Structural Genomics Consortium

Readme: SGC Epigenetic Chemical Probe Collection

Contents

Concentration Range, Storage and Handling Recommendations

1. The Cell Assay Parameters spreadsheet lists guidelines for performing a <u>cellular biomarker assay</u> and the recommended concentration (IC_{90}) for a <u>biomarker or phenotypic assay</u>. The guidelines include the compound concentration at which significant biomarker activity of the cellular target is observed, duration of the assay, and ***in vitro*** off-target proteins. **Important**: The 'recommended concentration' in the spreadsheet is designed to avoid off-target toxicity and/or off-target activity. Using concentrations that are higher than recommended could lead to false positive or confounding results.
2. Please refer to the MW listed in the Cell Assay Parameters spreadsheet.
3. Dissolve in DMSO in the range 10-20 millimolar, make aliquots and store in a -80°C freezer in a microtiter plate or in vials. If the probe does not dissolve readily use gentle heating and/or sonication and/or vortexing. (The aliquots can be stored for 6 months at this temperature. Please use only 1 freeze/thaw cycle per aliquot.)
4. Add fresh inhibitor at the same concentration with every cell split, media change.

Overview of the SGC Epigenetic Chemical Probe Collection

Chemical probes are well-characterized drug-like small molecules that potently and selectively inhibit/antagonize the target protein *in vitro* with a defined mode of action (MOA) (Frye, 2010). These probes enable the researcher to link selective inhibition/antagonism of a specific protein target with a biological and disease phenotype in cell-based assays with high confidence. These compounds are primarily intended for initial target validation or phenotypic profiling studies in cell lines or primary patient samples cultured *in vitro*. As such, each probe has been confirmed to be cell-permeable and stable in cells, but may not necessarily have favourable pharmacokinetic properties for *in vivo* studies. Importantly, each probe has also been shown to bind to and inhibit/antagonize the intended target in the cell at low μM concentrations. Please see the original publications for more details on individual chemical probes; the PubMed identifiers are listed in Appendix A.

The SGC Epigenetic Chemical Probe Library currently comprises more than thirty well-characterized compounds that selectively and potently inhibit/antagonize specific chromatin regulatory proteins or domains including protein methyltransferases, demethylases, and bromodomains. This collection will grow by about six probes per year. Consult our website, http://www.thesgc.org/chemical-probes/epigenetics, for periodic release of new chemical probes. Most chemical probes are matched with a 'control compound' that is structurally similar to the active probe, but is inactive or much less active at inhibiting/antagonizing the target protein. Such compounds are important reagents to control for potential off-target effects and should be used as 'negative controls' to confirm that the activity you see in cells is due to the biochemical inhibition of the intended target (much as one would use a 'scrambled shRNA' as a control in an shRNA knockdown experiment). If the control compound gives the same or similar response to the chemical probe, then the observed cellular response cannot be due to inhibition/antagonism of the target protein. Information on control compounds is also listed on the SGC website; these are available upon request.

Use of the Epigenetic Chemical Probe Collection in Phenotypic Assays

Screen Design and Implementation

Epigenetic chemical probes have the potential to selectively alter gene expression programs or affect other genome associated processes (stability, replication, silencing, etc.) thereby potentially reprogramming cellular states. The molecular nature of epigenetic regulation often results in longer timeframes for observation of

resultant phenotypic effects compared to, for example, modulation of phosphorylation signalling. Because cell division is often required (allowing for 'dilution' of chromatin methyl marks and/or resultant changes in chromatin, for example), several days may be required to observe phenotypic effects. General guidelines and best practises for designing phenotypic assays have been published (Inglese et al., 2007; Mark-Anthony Bray, 2012), however, for the purpose of using the SGC's library in phenotypic assays, we would like to provide the following recommendations.
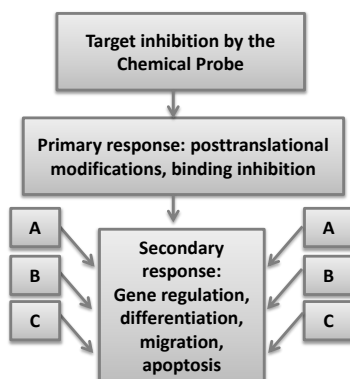
- Use DMSO to make a stock solution for each chemical probe with sufficient volume to replenish it at every change of media or splitting of cells. This will maintain a constant concentration of the chemical probe in the media. Reference the <u>Cell Assay Parameters</u> spreadsheet for the IC_{90}. A snapshot of the spreadsheet is in Appendix B on page 7.

