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CASE STUDY

APPROACH TO RATIONAL IDENTIFICATION OF LEAD MOLECULAR GLUE DEGRADERS FOR CASEIN KINASE 1α

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ABSTRACT

There has been a tremendous explosion of interest in the targeted protein degradation (TPD) in drug discovery in recent years. One approach that has been widely exploited is the use of proteolysis targeting chimeras (PROTACs). Here, a heterobifunctional compound with 2 ligands linked by a flexible/rigid spacer has one ligand that binds to an E3 ligase while the other binds to a protein of interest (POI). This action brings the POI in close proximity to the E3 ligase for polyubiquitination and subsequent degradation by cellular proteasome machinery. The limitations of this approach are that it has usually led to the identification of compounds that are outside of the rule of five, have molecular weights >500 and in most cases, are not orally bioavailable. An alternative TPD approach involves the use of molecular glue degraders (MGD). To date there are few examples of cases where a rational approach has been used to identify potent MGDs and most MGDs have been discovered by serendipity or by happenstance. Unlike PROTACs, MGDs generally have molecular weights of <500, satisfy the rule of five and are orally bioavailable. MGDs could be exploited to target a plethora of proteins that do not have a well-defined binding pocket (currently considered undruggable) where traditional small molecule approaches fail.

CRBN and CK1**α** have a relatively weak native protein-protein interaction with Kd of about 2 mM. These types of interactions, between the domain of one protein with the sequence motif of the other, are generally weak as they possess only a small buried surface area (BSA). The goal of a MGD is to increase these interactions (if present) and stabilize the resultant ternary complex. Indeed, when Lenalidomide binds to CRBN, it increases its affinity for CK1**α** and the resultant ternary complex has a Kd of about 75 nM. This could also be viewed as Lenalidomide binding to the binary complex of CRBN-CK1**α** and strengthening the interaction within the ternary complex by increasing the BSA interactions between CK1a and CRBN. In a recent report1, a series of MGDs have been designed based on the stabilization of the ternary complex, in silico.

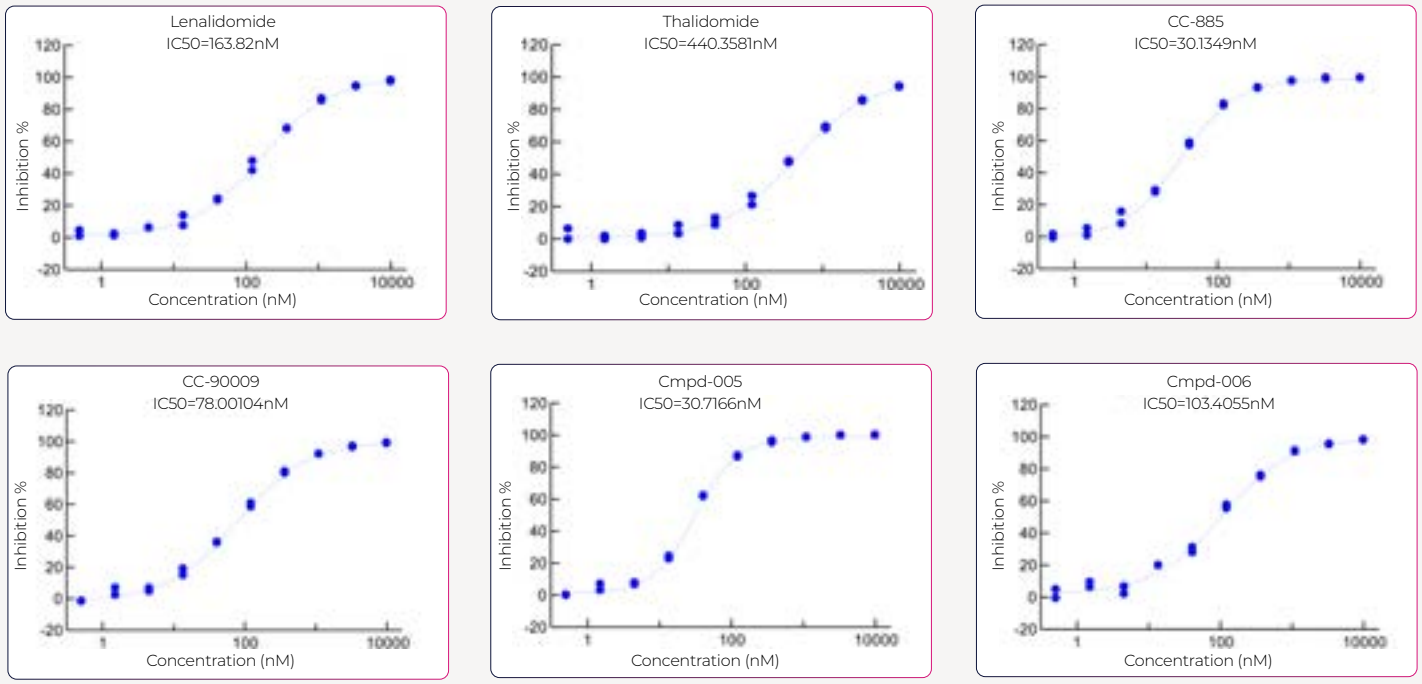
In this poster we report our findings on the binary and ternary complex formation, cellular target engagement, target ubiquitination and CK1**α** degradation. After screening compounds in these assays, two potent CK1**α** degraders, CC-885 and cmpd-006 have been identified.

