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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 (**Fig. 1**) 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 CK1α have a relatively weak native protein-protein interaction with Kd of about 2 μM. 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 (**Fig. 1**). Indeed, when Lenalidomide binds to CRBN, it increases its affinity for CK1α 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-CK1α and strengthening the interaction within the ternary complex by increasing the BSA interactions between CK1α and CRBN.

RESULTS AND DISCUSSION

The scientists at ChemPartner are actively screening compound libraries for several customers looking for novel molecular glues. As an internal validation project, we developed and tested a screening tree for the interaction of the E3 ligase, CRBN with target protein CK1α (**Fig. 2**). The screen tree starts with an HTRF assays formatted for 384 well plates. The HTRF assays test for both binary and ternary complex formation. The screening tree includes selectivity testing, cellular target engagement and a cell-based measurement of the degradation of CK1α.

Cmpd-001 - **004** were chosen based on the substitution pattern and the commercial availability of the analogs. Docking of the **cmpd-001** in the CRBN-Lenalidomide-CK1α crystal structure[1] (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[1] (PDB: 5FQD). **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. 3**). 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 IC_{50} of 93 nM.

Then the compounds were evaluated in ternary complex CRBN-Cmpd-CK1α formation assay. The dose response curves are shown in the **Fig. 4**. Relative and absolute EC_{50} values in nM are shown in **Table 1**. CC-885 and **cmpd-006** were the most potent in the assay with EC_{50} 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 EC_{50} of 33 nM the top activation was only 54%. Thalidomide and **cmpd-001 – 004** did not form ternary complex.

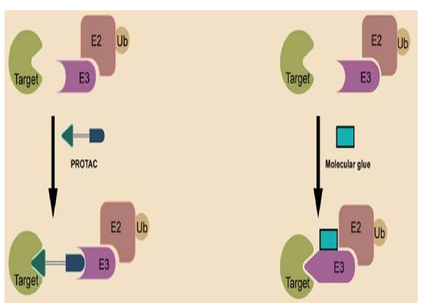
Ternary complex formation, in silico, was visualized in MOE. The final frames of a 15ns molecular dynamics simulation of ternary complexes of CC-885, **cmpd-005** & **006** were shown in **Fig. 5**. Glutarimide and isoindolone moieties of the ligands interact with CRBN H378 and N351, respectively. The sidechains of CC-885 and **cmpd-005** engaged in hydrogen bonding acceptor interaction with the K18 sidechain amine of CK1α and the sidechain of the **cmpd-006** had hydrogen bonding donor interaction with the E377 sidechain carbonyl of CRBN.

In the cellular target engagement studies (**Fig. 6**), all compounds except **cmpd-003** & **006** had better membrane permeability than Lenalidomide. **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 IC_{50} 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.

Western blot was done to detect the effects of the compounds on *in vitro* ubiquitination of CK1α. Lenalidomide and CC-885 promoted ubiquitination of CK1α after 24 hours of reaction, more obvious after 48 hours (**Fig. 7**). Ubiquitination was also observed with **cmpd-005** & **006**. Thalidomide, CC-90009 and **cmpd-001 – 004** showed minimal ubiquitination.

In the cellular CK1α degradation assay, CC-885 and **cmpd-006** were effective (**Fig. 8**). CC-885 also showed dose dependent degradation of CK1α 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 CK1α 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.



