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Combining Artificial Intelligence and Human Expertise in Pursuit of Novel PolQ Inhibitors

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Introduction

Application of Iktos' Makya generative AI platform, synergistically with traditional knowledge-based approaches, has expedited the discovery of small molecule PolQ polymerase domain inhibitors. Following the synthesis and testing of a diverse range of ligands proposed by Makya, we decided to focus our attention on a novel oxime template. Although the initial hit showed modest biochemical potency, substitution off the oxime, a vector unexplored by related ligands, was considered a route to increased biochemical activity and a means to deliver a compound with a suitable ADME profile. Gratifyingly, using this strategy we have delivered a highly potent array of functionalised oxime PolQ inhibitors displaying good initial ADME properties.

1 PolQ Biology

DNA damage response factors such as DNA polymerase theta (POLθ or POLQ) show synthetic lethality with homologous recombination (HR) factors such as BRCA1 and BRCA2. Using this precision oncology approach, PolQ inhibitors can selectively target HR-deficient cancers that occur in the breast, ovary, prostate and pancreas.[1]

This unique oncogenic fusion protein comprises both DNA polymerase (Pol) and DNA helicase domains that function together to facilitate multiple DNA repair mechanisms (**Figure 1**).[2] Small molecule inhibitors targeting both domains have been described throughout the last decade and have shown potent and selective HR-deficient tumor cell death *in vitro* and *in vivo*. Despite the growing number of advanced PolQ polymerase domain inhibitors reported, significant ADME liabilities remain associated with these scaffolds.[1-2] New approaches, such as the application of generative AI, present the opportunity to solve these problems.

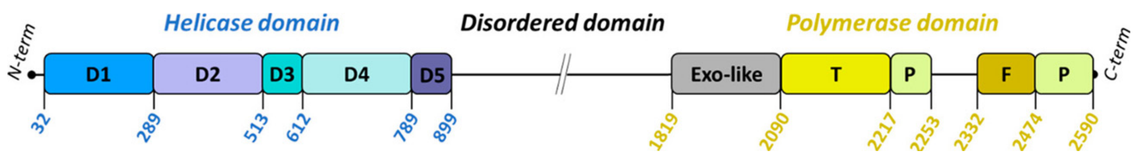


Figure 1 Schematic of the full-length POLQ domains and their spatial arrangement on POLQ structure.[2]

2 Makya AI Hit Finding

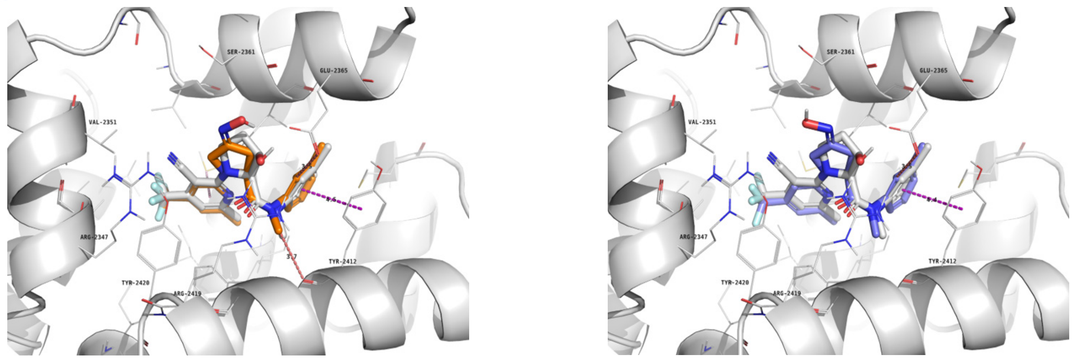


Figure 2 Schematic of the full-length POLQ domains and their spatial arrangement on POLQ structure.[2]

Rational design of new PolQ ligands was performed using Makya, an Iktos proprietary Generative AI software using fragment growing and linking methods. New molecules were generated starting from available fragments and known chemistry steps, ensuring synthesizable new compounds.[3] During this iterative process, we focused on optimizing key contacts and docking score within the PolQ binding site while closely monitoring the predicted physicochemical properties in order to ensure developability perspectives.

In the hit finding stage, generations covering seven distinct chemotypes were run. Out of 247 selected molecules, 86 were synthesized. Early results identified oxime **3** (**Figure 2**) as a potent hit (PolQ IC_{50}= 50 nM). This molecule contains similar pharmacophoric elements as **ART-558**, retaining a key H-bond with Glu-2365 through the oxime.

