Characterization of a novel series of highly selective PARP1 inhibitors

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SCAN ME

Background

First-generation PARP1 inhibitors have provided significant therapeutic benefit to patients whose tumours exhibit homologous repair deficiencies, including BRCA mutations, however their use has been associated with haematological toxicities that have restricted their application, particularly in combination with standard-of-care chemotherapy. All four FDA-approved PARP1 inhibitors are largely non-selective for the closely related enzyme PARP2, inhibition of which has been shown to drive haematotoxicity. Hence, the development of second-generation molecules highly selective for PARP1 over PARP2 offers a significant opportunity to

1) dramatically enhance therapeutic index,
2) enable additional precision medicine / combination approaches with chemotherapy, radiotherapy, immunotherapy and targeted agents and
3) expand the addressable patient population to those whose tumours harbour additional DDR defects.

We have discovered a novel series of PARP1-selective inhibitors using X-ray crystallography and structure-based design. Herein we describe the characterization of these compounds, exemplified by DSB1522.

In vitro Potency & ADME

		DSB1522	Olaparib
PARP1 Biochemical Binding (PARP2 Selectivity)	K_D (nM)	0.5 (85-fold)	0.8 (0.6-fold)
PARP1 NanoBRET (PARP2 Selectivity)	K_D (nM)	0.62 (288-fold)	3.0 (0.7-fold)
PARP1 Cell-free Trapping	IC_{50} (nM)	6	58
MDA-MB-436 (BRCA1m)			
7 Day Viability (Cell Titer Glo)		6	26
14 Day Colony Forming Unit Inhibition	IC_{50} (nM)	0.2	0.5
P-γH2AX Induction	IC_{50} (nM)	6	26
PARylation Inhibition	IC_{50} (nM)	8	34
DLD1 BRCA2$^{-/-}$			
7 Day Viability (Cell Titer Glo)		8	65
14 Day Colony Forming Unit Inhibition	IC_{50} (nM)	0.5	2.5
P-γH2AX Induction	IC_{50} (nM)	4	-
PARP1 Chromatin Trapping	IC_{50} (nM)	3	-
Mics CLint (μL/min/mg) / Heps CLint (μL/min/10^6 cells)		<7.4	<1.9
Plasma Stability $T_{1/2}$ (mins) / Plasma Protein Binding (%)		>373	88.7
CYP Inhibition, 5 isoforms (μM)		>50	
CYP Time-Dependent Inhibition (μM)		>50	
Caco-2 (10^{-6} cm/s) A→B / Efflux Ratio		21.9	0.6
MDCK-MDR1 (10^{-6} cm/s) A→B / Efflux Ratio		24	1.2

Table 1. DSB1522 is a potent inhibitor of PARP1 in biochemical and cell-based assays with high selectivity over PARP2, while demonstrating excellent *in vitro* ADME.

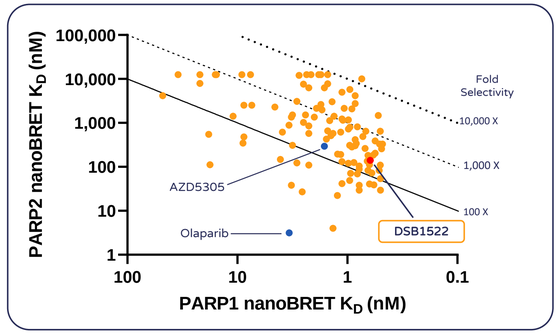


Fig. 1. DSB1522 demonstrates high PARP1 selectivity over PARP2 in a cell-based NanoBRET target engagement assay.

Table 2. DSB1522 demonstrates excellent *in vivo* PK

	Mouse	Rat	Dog
Vss (L/kg)	1.55	3.01	5.23
IV CL (mL/min/kg)	14	13.7	29.8
IV T1/2 (h)	2.23	2.97	2.5
Oral T1/2 (h)	2.48	2.94	3.6
Oral Bioavailability (F%)	96	84.9	79.7

Table 3. DSB1522 exhibits excellent selectivity over other PARP proteins in NanoBRET assays.

