Development of DGK Inhibitors, hit validation of initial HTS

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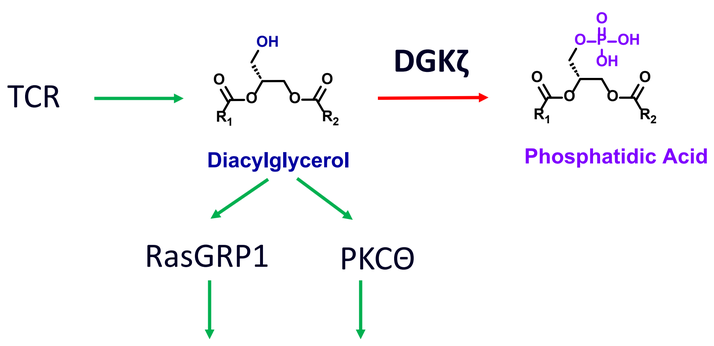
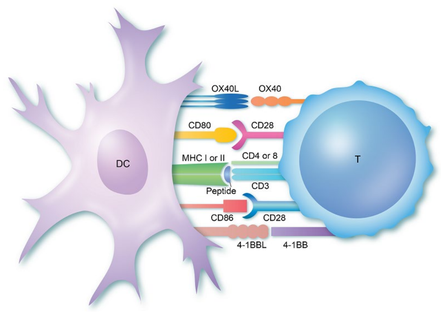
TARGET HYPOTHESIS

Disease setting: Immune oncology, solid tumours.

- **DAG regulates immune cell signalling and activation** (T-cells and NK cells).
- Diacylglycerol kinase zeta (**DGKζ**) catalyses the phosphorylation of DAG to PA.
- DGK has 10 isoforms, ζ and α are those mostly expressed in the immune system.
- **Limiting the pool of DAG effects a positive signalling through T-cells.**





Schematic of early T cell activation through binding to a dendritic cell (DC). Subsequently, co-stimulation occurs through DC-bound CD86, CD80, OX40L and 4-1BBL. This induces full activation and effector function in the T cell.[1]

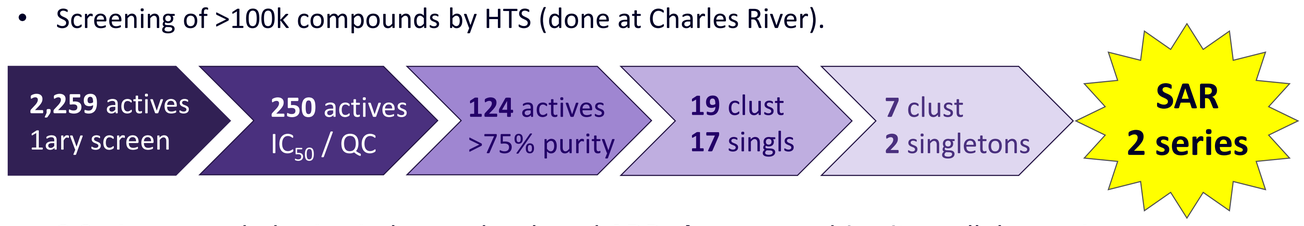
T-cell activation

OBJECTIVES and METHODOLOGY

- Tumour evasion of immune responses linked to **DGKζ and DGKα deregulation**.
- **Inhibition** of both DGKζ and DGKα to maintain higher levels of DAG, promote T cell proliferation and anti-tumour activity.
- Thus, **activating the immune response in "cold" tumours, resulting in activation of cytokine and inflammatory responses to stimulate the immune system to kill tumours**.

- Potent mixed inhibitors targeting DGKζ and DGKα identified at the onset of the project, the specific effects of selective inhibition remained unexplored.

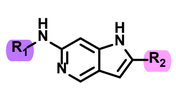
- **Finding a "first-in-class" oral inhibitor of DGKζ.**
- Screening of >100k compounds by HTS (done at Charles River).

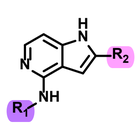


- 2 Series expanded using in-house developed **ADP-glo assay** and ***in vitro*** cellular testing.

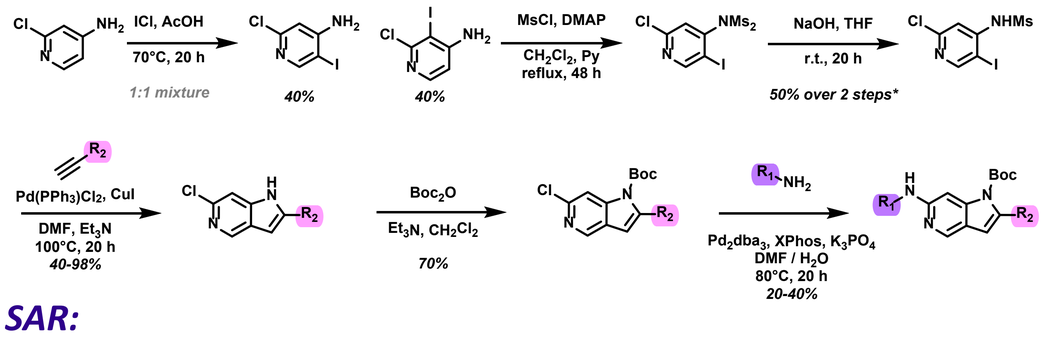
AZAINDOLE SERIES

- **From initial triage, cluster of 40 compounds with IC_{50} < 10 µM**.
- SAR-study around two regioisomers was carried out.