   o For the initial screen, do NOT exceed the concentration specified in the IC_{90} column.

- We recommend using two controls for the initial screen: a media only control and a DMSO control. For example, if the stock solution of the chemical probe is 20 millimolar and the final concentration required for the assay is 10 micromolar, then use a 0.2% DMSO control. (Many bromodomains are weakly inhibited by DMSO and we recommend 0.2% as a highest concentration in the assay.)

- <u>Design the assay to capture the expected length of time required to observe the effect of a given compound, see column "Minimum time required to observe a phenotypic change".</u> Selecting the appropriate duration is an important requirement for capturing full effects such as cell differentiation and other reprogramming events. For example, histone methyltransferases require several days for a primary response (change in level of the histone methyl mark) and a week or more for a phenotypic response (Daigle et al., 2011; M Vedadi 2011; McCabe et al., 2012; W Yu, 2012).

- When the initial screen is complete, note the chemical probes for which there was a response and confirm these observations with the following dose-response experiments.

   o Repeat the assay over a range of probe concentrations that spans at least 2 orders of magnitude centered around the cellular EC_{50} value, as well as media and DMSO-only controls
   o For each 'active' chemical probe perform the same dose-response experiment with the related inactive 'control' compound if available. The control compound should give no response or a much weaker response if the phenotype is related to inhibition of the target.
   o Even if there is a control compound, we recommend using (if available) two or more chemical probes for the same target. The best case scenario is that the probes have different chemotypes, for example (+)-JQ-1 and PFI-1 for BET bromodomains. If these are used in parallel, there are two possible outcomes. If both probes yield a similar phenotypic response then it is more likely that inhibition of the target is responsible for the observed phenotype (Pappano et al., 2015). If the phenotypic response is not in the same direction, there could be confounding off-target or toxic effects from one but not the other probe.

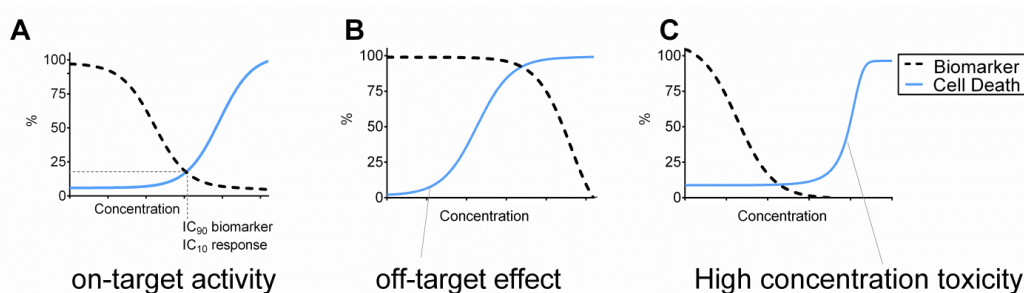## Mechanistic follow-up to confirm on-target activity

For a candidate chemical probe/target of interest, use the index publication (see Appendix) and the spreadsheet to identify 'maximum recommended concentration', the 'minimum time required for full reduction of in-cell biomarker activity', and the "closest *in vitro* off-target". With these parameters in mind, design a follow-up experiment using the principles described in the following example.

