Structure-Based Design of small macrocyclic CDK9 degraders as chemical biology tools and beyond

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1 CONTEXT & OBJECTIVES

• Background:

Small molecule macrocyclic kinase inhibitors have attracted significant attention in drug discovery over the past years with drug approval such as Lorlatinib and several on-going advanced clinical trials demonstrating the clinical relevance of this approach.
Over the years, we developed our expertise to further optimize those cyclic molecules. Having low molecular weight, they favorably alter the biological and physiochemical properties as well as selectivity, as compared to their linear parent, yielding high-quality drug candidates.

The cyclin-dependent kinases (CDKs) are a family of more than 13 serine-threonine kinases. CDK1-4, CDK6 and CDK11 regulate cell cycle, while CDK7-9 are involved in transcription regulation. Over the past two decades, despite the successful registration of CDK4/6 inhibitors (abemaciclib, palbociclib, and ribociclib) for HR-positive, HER2-negative advanced or metastatic breast cancer, the development of CDKs inhibitors remains a high challenge due to selectivity and off-target dose limiting toxicities. Consequently, novel approaches need to be considered to address the development of CDK inhibitors.

• Objectives:

Current CDK9 inhibitors are reversible and require continuous target occupancy to maintain CDK9 inhibition. Degradation and eradicating protein have specific advantages over just inhibiting the kinase active site especially with kinases where scaffolding function is important to biological outcomes *in vitro* and *in vivo*. CDK9 is present in distinct mTOR-like (CTOR) complexes and pharmacological targeting of CTORC complexes results in suppression of growth of primitive human AML progenitors in vitro and elicits strong antileukemic responses in AML xenografts in vivo.
With small macrocyclic “probes” from our proprietary library in the low nanomolar range IC50, we turned our attention to CDK9 inhibitors with good selectivity profile against CDK1/2/5/7 as a starting point for building bifunctional small macrocyclic kinase degraders. Although the ATP binding site of the CDKs are structurally similar, developing a small macrocyclic PROTAC degrader could enhanced the selectivity of the parent molecule by preferential ubiquitination of lysine exposed residues among the different CDKs.

2 RESULTS

• Design of small macrocyclic PROTAC® CDK9 degraders

By analogy to a previous co-crystalized X-Ray of one of our small macrocyclic kinase inhibitor (ODS’309), we carried out a simple molecular docking and minimization using MOE (Chemical Computing Group) in the structure of CDK9 (4BCG). We found that the morpholine ring could be exposed to the solvent area (See Figure 1) and the adjacent phenyl ring could yield two additional exit vectors for chemical modification and linker analogues synthesis.

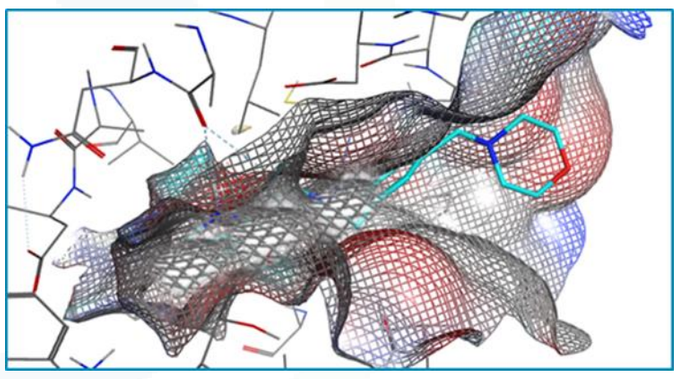

Figure 1: Molecular modeling of ODS’309 in CDK9 structure (4BCG)

Having defined two possible substitution patterns, we rapidly synthesized and tested three new small macrocyclic structures. ODS’152-1 was selected on the basis of its biochemical activity, selectivity and solubility as compared to ODS’171 & 172 (table 1). In addition, ODS’152-1 is bearing a piperidine moiety amenable to further derivatization for linker coupling.

		Biochem IC_{50} (nM)					Sol.PBS/FaSSIF (µM)	Chrom LogD @pH 7.4
Compounds	CDK9 Kd (nM)	CDK9 cyclinT1	CDK1 cyclinB	CDK2 cyclinA2	CDK5 p35	CDK7 cyclinH		
ODS’152-1	2	15	1587 (x106)	3000 (x200)	263 (x17)	77 (x5)	229 / 240	2
ODS’171-1	6.4	11	103 (x10)	357 (x32)	24 (x2)	98 (x9)	227 / 233	2.2
ODS’172-1	0.33	9	11 (x1)	12 (x1)	5 (x1)	135 (x15)	8 / 14	2.9

Table 1: Physico and biochemical profile of newly synthesized small macrocyclic kinase inhibitors

