#5164 Exploration of Novel E3 Ligase to Support Targeted Protein Degradation (TPD) Drug Discovery

Zuyuan Shen[1], Yunyun He[1], Andreas Schoop[2], Rui Wang[1], Nengwei Xu[1], Weixiao Song[1], Hao Chen[1], Peichuan Zhang[1], Lingbing Sun[1], and Wenji Su[1]

[1]WuXi Biology, WuXi AppTec, Shanghai, China

[2]Crelux GmbH, WuXi Biology, WuXi AppTec, Gräfelfing, Germany

WuXi AppTec

WuXi Biology

Abstract

Targeted protein degradation via the ubiquitin-proteasome system relies on specific E3 ligase complexes. To look for novel E3 ligases that can be used for proteolysis-targeting chimeras (PROTACs), we conducted DNA-encoded library (DEL) screening and surveyed the ligandability of a small panel of E3 ligases (*not shown*).

We identified proprietary molecules that bind GID4, a substrate receptor of the CTLH complex. We further optimized the GID4-binding molecules and generated PROTACs for targeting BRD4. We demonstrated that these GID4-BRD4 PROTACs can mediate biochemical ternary binding and induce BRD4 degradation in cancer cell lines To look into potential working mechanism, we also solved the crystallography structure of the ternary complex for one top PROTAC molecule. Furthermore, using the direct-to-biology (D2B) high-throughput approach, we recently have optimized the PROTACs and identified structures that can enhance degradation efficacy.

Taken together, we demonstrated that, as a proof-of-concept, DEL selection can help identify novel E3 that can be harnessed to support TPD discovery.

Background

A PROTACtable E3 ligases

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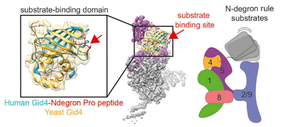
Also, see DOI: 10.1016/j.bmc.2024.117718

B GID4 as a candidate

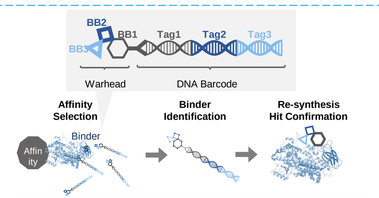


DOI: 10.1016/j.molcel.2019.10.009
Also, see DOI: 10.1038/s41594-025-01537-1

Homolog of yeast glucose-induced degradation protein 4
- Substrate receptor of the CTLH E3 complex
- Recognition of **Pro/N** degron motif
- Showing promising ligandability as suggested by a previous study using DELopen (*J. Med. Chem.* 2022)

Figure 1. The landscape of E3 for TPD

Results



Condition	1	2	3
Target	His-GID4	His-GID4	NTC (His)
Additive	-	100 μM substrate peptide	-

Enrichment

No.	Nsynthon ID				Copy			Enrichment		
	Library	BB1	BB2	BB3	C1	C2	C3	C1	C2	C3
1	10144	43	668	7	256	1	0	308673.37	/	/
2	10144	173	626	7	238	4	0	286969.77	/	/
3	10144	173	5	7	221	2	0	266471.93	/	/
4	10144	43	652	7	202	2	0	243562.58	/	/
5	10144	43	220	7	189	1	0	227887.76	/	/
6	10144	43	113	7	172	2	0	207389.92	/	/
7	10144	173	733	7	167	3	0	201361.14	/	/
8	10144	43	376	7	155	1	0	186892.08	/	/
9	10144	43	638	7	134	1	0	161571.22	/	/
10	10144	43	604	7	124	1	0	149513.66	/	/

Figure 2. DEL screening identified potential ligands for GID4 E3 ligase

Results

SPR evaluation of synthesized compounds

Hit validation:
- 63 off-DNA compounds synthesized

Hit optimization:
- 34 truncates & fragments

- Cluster Library 10071: MW: 599 – 614, K_D: 47 – 170 μM
- Cluster Library 10097: MW: 308 – 833, K_D: 0.29 – 9.7 μM
- Cluster Library 10144: MW: 311 – 595, K_D: 0.12 – 1.1 μM

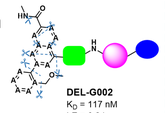
Figure 3. Synthesized compounds were confirmed by SPR for GID4 binding.

A Profiles of 3 representative binders

Annotation: Poor – Ideal

Nsynthon ID		DEL-G001	DEL-G002	DEL-G003
DEL screen	Copy	155	238	184
	Enrichment	186,892	286,970	34,272
Physicochemical properties	MW	541.6	538.6	762.3
	clogP	3.06	3.82	6.27
	PSA	122.1	108.5	129.6
	HAC	40	40	55
K_D measurement by SPR		123 nM	117 nM	116 nM
Ligand efficiency (LE)		0.24	0.24	0.17

B Fragmentation derivatives



DEL-G002
K_D = 117 nM
LE = 0.24

G002-T05	G002-T09	G002-T02
K_D = 42 nM	K_D = 76 nM	K_D = 158 nM
MW = 494	MW = 460	MW = 445
LE = 0.27	LE = 0.29	LE = 0.28

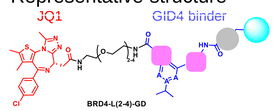
MedChem optimization aims:
- To improve physiochemical properties
- To maintain or enhance affinity (K_D < 0.5 uM)
- To increase ligand efficiency (LE > 0.3)
- To decrease molecular size (MW < 400)

C G002-T09 derivatives for SAR evaluation (~20)

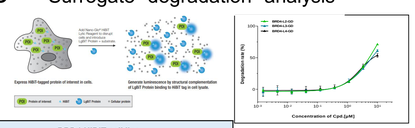
G002-T09-1	G002-T09-4	G002-T09-7	G002-T09-8
K_D = 2,806 nM	K_D = 39 nM	K_D = 357 nM	K_D = 159 nM
MW = 311	MW = 472	MW = 365	MW = 395
LE = 0.35	LE = 0.29	LE = 0.33	LE = 0.32

Figure 4. Primary hits were optimized for their physicochemical profiles.