RESULTS AND DISCUSSION

Cmpd-001 - 004 were chosen based on the substitution pattern and the commercial availability of the anlogs. Docking of the cmpd-001 in the CRBN-Lenalidomide-CK1**α** crystal structure2 (PDB: 5FQD; 2.45 Å resolution) revealed that absence of glutarimide hydrogen bonding and the steric clash of N-Me with W386 indole weakened its interaction in the Lenalidomide pocket. This apparently resulted in loss of CRBN binding in the binary assay and hence no ternary complex could form. Virtual screening was carried out using Enamine Lenalidomide library after prepping the crystal structure (PDB: 5FQD) as described in the materials and methods section. Cmpd-005 was chosen from the top ranked sulfonamide cluster and cmpd-006 from the overall top ranked compounds and that are commercially available.

The reference compound Lenalidomide along with Thalidomide, CC-885, CC-90009, cmpd-001 – 006 were evaluated for affinity in E3 ligase, Cereblon (CRBN) assay (Fig. 1) as described briefly in materials and methods section. Lenalidomide had an affinity of 73 nM. Cmpd-005 had the highest affinity of the evaluated compounds at 10 nM followed by CC-885 at 12 nM and CC-90009 at 33 nM. These 3 compounds had higher affinity for CRBN than Lenalidomide. Cmpd-001 had no detectable affinity for the ligase. The rest of the compounds shown in Table 1 had relatively low affinity in the sub micromolar range except cmpd-006 that had an IC50 of 93 nM.

FIG.1 CRBN-CMPD-CK1α BINARY COMPLEX FORMATION ASSAY_HTRF ASSAY



Then the compounds were evaluated in ternary complex CRBN-MG-CK1**α** formation assay. The dose response curves are shown in the Fig. 2. Relative and absolute EC50 values in nM are shown in Table 1. CC-885 and cmpd-006 were the most potent in the assay with EC50 of 27 nM and 38 nM, and top activation of 99% and 182%, respectively. Both the compounds were more potent in the ternary complex formation than Lenalidomide. Though the cmpd-005 had a relative EC50 of 33 nM the top activation was only 54%. Thalidomide and cmpd-001 – 004 did not form ternary complex.

