SGC-iMLLT: A chemical probe for the YEATS domains of MLLT1/MLLT3

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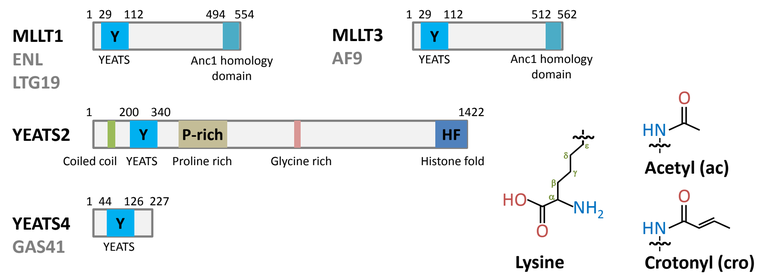
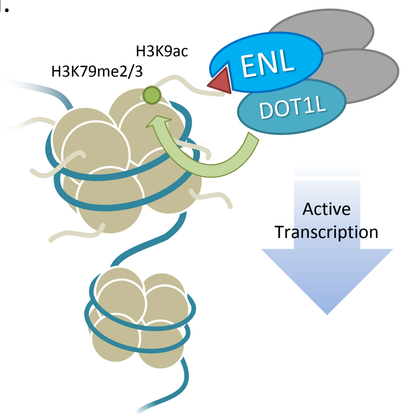
Introduction

In recent years, YEATS (Yaf9, ENL, Af9, Taf41, Sas5) domains have emerged as readers of histone post-translational modifications (HPTM) alongside bromodomains, PHD fingers and others. Like bromodomains, they recognise acylation on the ε-carbon atom of lysine and human YEATS domain containing proteins have been implicated as actors in a range of cancer types.



The paralogs MLLT1(aka ENL) and MLLT3(aka AF9) have long been known for their role in mixed lineage leukaemia (MLL) as fusion partners of KMT2A/MLL. However, the role of functional YEATS domains in MLL remains poorly understood.

Here, report the discovery of a selective inhibitor with sub-micromolar affinity for MLLT1/MLLT3 through a library screen and its development into a chemical probe for the YEATS domains of MLLT1 and MLLT3.



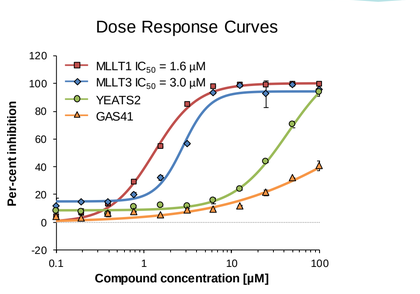
SGC-iMLLT is a potent inhibitor of both YEATS domains (K_d ≈ 100 nM, in vitro IC_{50} < 500 nM and cellular activity at < 1 μM for both MLLT1 and MLLT3) and selective over the other human YEATS domain containing proteins as well as bromodomains.

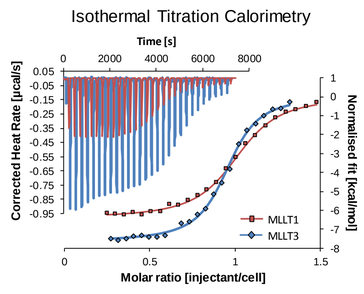
Library Screen

Using a peptide displacement assay for MLLT1 based on AlphaScreen® technology, we screened a set of 40,000+ compounds from our internal bromodomain inhibitor library and the diversity set of the Ontario Institute for Cancer Research and further characterised the hits, yielding one selective hit with an IC_{50} at around 1 μM.

