

Technical Bulletin

QuicTPD™ Amine Screening Set

QTPDAMINE

Storage Temperature: 2-8 °C

Product Description

PROTACs (Proteolysis Targeting Chimeras) represent a groundbreaking approach in targeted protein degradation, leveraging the ubiquitin-proteasome system to selectively eliminate unwanted proteins within cells. By designing bifunctional molecules that bind both the target protein and the E3 ligase, PROTACs facilitate the recruitment of the proteasome to the target protein, leading to its ubiquitination and subsequent degradation. This targeted approach not only enhances the specificity of protein degradation but also minimizes off-target effects, making PROTACs a promising avenue for drug development.

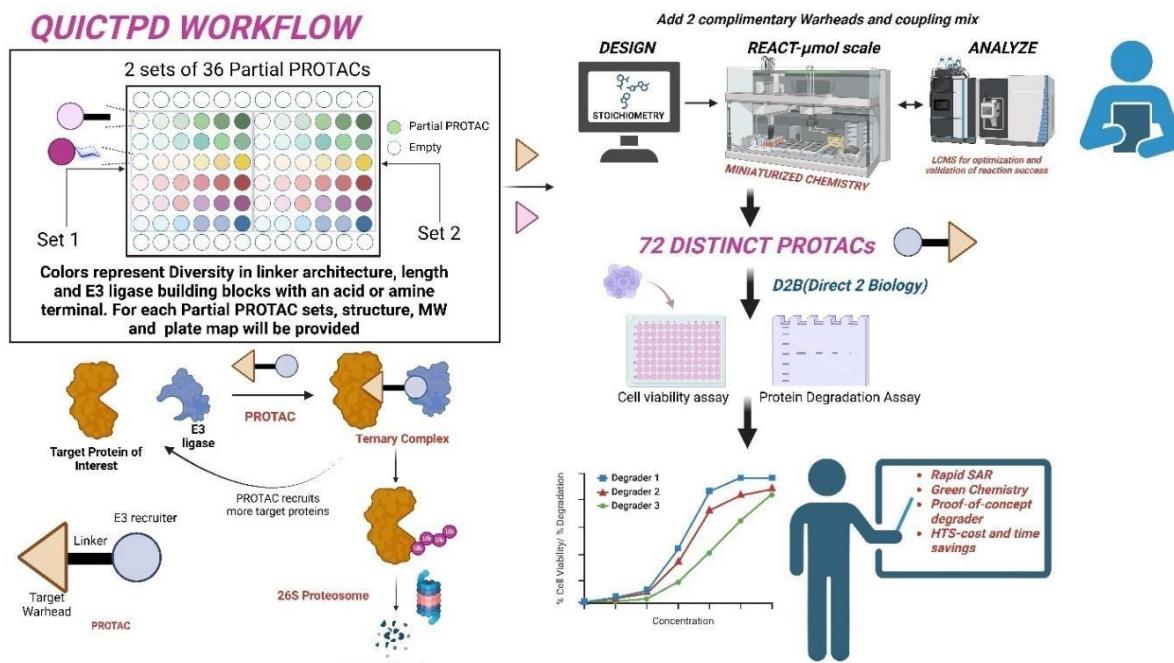
This innovative technology holds immense potential for therapeutic applications, particularly in oncology and other diseases where protein dysregulation is a key factor¹. Additionally, PROTACs have applications in other diseases characterized by protein misfolding or aggregation, such as neurodegenerative disorders. As research in this area continues to evolve, PROTACs are expected to pave the way for novel therapeutic strategies that can address previously undruggable targets, ultimately transforming the landscape of drug discovery and development.

QuicTPD™ Amine Screening Set is a versatile collection of 36 Partial Protacs/E3-ligase-linker conjugates, expertly designed with a variety of linkers with different architectures and lengths, featuring a convenient amine terminal. This set is ideal for researchers looking to develop proof-of-concept PROTAC degraders for their targets of interest. This set specifically targets Cereblon (CRBN) and von Hippel Lindau (VHL) E3 ligases, both of which play crucial roles in the ubiquitin-proteasome system and are pivotal in PROTAC discovery for targeted protein degradation.

Features and Benefits

- The QuicTPD™ Amine Screening Set enables seamless reactions with warheads for targets of interest that have solvent-exposed, amine-reactive handles, such as acids, through peptide coupling reactions.
- Each Partial PROTAC (E3 Ligase + Linker) is conveniently plated in micromole scale within glass shell vials, facilitating a ready-to-react workflow that accommodates various reaction conditions—including different coupling reagents, bases, and temperatures.
- With the ability to react with at least two distinct warheads, researchers can create 72 or more unique small molecule degraders for further screening in a Direct to Biology (D2B) approach²⁻⁶. This miniaturized synthesis approach not only accelerates PROTAC discovery but also promotes sustainability by reducing the use of solvents, reagents, water, and energy.

- Partial PROTACs are based on Lenalidomide, Pomalidomide, Piperidinedione, and VH032 with a rich diversity in linkers for hydrophilicity, length, and rigidity to maximize your SAR discovery campaign.