Classify primary and secondary cellular responses – compare primary (biochemical) dose response to downstream functional and phenotypic events also measured in a dose dependent manner (Figure 1). This data will establish the causal relationship between the target and the phenotype. The primary response can often be monitored by a suitable biomarker (such as the cellular levels of a histone methyl mark deposited by an enzyme that is inhibited by the probe). Secondary functional readouts such as cell viability are often dependent on many

other factors including those that are activated in response to a general toxic response. A general toxic response can be elicited by many chemotypes and may not necessarily be related to direct inhibition of the target. Three scenarios of cellular responses to chemical probes are outlined in Figure 2.

Figure 1. Chemical probes response cascade. Arrows on boxes A-C represent contributions to the secondary responses that are unrelated to the probe.

Figure 2. Dose response scenarios for the response biomarker and secondary events. A. Correlated and likely causal relationship of biomarker and viability responses. B. Viability changes occurring at lower concentrations than the target inhibition as measured by the biomarker indicates no-causal relationship. C. Dose response relationship where full biomarker inhibition does not elicit changes in cell viability likely indicates non-causal relationship.

In cancer research cell viability is often a parameter of interest. If an enzyme is 90% inhibited at a particular concentration of the probe (as measured by a biomarker) and cell viability starts to decrease at this concentration (Figure 2A), then this is likely to be a target-specific cell death. Such a conclusion can be further supported by genetic knockdown of the target. In another scenario, if the cell death occurs at probe concentrations lower than that required for target inhibition (measured by a biomarker), then the response is most-likely not target related (Figure 2B), and may be due to off-target or general cytotoxic effects of the probe. The use of chemically similar, but target-inactive (or less active) 'control' compounds is very helpful here. The shape of the cell viability curve can also reveal off-target effects, as excessively steep or shallow slopes have been associated with polypharmacology, population response heterogeneity or non-specific toxicity (Fallahi-Sichani et al., 2013). The third scenario is one in which the apparent functional effect occurs at much higher concentrations than target inhibition (Figure 2C) and most often this kind of response is caused by non-specific compound toxicity at high concentrations. Again the slope of the toxicity curve can be revealing and negative control compounds are important. A complementary strategy is to use two or more chemically unrelated probes for the same target; it is highly unlikely that two different compounds will show the same off-target activity.

Future Directions

We hope you find the SGC chemical probes useful in your research. During the discovery and development of these compounds the SGC scientists have gained extensive experience with these compounds and their targets. In many cases additional reagents have been developed; these include cell lines over-expressing the target, or its inactive mutants, protocols for assaying the biomarker of a target, biotinylated derivatives of the chemical probes for use in chemoproteomics or ChIP experiments. The SGC has also developed recombinant antibodies to many chromatin proteins including targets of many of our chemical probes. Feel free to contact us to discuss potential further collaborations based on your use of our chemical probes (Arrowsmith et al., 2015; Bunnage et al., 2013; Drewry et al., 2014; Edwards et al., 2015; Edwards et al., 2009; Knapp et al., 2013; Workman and Collins, 2010).