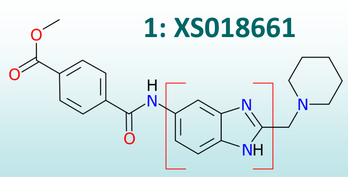
OICR Ontario Institute for Cancer Research + SGC

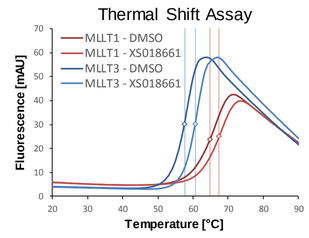
Single concentration screen → **Structure based selection** → **Biophysical characterisation of best hit**





1: XS018661



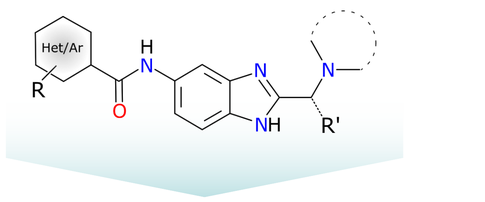


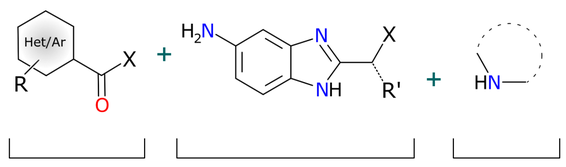
"SAR-by-catalogue" around the [core] with purchased analogues revealed that both sides around the core are required for binding.

	MLLT1	MLLT3
IC_{50}	1.6 μM	3.0 μM
ΔT_m	1.9 °C	3.7 °C
K_d (BLI)	530 nM	N/T
K_d (ITC)	754 nM	523 nM
ΔH (kcal/mol)	-6.4	-7.6
ΔS (cal/mol·K	6.2	3.1

Medicinal Chemistry

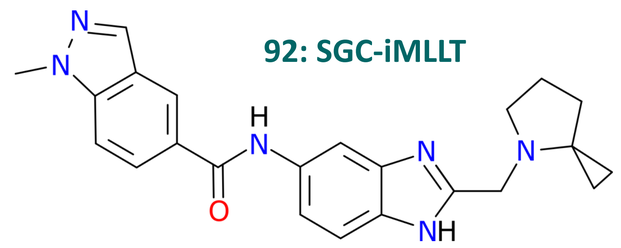
Following "SAR-by-catalogue" studies on **XS018661** , we utilised a "poised" approach to ligand design: Compound **XS018661** was disconnected into synthons for rapid diversification, culminating in synthesis and screening of >200 lead generation analogues.





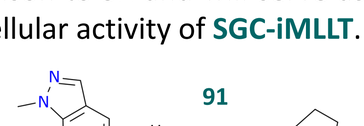
All analogues were tested in full dose response curves in the peptide displacement assays. Compound 92 was chosen as the final probe, **SGC-iMLLT**.

92: SGC-iMLLT



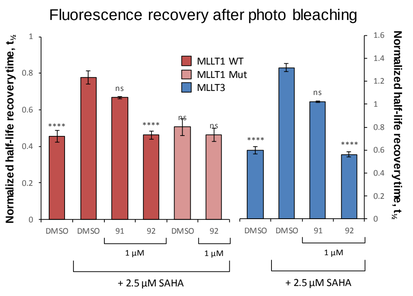
During the screening of the chemical series, compound 91, an isomer of compound 92, showed significantly reduced activity in comparison to 92 and will serve as a reference compound for cellular activity of **SGC-iMLLT**.