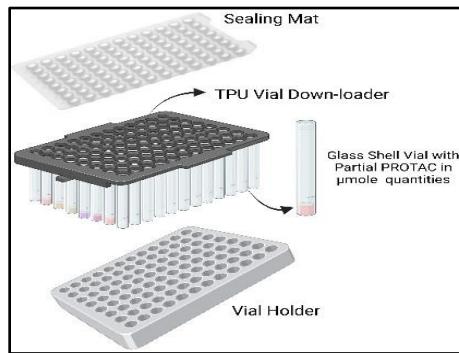


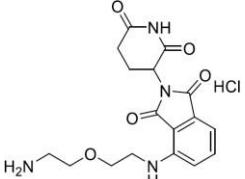
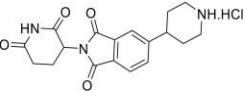
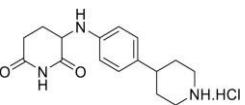
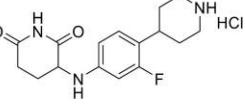
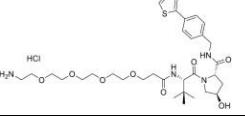
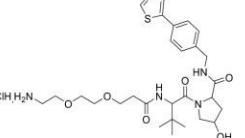
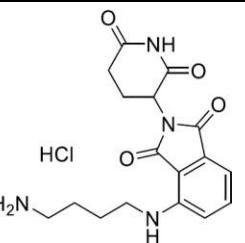
Figure 2: shows the assembly of QuicTPD Amine Screening Set

Table 1: Structures of Partial PROTACs in the QuicTPD™ Amine Screening Set.

Catalog No	Name & SMILES	Structure
<u>901495</u>	Pomalidomide-PEG₃-NH₂ hydrochloride O=C(C(CC1)N(C2=O)C(C3=C2C=CC=C3NC(COCCOCCOCCN)=O)=O)NC1=O.Cl	
<u>901513</u>	Pomalidomide-PEG₂-NH₂ hydrochloride O=C(C(CC1)N(C2=O)C(C3=C2C=CC=C3NC(COCCOCCN)=O)=O)NC1=O.Cl	
<u>901488</u>	(S,R,S)-AHPC-PEG₂-NH₂ hydrochloride O=C(NCC1=CC=C(C2=C(C)N=CS2)C=C1)[C@H](C[C@@H](O)C3)N3C([C@H](C(C)(C)C)NC(COCCOCCN)=O)=O.Cl	
<u>901511</u>	(S,R,S)-AHPC-PEG₃-NH₂ hydrochloride O=C(NCC1=CC=C(C2=C(C)N=CS2)C=C1)[C@H](C[C@@H](O)C3)N3C([C@H](C(C)(C)C)NC(COCCOCCOCCN)=O)=O.Cl	
<u>901831</u>	Pomalidomide-PEG₅-NH₂ hydrochloride O=C(C(CC1)N(C2=O)C(C3=C2C=CC=C3NC(COCCOCCOCCOCCN)=O)=O)NC1=O.Cl	
<u>901516</u>	Pomalidomide-PEG₁-NH₂ hydrochloride O=C(C(CC1)N(C2=O)C(C3=C2C=CC=C3NC(COCCN)=O)=O)NC1=O.Cl	
<u>901850</u>	(S,R,S)-AHPC-PEG₅-NH₂ hydrochloride O=C(COCCOCCOCCOCCOCCN)N[C@H](C(N1[C@H](C(NCC2=CC=C(C3=C(C)N=CS3)C=C2)=O)C[C@@H](O)C1=O)C(C)(C)C.Cl	

<u>901493</u>	(S,R,S)-AHPC-PEG₁-NH₂ hydrochloride O=C(NCC1=CC=C(C2=C(C)N=CS2)C=C1)[C@H](C[C@@H](O)C3)N3C([C@H](C(C)(C)C)NC(COCCN)=O)=O.Cl	
<u>901848</u>	(S,R,S)-AHPC-PEG₄-NH₂ hydrochloride O=C(COCCOCCOCCOCCN)[C@H](C(N1[C@H](C(NCC2=CC=C(C3=C(C)N=CS3)C=C2)=O)C[C@@H](O)C1)=O)C(C)(C)Cl	
<u>905224</u>	Pomalidomide-PEG₆-butyl amine hydrochloride O=C(C(CC1)N(C2=O)C(C3=C2C=CC=C3NC(COCCOCCOCCOCCOCCOCCN)=O)=O)NC1=O.Cl	
<u>905232</u>	(S,R,S)-AHPC-C₆-PEG₃-butyl amine hydrochloride NCCCCCOCCOCCOCCCCCCC(N[C@H](C(N1[C@H](C(NCC2=CC=C(C3=C(C)N=CS3)C=C2)=O)C[C@@H](O)C1)=O)C(C)(C)C)=O.Cl	
<u>905275</u>	(S,R,S)-AHPC-PEG₆-butyl amine hydrochloride NCCCCCOCCOCCOCCOCCOCC(N[C@H](C(N1[C@H](C(NCC2=CC=C(C3=C(C)N=CS3)C=C2)=O)C[C@@H](O)C1)=O)C(C)(C)C)=O.Cl	
<u>906123</u>	(S,R,S)-AHPC-PEG₂-butyl amine hydrochloride NCCCCCOCCOCC(N[C@H](C(N1[C@H](C(NCC2=CC=C(C3=C(C)N=CS3)C=C2)=O)C[C@@H](O)C1)=O)C(C)(C)C)=O.Cl	
<u>911739</u>	C5 Lenalidomide-PEG₁-NH₂ hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(COCCN)=O)=CC=C31.CI	
<u>915572</u>	Pomalidomide-dipiperazine-NH₂ hydrochloride O=C(C1=C2C=CC=C1N3CCN(CC3)C(N4CCN(CC4)CCN)=O)N(C2=O)C5CCC(NC5=O)=O.Cl	
<u>911720</u>	C5 Lenalidomide-C₉-NH₂ hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(CCCCCCCCCN)=O)=CC=C31.Cl	
<u>920754</u>	C5 Lenalidomide-benzyl-piperazine hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(C4=CC=C(CN5CCNCC5)C=C4)=O)=CC=C31.Cl	
<u>920797</u>	C5 Lenalidomide-pyridine-PEG₁-piperazine hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(CC4=CC=C(OCCCN5CCNCC5)N=C4)=O)=CC=C31.Cl	

