirement that assay results be released into the public domain for the benefit of the entire research community. Examples from the efforts of several collaborators are summarized.

Keywords: Open access chemical biology, kinase inhibitor probe.

INTRODUCTION

Open access chemical biology has been proposed as a strategy to increase our understanding of fundamental physiology and pathology and, in turn, facilitate drug discovery [1]. The release of high quality chemical probes to the research community dramatically increases knowledge and creates opportunities for proprietary value creation to an extent which would not be possible otherwise [2]. For example, the release of chemical probes for several orphan nuclear receptors led to a large increase in research on them as targets for drug discovery [2]. In another recent example, the impact of quality chemical probes was illustrated by the unencumbered access to the BET family bromodomain probes JQ1, iBET, and PFI-1 [3]. These compounds, accompanied by structurally related inactive negative control compounds, enabled researchers to interrogate BET family function [4] in diverse areas such as oncology, inflammation, and male contraception [5, 6]. Several pharmaceutical companies have initiated programs targeting BET proteins, and tangible value has already been created in the form of startup companies to capitalize on these discoveries [7].

Open access chemical probes have the potential to aid in target validation and exploration of cell signaling pathways. However, despite the promise of precompetitive chemical biology, there are three important factors that limit its implementation. First, there is a dearth of high quality chemical probes [2]. In part, the paucity of probes can be ascribed to the technically challenging, resource intensive, and cross-disciplinary process required for generation of potent, selective, bioavailable compounds. Pharmaceutical companies are well organized for this task, but academia less so. Second, intellectual property (IP) concerns around sharing compounds can overshadow potential value, particularly if the associated research is exploratory rather than directed to a particular, defined commercial endpoint. Surprisingly, the third factor is the aversion to high risk research in both academia and industry, which both have track records of funding those projects that focus on well characterized and precedented targets. This bias has been exemplified by research on the human kinome, where the majority of effort has been directed at a small number of kinases even though several unbiased studies of siRNA or driver mutations suggest the biological potential is broadly distributed across the >500 gene products [8]. The reluctance to pursue untargeted kinases comes despite the track record of this class of enzymes as targets for drug discovery. Over 15 medicines targeting the conserved ATP-binding site have been approved over the past decade.

THE GSK PUBLISHED KINASE INHIBITOR SET (PKIS)

We devised an approach to enable open access chemical biology studies into the kinome with small molecules while at the same time addressing the aforementioned limiting factors. The majority of kinase inhibitors compete with the ATP substrate for binding to a common enzyme active site. This "chemical connectivity" results in cross-activity of inhibitor chemotypes across multiple kinases, which we chose to exploit for the identification of inhibitors of previously unstudied kinases. We selected a set of kinase inhibitors representing 31 chemotypes, within which there was a range of structure-activity against previously targeted kinases. These compounds would aid the research community by [1] serving as a focused screening set against new kinase enzymes, and [2] serving as a set for broad profiling in cellular systems to annotate the phenotype of kinase inhibition.

The GSK Published Kinase Inhibitor Set (PKIS) is a collection of 367 kinase inhibitors previously published by GSK scientists. In assembling the set we applied a number of filters, including availability of the compound, reduction of overrepresented chemotypes, and the diversity of their origi-

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nal kinase targets. The resulting set included 31 chemotypes, each exemplified by between 1 and 31 compounds. The presence of multiple analogs within a chemotype was expected to aid deconvolution of phenotypic screening data by defining a relationship between chemical structure and kinase inhibition profile. Some limited annotation of the PKIS compounds was available from the original literature reports. However, this information was spread across over sixty publications. The data was sparse across the set, heterogeneous, and not readily accessible to scientific investigators. We decided to address the kinase annotation by screening the PKIS across over 200 kinase inhibition assays and making the data readily available at ChEMBL (https://www.ebi.ac.uk/chembl/) [9].

Our plan to promote precompetitive kinase chemical biology would have limited prospects for success if the language accompanying a conventional Material Transfer Agreement (MTA) restricted the disclosure of compound structures and sharing of screening data. However, by limiting the members of the PKIS to kinase inhibitors whose chemical structures had already appeared in the scientific literature, we were able to mitigate many of the concerns within GSK over control of public disclosure. Pharmaceutical companies typically have established publication approval processes which involve vetting of each and every chemical structure which will appear in a manuscript. In our case, the set was constructed such that the individual compound structures had already been subject to an IP decision on composition of matter as part of the approval process for the original scientific publications. Next, we established an abbreviated MTA that did not restrict the use of the compounds as research tools and provided GSK with no rights to the data or results. The only significant stipulation of the MTA was that researchers make publicly available any screening data resulting from use of the set. The process for request and transfer of the set was now straightforward and allowed for expedient execution of agreements with collaborators (Box **1**).