FIG.2 CRBN-CMPD-CK1A TERNARY COMPLEX FORMATION ASSAY_HTRF ASSAY

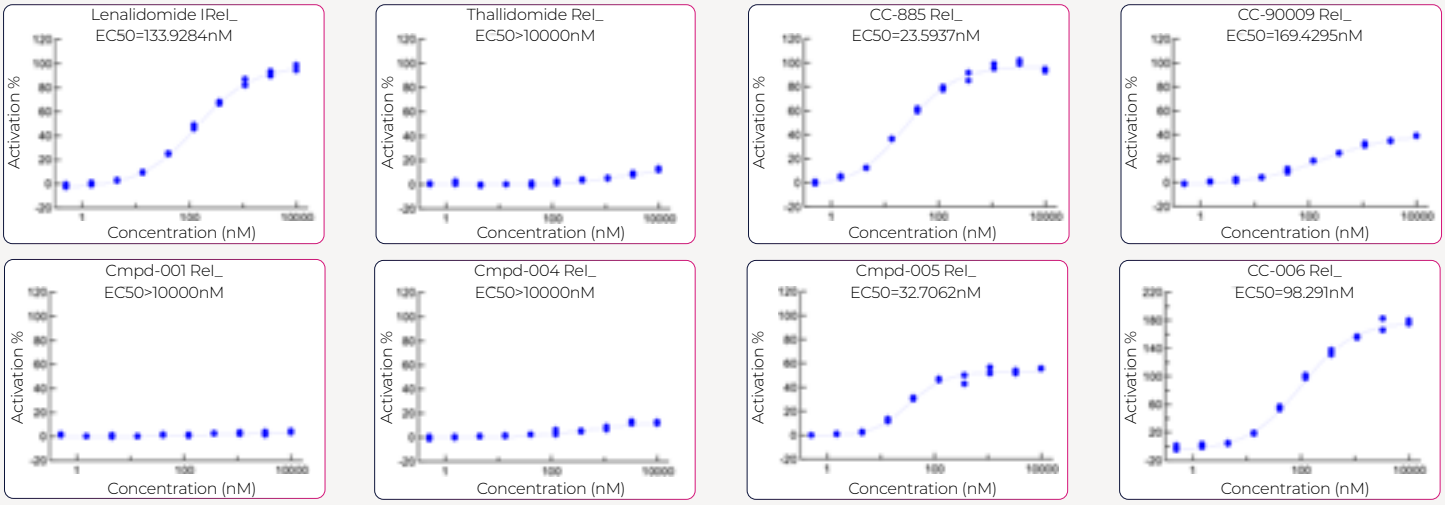
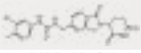
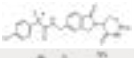
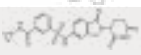
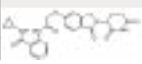
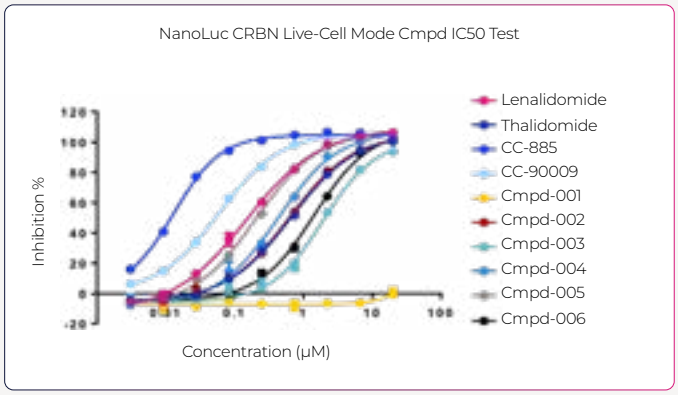