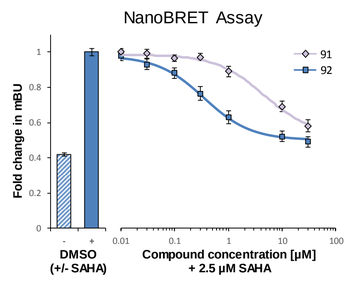
91

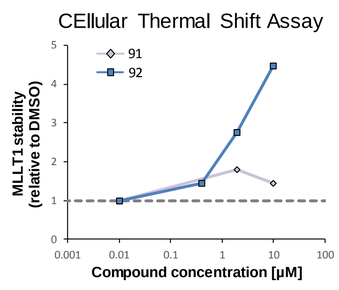


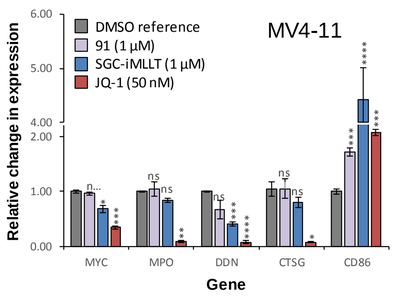
Cellular activity

To assess the cellular activity of **SGC-iMLLT**, we directly measured its ability to displace MLLT1 and MLLT3 from chromatin (FRAP assay and NanoBRET). Its ability to stabilise native MLLT1 in cells was assessed using Cellular Thermal Shift Assay (CETSA). Using the known MYC down-regulator JQ-1 as a reference compound, we investigated the probe's ability to affect the expression of several genes regulated by MLLT1/MLLT3 in MV4-11 cells via qPCR.

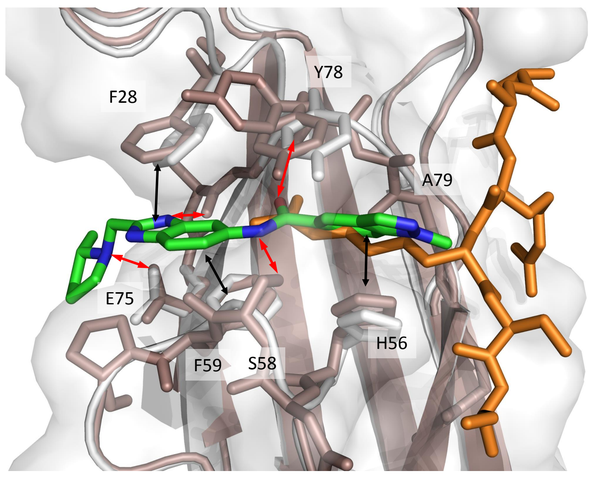








Crystal Structure

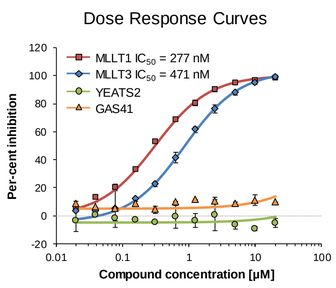


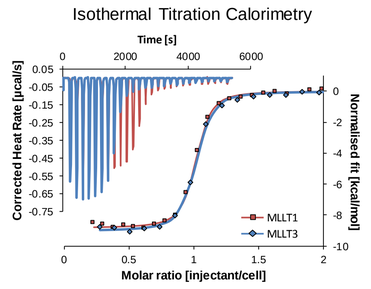
Detailed interactions of **SGC-iMLLT** (green sticks) with the peptide binding site of the MLLT1 YEATS domain (red, PDB ID pending) overlaid with MLLT1 YEATS domain (grey) and H3K9ac peptide (orange sticks) from PDB 5J9S (Wan et.al, 2017).

Y78 adopts two conformations - an 'in' pose where a π-stacking interaction with the amide of **SGC-iMLLT** can take place along with an 'out' pose in which the Y78 side chain is now displayed edge-to-face with the adjacent side chain of F28.

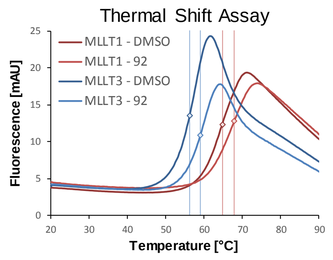
Biophysical Characterisation

SGC-iMLLT was then further characterised. In addition to thermal shift assays, ITC and BLI, the IC_{50} were re-determined (n >> 3) using both the AlphaScreen® based displacement assay as well as a HTRF (Cisbio) based version as an orthogonal technique. Both assays are in good agreement and the mean values are shown. The probe showed excellent selectivity over the other two human YEATS domain containing proteins as well as a small panel of bromodomains (not shown).





	MLLT1	MLLT3
IC_{50}	277 nM	471 nM
ΔT_m	4.0 °C	3.5 °C
K_d (BLI)	192 nM	N/T
K_d (ITC)	113 nM	103 nM
ΔH (kcal/mol)	-8.8	-9
ΔS (cal/mol·K)	2.2	1.7



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