Picture from biochempeg.com

Fig.1 Schematic diagram for PROTAC and molecular glue interactions

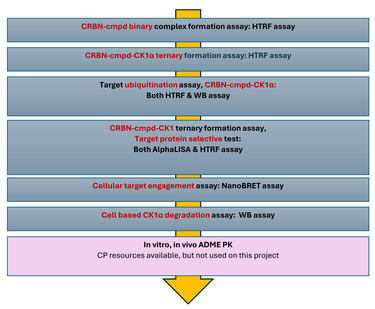


Fig.2 Screening tree for molecular glues at CP

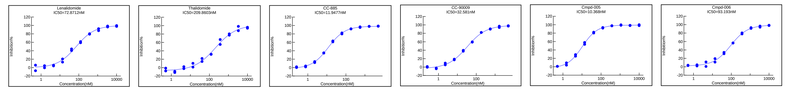


Fig.3 CRBN-cmpd binary complex formation HTRF assay

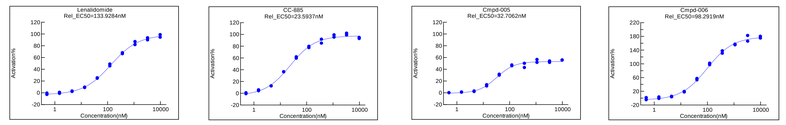


Fig.4 CRBN-cmpd-CK1α ternary complex formation HTRF assay

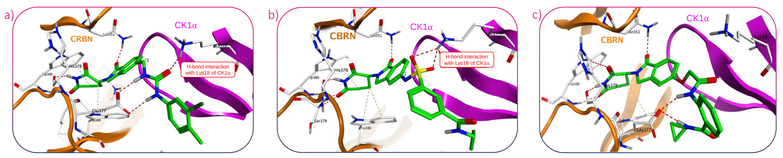


Fig.5 Ternary complexes final frame at 15 ns, CRBN in brown, CK1α in pink, ligand in green a) CC-885, b) **Cmpd-005** and c) **Cmpd-006**

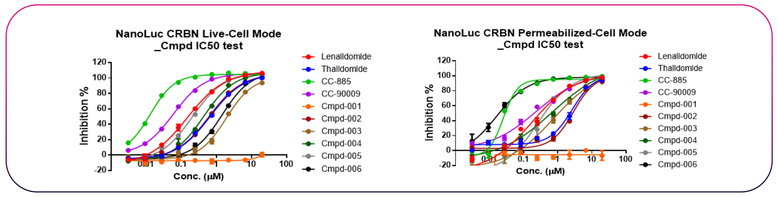


Fig.6 Cellular target engagement NanoBRET assay

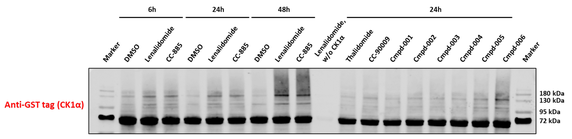


Fig.7 CK1α ubiquitination WB assay

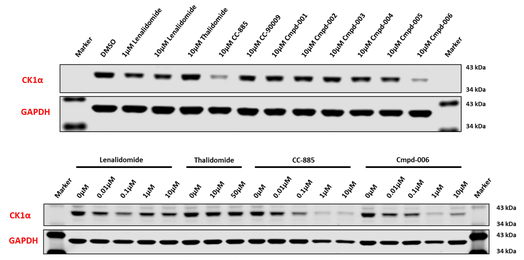
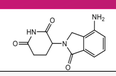
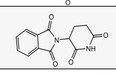
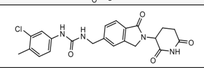
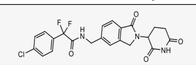
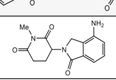
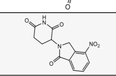
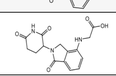
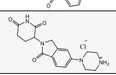
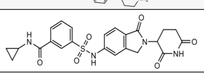
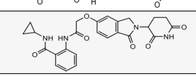


Fig.8 Cell based CK1α degradation WB assay

CONCLUSIONS

Cereblon modulator CC-885 was a known MG degrader for GSPT1[2], BNIP3L[3], PLK1[4] and CDK4[5]. **Cmpd-006** was a potent degrader of CK1α. Both CC-885 and **cmpd-006** were more efficient in degrading the target CK1α than the known degrader Lenalidomide. **Cmpd-006** despite its poor cellular permeability showed very good potency in CK1α 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.

Compound	Structure	Binary Complex (CRBN-Cmpd) IC_{50} (nM)	Ternary Complex (CRBN-Cmpd-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	0.172	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	0.602
CC-90009		32.58	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

a) RBA (Relative-Binding Affinity) = $IC_{50,\ live\text{-}cell\ mode}$ / $IC_{50,\ permeabilized\text{-}cell\ mode}$ b) AI (Availability Index) = RBA_{ligand} / $RBA_{permeable\ control}$

Table 1 Structures and Assay Results

REFERENCES

1) Petzold, *et.al.* Nature, **2016**, *532*, 127-30; 2) Hansen *et.al.* J Med Chem **2018**, *61*, 492-503; 3) Hao, *et.al.* Acta Pharmacologica Sinica **2020**, *41*, 1246-54; 4) Li, *et.al.* Mol Ther Oncolytics **2020**, *18*, 215-25; 5) Zhao, *et.al.* Biochem Biophys Res Comm **2021**, *549*, 150-56.