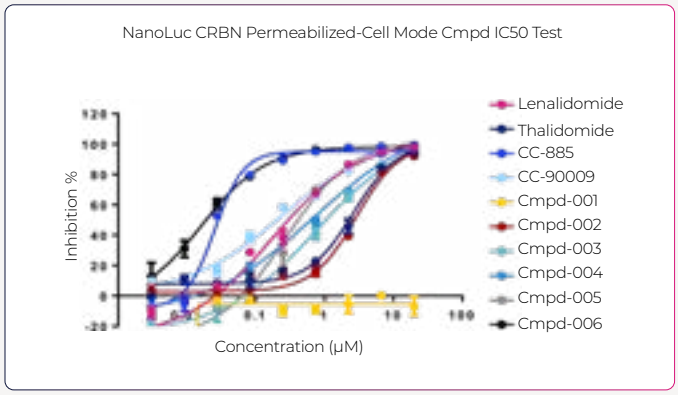
TABLE 1 STRUCTURES AND ASSAY RESULTS

Compound	Structure	Binary Complex (E3)_ HTRF IC_{50} (nM)	Ternary Complex (CRBN-MG-CK1α) EC_{50} (nM)		Top Activation	Cellular Target Engagement (NanoBRET) IC_{50} (μM)			
			Relative	Absolute		Live Cell	Permeabilized Cell	RBA[a]	AI[b]
Lenalidomide		72.87	133.93	149.69	100.00%	0.147		0.855	1
Thalidomide		209.86	>10000	>10000	13.30%	0.660	2.645	0.249	0.292
CC-885		11.95	23.59	26.85	99.09%	0.013	0.025	0.515	.0602
CC-900009		32.85	169.43	>10000	41.32%	0.059	0.234	0.254	0.297
Cmpd-001		>10000	>10000	>10000	3.35%	NA	NA	NA	NA
Cmpd-002		340.28	>10000	>10000	24.87%	0.619	2.867	0.216	0.252
Cmpd-003		320.85	>10000	>10000	20.51%	1.899	0.767	2.477	2.896
Cmpd-004		126.66	>10000	>10000	13.10%	0.427	0.693	0.616	0.721
Cmpd-005		10.37	32.71	288.42	53.91%	0.204	0.262	0.774	0.905
Cmpd-006		93.19	98.29	37.91	181.83%	1.435	0.017	86.655	101.303

In the cellular target engagement studies (Fig. 3), all compounds except cmpd-001, 003 & 006 had better membrane permeability than Lenalidomide. The inactivity of cmpd-001 was due to its inability to form ternary complex as mentioned above. Cmpd-003 had a poor cellular permeability as shown by their availability index (AI) value of 2.89. Notably, though cmpd-006 showed the least cellular permeability (AI = 101.3), the IC50 in permeabilized cell is 17 nM (Table 1). This indicates that cmpd-006 has a strong affinity for CRBN in permeabilized cell mode, which is consistent with the results of the ternary complex formation assay.

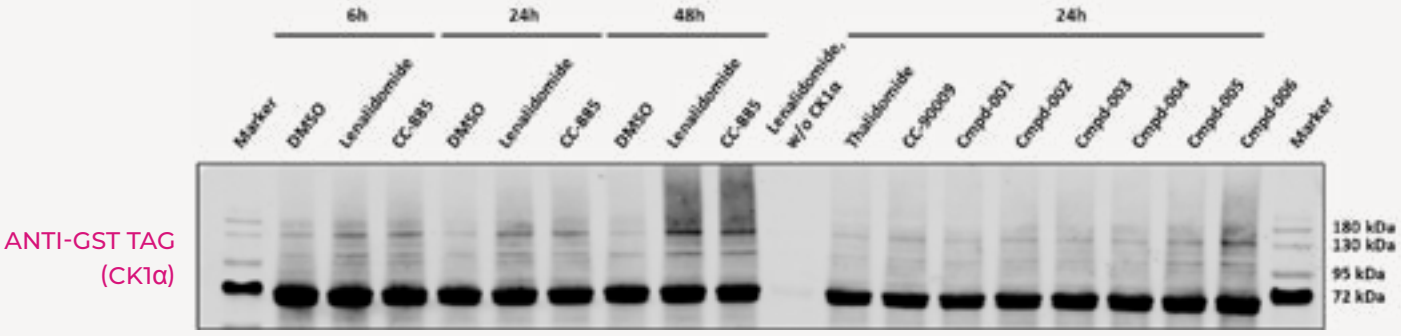
FIG.3 CELLULAR TARGET ASSAY _NANOBRET ASSAYENGAGEMENT





Western blot was done to detect the effects of the compounds on in vitro ubiquitination of CK1**α**. Lenalidomide and CC-885 promoted ubiquitination of CK1**α** after 24 hours of reaction, more obvious after 48 hours (Fig. 4). Ubiquitination was also observed with cmpd-005 & 006. Thalidomide, CC-90009 and cmpd-001 – 004 showed minimal ubiquitination.