3 Biochemical Screening

Biochemical activity of PolQ polymerase domain (1820-2590), generated in house, was measured using a PicoGreen primer extension assay. The assay format was amenable to high-throughput screening of compounds, and adaptable for mechanism of inhibition profiling. A total of 133 compounds were profiled in the assay, in a 10-point concentration response format (n=2). Early screening identified oxime **1** as having moderate potency against PolQ.

Initially screened as a mixture of geometric isomers, this oxime hit ranked moderately amongst other series but possessed a promising vector to grow and pursue with a targeted follow-up library. Separation of the isomer pair highlighted the *E* geometry **3** as preferential with a 10-fold potency jump over *Z* isomer **2**.

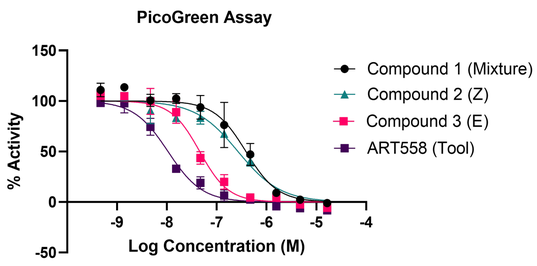
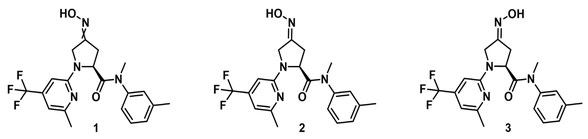


Figure 3 Normalised concentration response curves for Compounds 1, 2 and 3 alongside ART558 control. Data plotted as mean +/- SD (n=3)

Table 1 Hit structures and SAR table for PolQ inhibition, clearance, kinetic solubility and measured LogD.

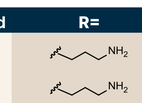
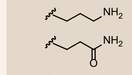
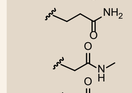
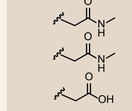
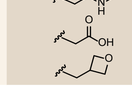
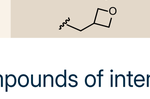
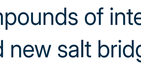


	Compound 1	Compound 2	Compound 3
PolQ Biochem pIC_{50} (LLE)	6.5 (-)	6.6 (-)	7.3 (3.2)
hHEP Cl_{int} (mL/min/10^6 cells)	-	-	104
Solubility (pH 7.4 (µM))	-	-	20 to 50
logD (pH 7.4)	-	-	4.1

4 Hit Optimisation and SAR

Assessment of the docking pose of these oxime ligands suggested the potential to target multiple amino acid residues by growth from the oxime. A library of oxime derivatives was designed to explore this vector and target these residues, to drive increases in potency through improved binding (**Table 2**). Further structure-guided refinements and library synthesis, using the Iktos Robotic platform, led to the identification of several substituents with improved potency and a continued preference for one isomer.

Table 2 Follow-up library SAR table and inhibition of PolQ

Compound	R=	Isomer	PolQ Biochem pIC_{50}
12		*Z*	5.7
13		*E*	6.8
14		*Z*	6.3
15		*E*	7.2
16		*Z*	7.5
17		*E*	6.7
18		Mixture	7.7
19		Mixture	7.0

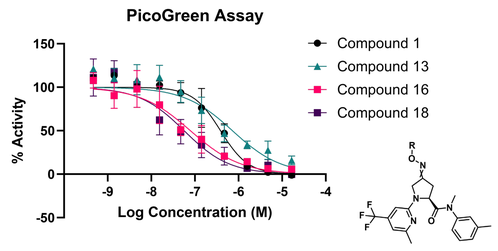
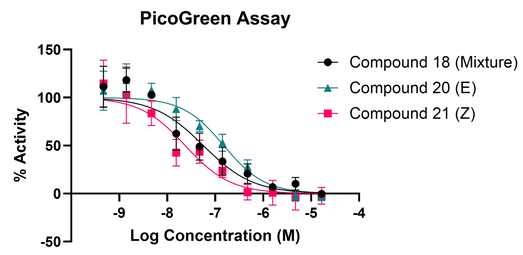


Figure 4 Concentration response curves displaying SAR based increases in potency through modification of Compound **1**.

Two compounds of interest were identified from the screen, amide **16** and acid **18**. Modelling each isomer of **18** indicated new salt bridge interactions being formed with residues Arg-2347 and Arg-2419 for only the Z isomer. To confirm this hypothesis, the *E* (**20**) and *Z* (**21**) isomers were separated and a 10-fold potency jump was observed for the Z isomer, confirming the correct binding pose observed in the molecule. Lipophilic ligand efficiency (LLE) was monitored, and improvements have been made from oxime **1** (LLE = 2.4) to lead **21** (LLE = 7.9).