Box 1. How to obtain GlaxoSmithKline PKIS: In order to obtain the GSK PKIS, prospective collaborators must agree to terms of a streamlined MTA, the most significant requirement of which is that data produced from use of the set be made publicly available. The MTA, which can obtained by email request to william.j.zuercher@gsk.com, is presented for consideration without modification. Changes requested to the template can lead to significant delays in consideration and run the risk of failure to establish a collaboration. The PKIS is provided in screening quantities (10 μL of a 10 mM DMSO solution) in 96- or 384-well plates.

As a result, in less than 18 months, the PKIS was distributed to >100 collaborators across the globe. These collaborations all fall into the general theme of advancing kinase science, but include a broad diversity of research, from oncology to neglected tropical diseases to developmental biology. The ability to share the compounds in an open manner not only allowed the set to be characterized in well established assays but also enabled access to expert scientists with deep knowledge in a particular area and, in many cases, the most advanced disease models that have been developed.

PKIS Kinase Activity Characterization*:* Initial activity characterization for the PKIS was addressed through progression through large panels of kinase inhibition assays at Nanosyn (using Caliper technology) and biophysical assays at the Structural Genomics Consortium (using differential scanning fluorimetry) [10]. These screens confirmed the high selectivity of previously reported inhibitors of CSFR, p38α, GSK3, and EGFR/ERBB2, and showed that other compounds in the PKIS have activity across a wide range of other kinases at 0.1 and 1.0 μM. In addition, the results substantiated the utility of the set in the identification of inhibitors of new kinase targets; chemical starting points were found for a number of previously untargeted kinases. In many cases, these chemical starting points showed encouraging potency values and selectivity profiles. Because they were originally generated in lead optimization programs, their optimization into high quality probes may be facilitated, for example, by extant cocrystal structures and/or known structure activity relationships. Moreover, the resulting full rank matrix data set can now serve as a guide for the interpretation of other phenotypic screening data.

PKIS Characterization in Non-Kinase Target Based Assays: Luciferase enzymes are some of the most common reporter enzymes employed in modern high throughput bioassays. Consequently, a thorough understanding of luciferase interactions is necessary for the valid interpretation of these assays. Inglese and coworkers at the NCATS obtained the PKIS and assayed the set against the commonly used luciferase reporter enzymes from the firefly *Photinus pyralis* (FLuc) and marine sea pansy *Renilla reniformis* (RLuc) [11]. A total of 22 compounds (approximately 6% of the PKIS) were found to inhibit the ATP-dependent FLuc with 10 compounds showing potencies ≤1 μM. The resulting data is useful in interpretation of results from assays employing these reporters, and moreover, the identification of FLuc inhibitors distinct from those previously observed confirmed the value of experimental validation of FLuc activity for compounds of interest.

PKIS Screening in Disease Models: Ependymoma is the third most common brain tumor in children. An ependymoma treatment is desperately needed. The malady is incurable in 40% of cases, and has a ten year survival rate of <50%. Gilbertson and coworkers generated an *in vivo* mouse model, mEP^{Ephb2}, which recapitulated histologic and transcriptomic markers of cereberal ependymoma subtype-D [12]. Cells isolated from mEP^{Ephb2} model were incorporated in the construction of a robust, high throughput assay system to assess compound effects in comparison with neural stem cells and nonependymoma tumor cells [13]. PKIS screening in this assay system identified several compounds which disrupted mEP^{Ephb2} cell proliferation. Significantly, the kinase activity annotation associated with the set helped to implicate several kinases for targeting which had not been previously suggested as drivers of ependymoma, including IGF1R, PLK1, CDK2, and JNK2/3. In cases where compounds are approved or in clinical trials, such screening of a highly annotated compound set enables therapeutic hypotheses which can be immediately translated to the clinic for testing.

These three examples illustrate the effectiveness of the PKIS as a vehicle to establishing collaborations to explore kinase inhibition. The data output from investigations which employ PKIS will be openly available at ChEMBL. As such, we are beginning to see an evolution from individual collaborations to an interactive network of collaborators where results from one group inform the studies of another. We expect the public annotation on this set of compounds across a range of diverse assays will generate insight into the patterns of kinase inhibition that yield favorable responses in phenotypic assays. It is expected that this annotation will enable unanticipated connections to new disease therapies that would otherwise remain unrealized.

CONCLUSION

We described a model involving the provision of published compounds to facilitate productive collaborative relationships with academic investigators. Key aspects of the approach include (1) a strategic set of compounds designed both for the identification of chemical starting points for untargeted and orphan kinases and for annotation of kinase signaling in cellular and phenotypic assays, (2) a streamlined material transfer agreement that requests data be made publicly available, and (3) open access to data derived from use of the set. Results to date substantiate that collaborations can be rapidly established, and that the compound set has been tested in a diverse range of screening assays.

The untargeted kinome provides an unrealized opportunity to demonstrate the potential of open access chemical biology, and the release of the PKIS is only a first step. It is hoped that this compound set will be only one component in a broader effort to identify therapeutic opportunity beyond the limited number of druggable proteins in order to create new medicines for human diseases [14].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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