Library Synthesis:



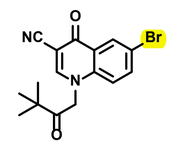
SAR:

IC_{50} µM	MeO	OMe	Cl	CF_3	Me	Me	Me-N-Me	MeO / MeO
	49	19	20	204				
	21	21	18*	23	37	37	32	34
	148	165	20*	95				
	50		>100		58	61	84	9
	44		85		42	70	76	71
	30		9		6	23	23	21

* Original hits from HTS, values are for retest in ADP-Glo assay (in house).

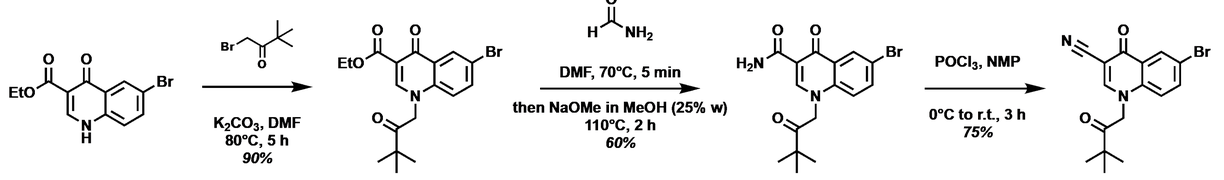
DIHYDROQUINOLINE SERIES

- Singleton choice with IC_{50} < 10 µM.
- **Br atom offers excellent synthetic vector for growing the fragment**.
- SAR around this region and around the "southern" part of the hit is shown below.



DGKζ IC_{50} = 8 µM
DGKα IC_{50} > 250 µM

Library Synthesis:



DGKζ IC_{50} > 250 µM

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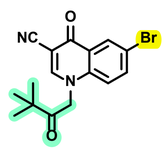
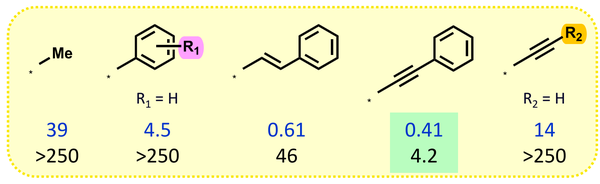
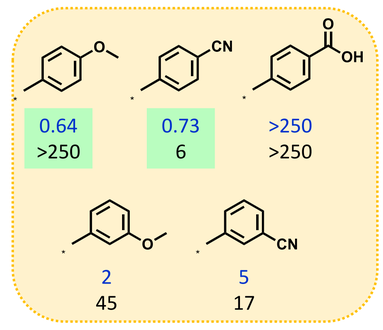
SAR:



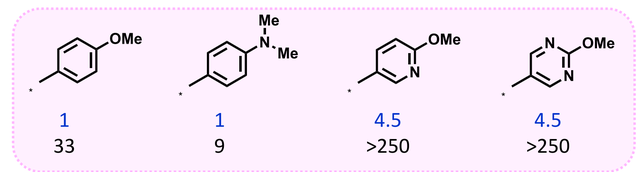
DGKζ IC_{50} (µM)
DGKα IC_{50} (µM)



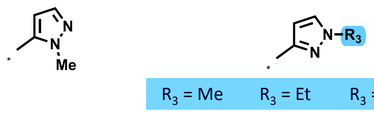
➢ R_1 substitution highly tolerated in *para* position, especially E.D.G.



- Other R_2 substituents not tolerated (*i.e.* pyridine, pyrimidines, bicycles, sat.)

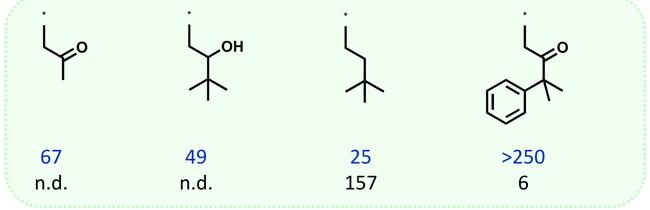


DGKζ IC_{50} (µM)
DGKα IC_{50} (µM)



	R_3 = Me	R_3 = Et	R_3 = iPr	R_3 = Bn	R_3 = Ph
DGKζ IC_{50} (µM)	4	36	>250	>8	99
DGKα IC_{50} (µM)	>250	>250	>250	50	89

- Expansion of 5-memb set, tolerability and selectivity.

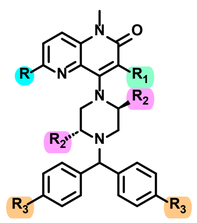


DGKζ IC_{50} (µM)
DGKα IC_{50} (µM)

- SAR on "southern" part = large bulky ketone helps with potency.
- Better interactions to be found?

COMPETITORS

- BMS has 2 optimized leads. Very potent but not selective. IC_{50} measured using biochemical lipid kinase assay.[2]



BMS-502 - R = CN, R_1 = NO_2, R_2 = H, R_3 = F
IC_{50} DGKζ = 0.002 µM, α = 0.005 µM

BMS-332 - R = CN, R_1 = H, R_2 = Me, R_3 = Me
IC_{50} DGKζ = 0.008 µM, α = 0.009 µM

CONCLUSION

- Extensive synthetic efforts to expand both series with SAR studies.
- **Azaindole series** hindered by lengthy synthesis and poor reactivity (*e.g.* Buchwald-Pd coupling in last step).
- **Dihydroquinolinone series** widely expanded and explored, **12-fold potency gain with ethynyl-linker** (Potential *hit-to-lead).*
- Unfortunately, no DGKα IC_{50} for all compounds due to assay format / protein production / purification.
- Competitors are in clinic with very potent (but not selective) candidates.

1. Cavanagh M and Findlay EG, BSI
2. Wichroski *et al.*, *Sci. Transl. Med.* **2023**, 15 (719)

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