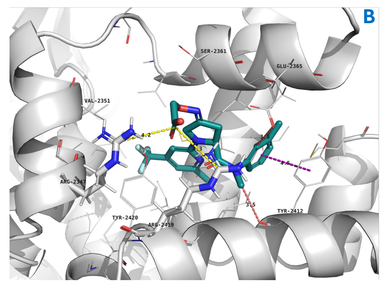


Figure 5A Concentration response curves for compound **18** (mixture) and Isomers 20 and 21 **(B)** Docking of **21** *Z* isomer, blue) with key contacts (salt bridge) with Arg-2347 and Arg-2419 highlighted in yellow.

5 Synthetic Lethality

Further profiling of compound **3** in a cellular proliferation assay demonstrated a reduction in cell growth in a DLD-1 BRCA2 (-/-) line over 6 days, comparable to tool compound **ART588.** No reduction in growth was observed in a control parental DLD1 line, indicating a synthetic lethal mechanism.

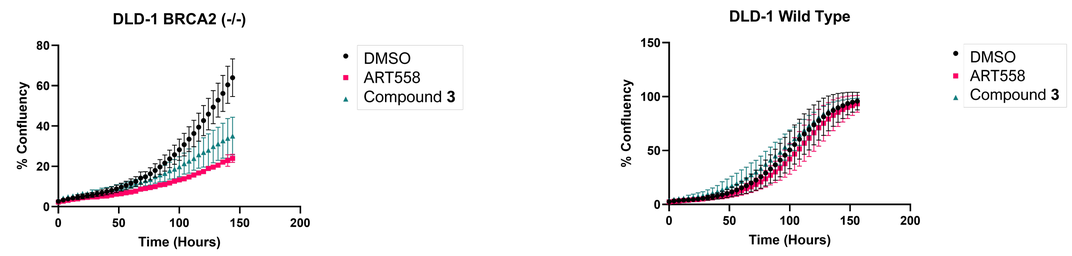
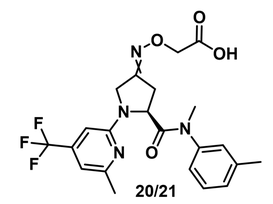
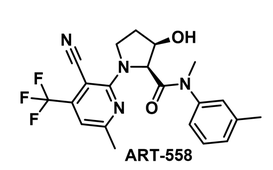


Figure 6 Cellular proliferation data for compound 3 tested at a concentration of 30 µM against DLD-1 isogenic pair lines. 30 µM ART558 data also plotted for comparison

6 *In Vitro* PK

With two compounds providing excellent biochemical potency against PolQ, oximes **16** and **21** for progressed for early ADME profiling alongside known literature tool compound, ART558, for comparison (**Table 3**). Improvements in human metabolic stability, measured in human hepatocyte clearance and measured kinetic solubility, were seen in comparison to hit **3**, through a substantial reduction in logD and addition of the ionizable acidic group. Increases to permeability remains the current focus for progression of our lead compounds whilst also reducing efflux liabilities associated with acid **21**.





Compound	Isomer	PolQ Biochem pIC_{50} (LLE)	hHEP Cl_{int} (mL/min/10^6 cells)	Solubility (pH 7.4 (µM))	CACO-2 (A to B pApp/10^{-6}cm/s; ER)	logD (pH 7.4)
16	*Z*	7.5 (3.8)	59	-	13.1 / 0.9	3.7
20	*E*	7.1 (-)	-	-	-	-
21	*Z*	8.3 (7.9)	8	>100	1.8 / 3.6	0.4
ART558 (22)	-	7.6 (4.2)	18	>100	26.0 / 0.9	3.4

Table 3 Structures and SAR table for PolQ inhibition, clearance, kinetic solubility, permeability and measured LogD.

7 Summary

In this work we have demonstrated the successful combination of artificial intelligence with a human knowledge-based approach to elucidate a novel oxime scaffold. Optimization in 3 DMTA cycles achieved ligands with low nanomolar biochemical potency (>100-fold improvement). A selection of our most potent inhibitors were progressed to *in vitro* ADME and have shown good overall profiles. Current efforts are aimed at further refinements of this scaffold to deliver a compound suitable for further *in vivo* assessment.

References 1. N. O'Neil et al, Nat Rev Genet, 2017, 18, 613–623. | 2. M. Chiara *et al*, J. Med. Chem.,2023, 66, 6498–6522. | 3. C. Descamps *et al*, ChemRxiv., DOI 10.26434/chemrxiv-2025-64bhb