Having defined, ODS’152-1 as the parent binder to CDK9, we rapidly synthesized and tested in a biochemical assay the novel “probes” vectorized with the pro-linker moieties. All probes demonstrated retention of biochemical activity as compared to the parent therefore confirming the orientation given by the modeling and exposition to the solvent area of the linker motif (Data not disclosed)

• Evaluation of small macrocyclic PROTAC® CDK9 degraders

ODS’152 was further derived into fully bifunctional molecules with eleven variations on the linker’s nature and length, and with thalidomide as the E3 ligase recruiter. All newly synthesized degraders maintained biochemical CDK9 activities as compared to the parent ODS’152 (Data not disclosed). The bifunctional compounds were further evaluated in an MTS assay against several cell lines (HCT116, Jurkat E6.2, MOLT-4 and MV4-11) to assess cell proliferation and viability. ODS’213-1 amongst other, demonstrated a level of activity comparable to the parent kinase inhibitor (Table2).

	MTS 96h IC_{50} (nM)			
Compounds	HCT 116	Jurkat E6.1	MOLT-4	MV4-11
ODS’152-1	33	70	67	50
ODS’197-1	27	40	41	33
ODS’199-1	32	33	33	18
ODS’200-1	47	59	56	27
ODS’201-1	121	239	284	75
ODS’202-1	132	172	152	26
ODS’203-1	285	146	86	55
ODS’208-1	142	289	145	34
ODS’210-1	55	61	50	27
ODS’211-1	131	220	165	28
ODS’213-1	33	28	25	20
ODS’215-1	149	171	141	41

Table 2: MTS assay of 11 Protac analogues derived from kinase inhibitor ODS’152-1

The E3 Ligase engagement was measured in a NanoBRET™ assay and cell permeability of the newly formed Protac measured by permeabilization of the cell membrane using Digitonin (a selection of results is presented in Table 3)

HEK-293 n=2	CTG 72H (c) IC50 (nM)	nanoBRET + **digitonin** EC50(nM)	nanoBRET wo digitonin EC50(nM)
ODS’201-1	229	191	>7500
ODS’211-1	394	154	942
ODS’213-1	35	37	76

Table 3: E3 target engagement and cell penetration

3 CONCLUSION

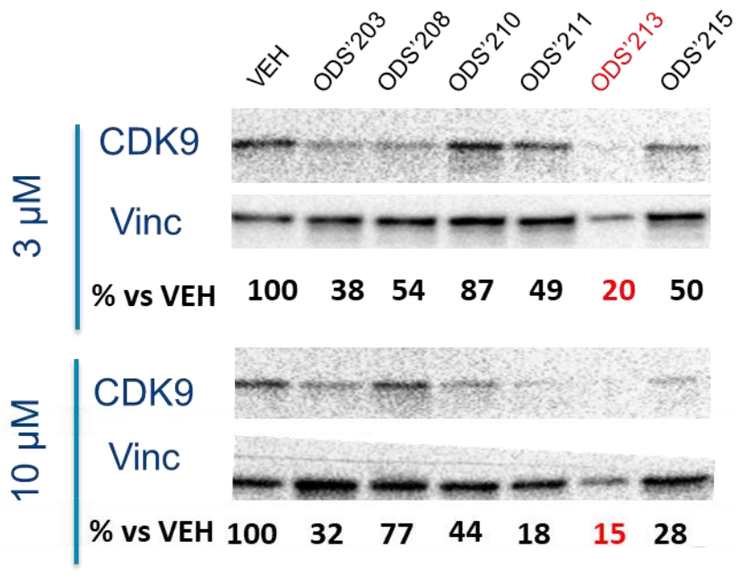

Figure 2: Degradation of CDK9 in MV4-11 after exposure to a selection of ODS’152 – Protacs at 10 and 3 µM over 20h treatment

Within the 11 Protac analogues of ODS’152 synthesized, ODS’213 demonstrated significant CDK9 protein degradation at 3 and 10µM. Further evaluations are on-going to characterized the potential of this series and its selectivity against other CDKs

• Small macrocyclic CDK9 degraders as chemical biology tools

Our findings support the rapid derivatization of small macrocyclic “probes” inhibitors into small macrocyclic-PROTAC as an advantageous strategy to generate early chemical biology tools with maintained potency as compared to their parent small macrocyclic inhibitor. **ODS’213** is standing-out of this series by demonstrating good biochemical activity, a robust MTS activity across different cell lines, good permeability and E3 target engagement confirmed by the proteolysis of CDK9 observed in MV4-11. Additional studies are on-going to assess the biophysical properties such as binary and ternary complexes formation along with dose response and selectivity of those bifunctional molecules between homologous targets such as CDK1/2/5/7 in addition to Ser2-CTD pol II.

Further optimization of the physico-chemical and ADME properties of those small macrocyclic-PROTAC degraders can potentially lead to drug candidates with distinct pharmacological effects as compared to the parent linear kinase inhibitors.