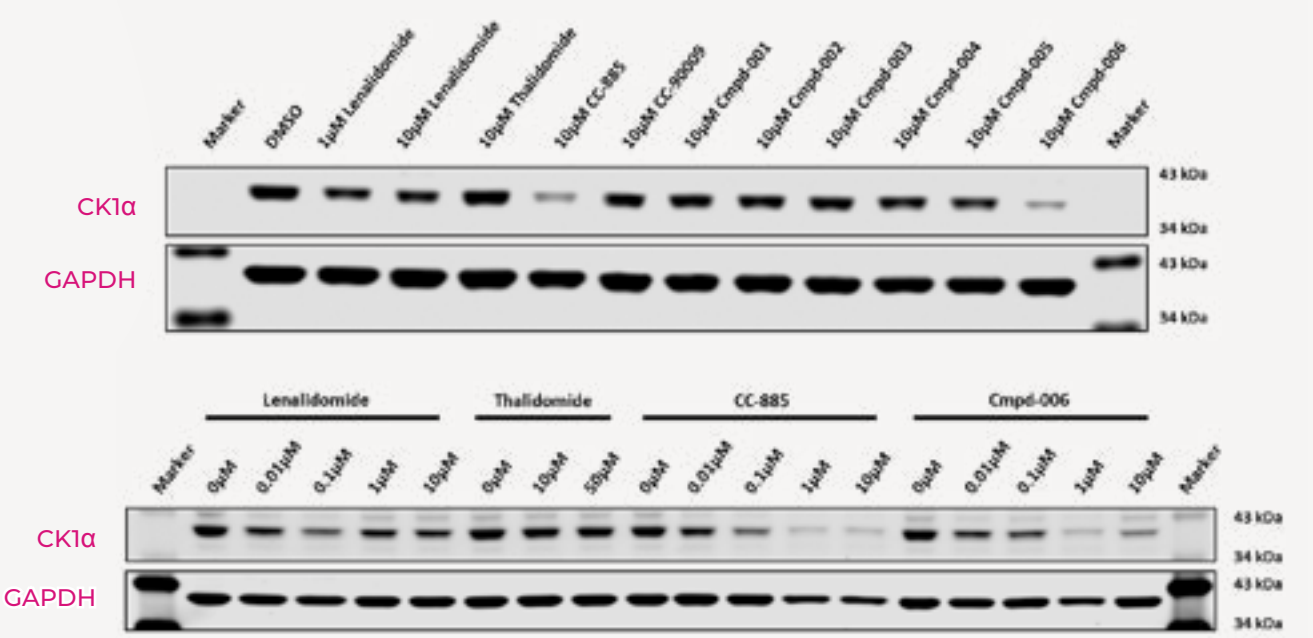
FIG.4 CK1α UBIQUITINATION ASSAY _WB ASSAY



In the cellular CK1**α** degradation assay, CC-885 and cmpd-006 were effective (Fig. 5). CC-885 also showed dose dependent degradation of CK1**α** starting at 0.01 µM to 10 µM. Like Lenalidomide, cmpd-006 also showed dose dependent degradation at 0.01 µM and 0.1 µM. Thalidomide, CC-90009, and cmpd-001 – 005 did not show any detectable CK1**α** degradation.

Thus we got a new lead compound, cmpd-006. Lead optimization by SAR as well as focusing on ADME/PK, would potentially yield more potent analogs of cmpd-006. Further preclinical development of these type of compounds could pave a path potentially to clinic.

FIG.5 CELL BASED CK1α DEGRADATION ASSAY_WB ASSAY



CONCLUSIONS

Cmpd-006 was a potent degrader of CK1**α.** Both CC-885 and cmpd- 006 were more efficient in degrading the target CK1**α** than the known degrader Lenalidomide. Moreover cmpd-006 despite its poor cellular permeability showed very good potency in CK1**α** degradation. Lead optimization by SAR as well as focusing on ADME/PK, would potentially yield more potent analogs of cmpd-006. Further preclinical development of these type of compounds could pave a path potentially to clinic.

REFERENCES

1) Weiss, et.al. J Chem Inf Model 2023, 63, 2382-92; 2) Petzold, et.al. Nature, 2016, 532, 127-30

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