A Representative structure

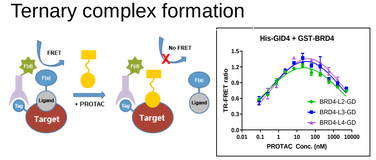


C Surrogate "degradation" analysis



BRD4-HiBiT cell line			
Compounds	BRD4-L2-GD	BRD4-L3-GD	BRD4-L4-GD
DC_{50} (μM)	5.32	6.49	7.23

B Ternary complex formation



TR-FRET assay			
Compound	BRD4-L2-GD	BRD4-L3-GD	BRD4-L4-GD
Hook point (nM)	24.55	31.33	40.74
EC_{50} (nM)	0.21	0.34	0.52

D Effects on endogenous BRD4 level

Cell line	S/B	Z factor	DC_{50} (μM)	Hill Slope	Max inh (%)
SW480	3.3	0.57	2.74	1.08	97.25
A204	2.2	0.60	0.57	3.03	131.97
U2-OS	1.4	-0.26	0.26	6.12	89.29

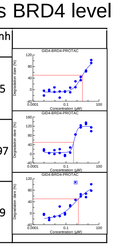


Figure 5. PROTACs, generated by conjugating optimized GID4 binders with a BRD4 ligand, were tested for their efficacy *in vitro*.

Results

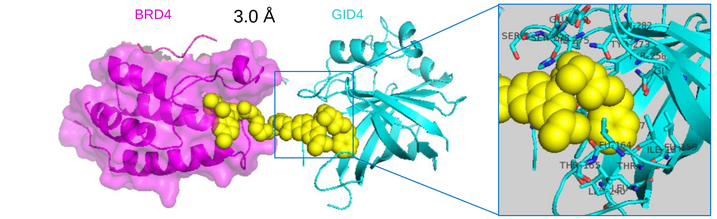


Figure 6. Structural analysis of the ternary complex suggested working mechanism for one top PROTAC. Enlarged diagram: binding of the warhead to a surface within a reported degron-binding pocket of GID4.

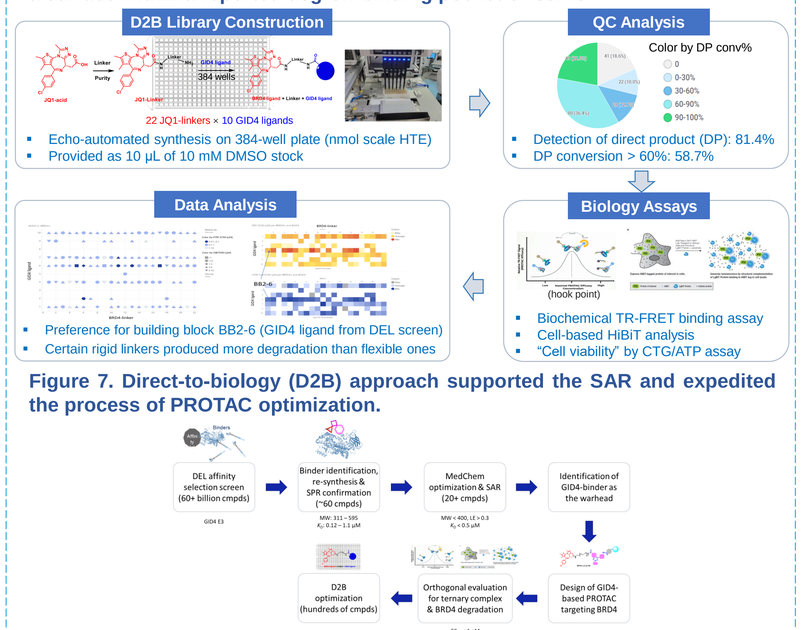


Figure 7. Direct-to-biology (D2B) approach supported the SAR and expedited the process of PROTAC optimization.

Summary

1. DEL-based selection helped identify small molecules that bind the GID4 E3 ligase.
2. Upon further optimization of physicochemical properties, several GID4-binding molecules were selected to produce PROTACs targeting BRD4 and characterized by biochemical and cell-based assays to confirm their degradation potency.
3. Crystallography revealed the mechanism by which one PROTAC molecule promotes formation of the ternary complex.
4. D2B approach further produced PROTACs that showed better potency *in vitro*.

Contact Us USA: mahnaz_arjomand@wuxiapptec.com EU/UK/Israel: dave_madge@wuxiapptec.com Japan: fumio_itoh@wuxiapptec.com Korea: sycho@wuxiapptec.com China/Singapore/Australia: xu_longji@wuxiapptec.com