	DSB1522	AZD5305	Olaparib
PARP1	1	9	17
PARP3	404	141	4
PARP4	2,120	821	13
PARP5a	750	218	178
PARP5b	340	91	45
PARP6	10,000	1,155	1,046
PARP7	752	99	23
PARP8	10,000	1,155	111
PARP10	10,000	433	325
PARP11	10,000	9	35
PARP12	2,276	4	45

PARP1 IC_{50} (nM): 1-9, 10-99, 100-999, >1000 — PARP1 Fold Selectivity

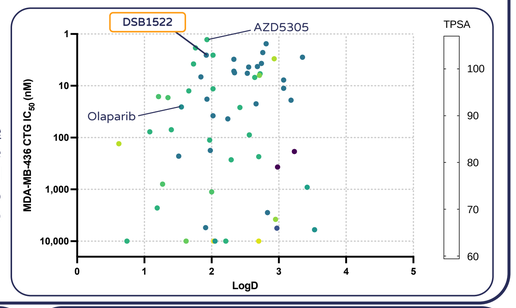


Fig. 2. DSB PARP1-selective inhibitors demonstrate excellent physicochemical properties with no correlation between mLogD and *in vitro* cellular potency.

Surface Plasmon Resonance

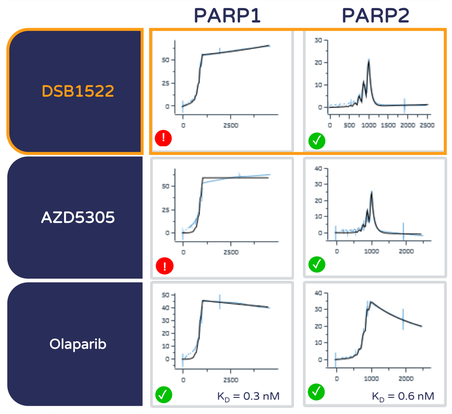


Fig. 3. DSB1522 demonstrates high affinity and prolonged residence time in surface plasmon resonance binding assays.

! High affinity with very slow off-rate (no dissociation observed within 1 hr), unable to obtain kinetic binding parameters

In vivo Efficacy

Fig. 4a

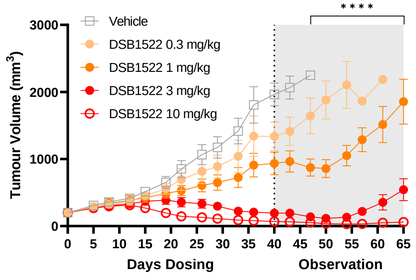


Fig. 4b

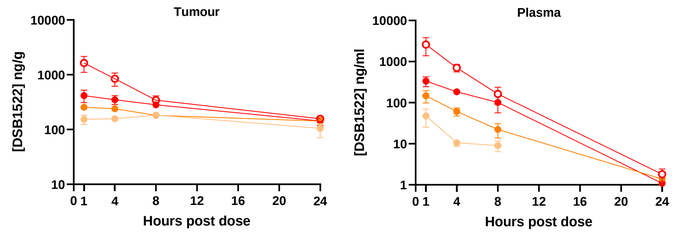


Fig. 4. DSB1522 drives rapid and durable tumour regression in a dose-dependent manner in a MDA-MB-436 breast cancer xenograft model (**Fig. 4a**), demonstrating prolonged tumour residence time at all doses tested (**Fig. 4b**).
****p<0.0001

Fig. 5. DSB1522 demonstrates significantly superior anti-tumour efficacy vs Olaparib, at 1/10th dose in a MDA-MB-436 xenograft model (**Fig. 5a**). Efficacy was comparable to AZD5305; DSB1522 demonstrates excellent tumour PK, with a high tumour:plasma ratio (**Fig 5b**).
****p<0.0001

Fig. 5a

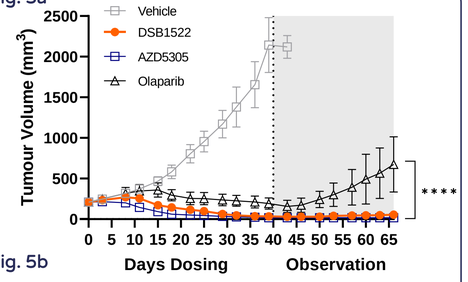
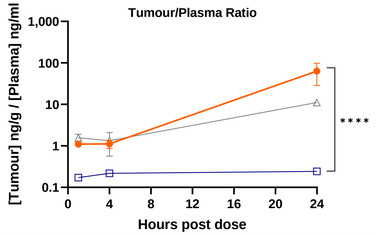


Fig. 5b



Summary

We describe the characterization of novel potent and selective PARP1 inhibitors. These molecules demonstrate excellent *in vitro* ADMET and *in vivo* PK, coupled with profound anti-tumour efficacy and tumour-targeting properties in a genetically-defined mouse model. Our data predict low therapeutic dosing with the potential to demonstrate improved efficacy and tolerability compared to marketed PARP inhibitors, supporting progression of these compounds into clinical studies.

Acknowledgements

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