Appendix A: A list of SGC's inhibitors and PubMed IDs

Domain: Protein target	Probe	Control	PubMed ID
BRD: ATAD2A/B	GSK8814	GSK8815	27530368
BRD: ATAD2A	BAY-850	BAY-460	
BRD: BET family	(+)-JQ1	(-)-JQ1	20871596
BRD: BAZ2A/2B	BAZ2-ICR		25719566
BRD: BAZ2A/2B	GSK2801	GSK8573	25799074
BRD: BRD9/7	BI-9564		26914985
BRD: BRD9/7	TP-472	TP-472N	
BRD: BRD9	I-BRD9		25856009
BRD: BRPF1/2/3	OF-1		
BRD: BRPF1/2/3	NI-57		
BRD: BRPF1B	GSK6853	GSK9311	27326325
BRD: BRPF2/TAF1	BAY-299	BAY-364	
BRD: CECR2	NVS-CECR2-1		
BRD: CREBBP/EP300	SGC-CBP30	BDOIA513	24946055
BRD: CREBBP/EP300	I-CBP112		26552700
BRD: SMARCA2/4	PFI-3	BDF25488524	26139243
DEHYDR: IDH1 mutant	GSK864		26436839
KDM: JMJD3/UTX	GSK-J4*/J1	GSK-J5	22842901
KDM: LSD1	GSK-LSD1		26175415
Kme: L3MBTL3	UNC1215	UNC1079	23292653
MT: DOT1L	SGC0946	SGC0649	23250418
Kme: EED	A-395	A-395N	
MT: EZH2	GSK343		24900432
MT: EZH2/H1	UNC1999	UNC2400	23614352
MT: G9a/GLP	A-366		24900801
MT: G9a/GLP	UNC0642		24102134
MT: PRMT type 1	MS023	MS094	26598975
MT: PRMT3	SGC707	XY-1	25728001
MT: PRMT4/6	MS049	MS049N	27584694
MT: PRMT4	TP-064	TP-064N	
MT: PRMT5	GSK591	SGC2096	26985292
MT: PRMT5	LLY-283	LLY-284	
MT: SETD7	(R)-PFI-2	(S)-PFI-2	25136132
MT: SMYD2	BAY-598	BAY-369	27075367
MT: SUV420H1/H2	A-196	SGC2043	
PAD: PADI4	GSK484	GSK106	25622091
WD40: WDR5	OICR-9429	OICR-0547	26167872

Appendix B: A snapshot of the data in the Cell Assay Parameters spreadsheet; maximum dose recommended is the IC_{90}

Target	Compound	Recommended concentration, IC_{90} (micromolar)
BAZ2A/B	GSK2801	1
BAZ2A/B	BAZ2-ICR	1
BET (BRD2, BRD3, BRD4, BRDT)	JQ1	1
BRD9/7	BI-9564	1
BRD9/7	TP-472	1
BRD9	I-BRD9	1
BRPF1, BRPF2, BRPF3	NI-57	1
BRPF1B	PFI-4	1
BRPF1B	GSK6853	0.5
BRPF2(1)/TAF1(2)	BAY-299	1
CBP, EP300 bromo	SGCCBP30	1
CBP, EP300 bromo	ICBP112	1
CECR2	NVS-CECR2-1	1
Dot1L	SGC0946	1
EED	A-395	1
EZH2, EZH1	UNC1999	3
EZH2	GSK343	3
G9a, EHMT1	UNC0642	1
G9a, EHMT1	A-366	1
JMJD3, UTX, JARID1B	GSK J4	5
IDH1 mutant	GSK864	1
L3MBTL3	UNC1215	1
LSD1	GSKLSD1	1
PAD4	GSK484	10
PRMT type I	MS023	1
PRMT4/6	MS049	5
PRMT3	SGC 707	1
PRMT4	TP-064	1
PRMT5	GSK591	1
SETD7	PFI-2	1
Smarca2 Smarca4, PB1	PFI-3	1
SMYD2	BAY598	1
SUV420H1/2	A-196	1
WDR5	OICR-9429	3

References

Arrowsmith, C.H., Audia, J.E., Austin, C., Baell, J., Bennett, J., Blagg, J., Bountra, C., Brennan, P.E., Brown, P.J., Bunnage, M.E., *et al.* (2015). The promise and peril of chemical probes. Nat Chem Biol *11*, 536-541.

Bunnage, M.E., Chekler, E.L., and Jones, L.H. (2013). Target validation using chemical probes. Nat Chem Biol *9*, 195-199.

Daigle, S.R., Olhava, E.J., Therkelsen, C.A., Majer, C.R., Sneeringer, C.J., Song, J., Johnston, L.D., Scott, M.P., Smith, J.J., Xiao, Y., *et al.* (2011). Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer cell *20*, 53-65.

Drewry, D.H., Willson, T.M., and Zuercher, W.J. (2014). Seeding collaborations to advance kinase science with the GSK Published Kinase Inhibitor Set (PKIS). Current topics in medicinal chemistry *14*, 340-342.

Edwards, A.M., Arrowsmith, C.H., Bountra, C., Bunnage, M.E., Feldmann, M., Knight, J.C., Patel, D.D., Prinos, P., Taylor, M.D., Sundstrom, M., *et al.* (2015). Preclinical target validation using patient-derived cells. Nat Rev Drug Discov *14*, 149-150.

Edwards, A.M., Bountra, C., Kerr, D.J., and Willson, T.M. (2009). Open access chemical and clinical probes to support drug discovery. Nat Chem Biol *5*, 436-440.

Fallahi-Sichani, M., Honarnejad, S., Heiser, L.M., Gray, J.W., and Sorger, P.K. (2013). Metrics other than potency reveal systematic variation in responses to cancer drugs. Nat Chem Biol *9*, 708-714.

Frye, S.V. (2010). The art of the chemical probe. Nat Chem Biol *6*, 159-161.

Inglese, J., Shamu, C.E., and Guy, R.K. (2007). Reporting data from high-throughput screening of small-molecule libraries. Nat Chem Biol *3*, 438-441.

Knapp, S., Arruda, P., Blagg, J., Burley, S., Drewry, D.H., Edwards, A., Fabbro, D., Gillespie, P., Gray, N.S., Kuster, B., *et al.* (2013). A public-private partnership to unlock the untargeted kinome. Nat Chem Biol *9*, 3-6.

M Vedadi , D.B.-L., F Liu, S Rival-Gervier, A Allali-Hassani, V Labrie, TJ Wigle, PA Dimaggio, GA Wasney, A Siarheyeva, A Dong, W Tempel, SC Wang, X Chen, I Chau, TJ Mangano, XP Huang, CD Simpson, SG Pattenden, JL Norris, DB Kireev, A Tripathy, A Edwards, BL Roth, WP Janzen, BA Garcia, A Petronis, J Ellis, PJ Brown, SV Frye, CH Arrowsmith and J Jin (2011). A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nature Chemical Biology *7*, 9.

Mark-Anthony Bray, A.C. (2012). Assay Guidance Manual. In Advanced Assay Development Guidelines for Image-Based High Content Screening and Analysis, C.N. Sittampalam GS, Nelson H, Arkin M, Auld D, Austin C, Bejcek B, Glicksman M, Inglese J, Iversen PW, Li Z, McGee J, McManus O, Minor L, Napper A, Peltier JM, Riss T, Trask OJ Jr., Weidner J, ed. (Eli Lilly & Company and the National Center for Advancing Translational Sciences).

McCabe, M.T., Ott, H.M., Ganji, G., Korenchuk, S., Thompson, C., Van Aller, G.S., Liu, Y., Graves, A.P., Della Pietra, A., 3rd, Diaz, E., *et al.* (2012). EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature *492*, 108-112.

Pappano, W.N., Guo, J., He, Y., Ferguson, D., Jagadeeswaran, S., Osterling, D.J., Gao, W., Spence, J.K., Pliushchev, M., Sweis, R.F., *et al.* (2015). The Histone Methyltransferase Inhibitor A-366 Uncovers a Role for G9a/GLP in the Epigenetics of Leukemia. PloS one *10*, e0131716.

W Yu, E.C., AK Wernimont, W Tempel, A Scopton, A Federation, JJ Marineau, J Qi, D Barsyte-Lovejoy, J Yi, R Marcellus, RE Iacob, JR Engen, C Griffin, A Aman, E Weinholds, F Li, J Pineda, G Estiu, T Shatseva, T Hajian, R Al-Awar, JE Dick, M Vedadi, PJ Brown, CH Arrowsmith, JE Bradner, M Schapira (2012). Catalytic site remodelling of the DOT1L methyltransferase by selective inhibitors. Nature Communications *3*.

Workman, P., and Collins, I. (2010). Probing the probes: fitness factors for small molecule tools. Chemistry & biology *17*, 561-577.