<u>917729</u>	C5 Lenalidomide-piperazine-pyridine-alkyne-NH₂ hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(CCCN4CCN(C5=CC=C(C#CCN)C=N5)CC4)=O)=CC=C31.Cl	
<u>929441</u>	C5-Pomalidomide-piperazine hydrochloride O=C1N(C(C2=CC=C(C=C21)N3CCNCC3)=O)C4CCC(NC4=O)=O.Cl	
<u>930652</u>	Pomalidomide-C₂-NH₂ hydrochloride O=C1NC(C(CC1)N2C(C3=C(C2=O)C(NCCN)=CC=C3)=O)=O.Cl	
<u>930660</u>	Pomalidomide 4'-PEG₃-amine hydrochloride O=C1NC(C(N2C(C3=C(C(NCCOCCOCCOCCN)=CC=C3)C2=O)=O)CC1)=O.Cl	
<u>930520</u>	Pomalidomide-PEG₂-C₂-NH₂ hydrochloride O=C1NC(C(N2C(C3=C(C(NCCOCCOCCN)=CC=C3)C2=O)=O)CC1)=O.Cl	
<u>901830</u>	Pomalidomide-PEG₄-NH₂ hydrochloride O=C(C(CC1)N(C2=O)C(C3=C2C=CC=C3NC(COCCOCCOCCOCCN)=O)NC1=O.Cl	
<u>911704</u>	C5 Lenalidomide-C₃-NH₂ hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(CCCN)=O)=CC=C31.Cl	
<u>901860</u>	(S,R,S)-AHPC-PEG₆-NH₂ hydrochloride O=C(COCCOCCOCCOCCOCCOCCN)N[C@H](C(N1[C@H](C(NCC2=CC=C(C3=C(C)N=CS3)C=C2)=O)C[C@H](O)C1)=O)C(C)(C)C.Cl	
<u>911712</u>	C5 Lenalidomide-C₆-NH₂ hydrochloride O=C1N(C2CCC(NC2=O)=O)CC3=CC(NC(CCCCCN)=O)=CC=C31.Cl	
<u>930687</u>	2,6-Piperidinedione, 3-[(3-aminophenyl)amino] hydrochloride O=C1NC(C(NC2=CC(N)=CC=C2)CC1)=O.Cl	
<u>934275</u>	Pomalidomide-piperazine O=C1NC(C(CC1)N2C(C3=CC=CC(N4CCNCC4)=C3C2=O)=O)=O.Cl	

<u>934305</u>	Pomalidomide-PEG1-C2-amine HCl Cl.O=C1NC(=O)C(N2C(=O)C=3C=CC=C(NCCOCCN)C3C2=O)CC1	
<u>934402</u>	2-(2,6-dioxopiperidin-3-yl)-5-(piperidin-4-yl)isoindole-1,3-dione hydrochloride O=C(N(C1CCC(NC1=O)=O)C2=O)C(C=C3)=C2C=C3C4CCNCC4.Cl	
<u>934429</u>	3-((4-(Piperidin-4-yl)phenyl)amino)piperidine-2,6-dione hydrochloride O=C1NC(C(CC1)NC2=CC=C(C3CCNCC3)C=C2)=O.Cl	
<u>936464</u>	3-(3-Fluoro-4-piperidin-4-ylphenylamino)piperidine-2,6-dione hydrochloride Cl.O=C1NC(=O)C(NC2=CC=C(C(F)=C2)C3CCNCC3)CC1	
<u>936618</u>	(S,R,S)-AHPC-CO-PEG4-C2-amine HCl C([C@H](NC(CCOCOCCOCCOCCN)=O)[C@](C)(C)C)(=O)N1[C@H](C(NCC2=CC=C(C=C2)C3=C(C)N=CS3)=O)C[C@H](O)C1.Cl	
<u>934399</u>	L-Prolinamide, N-[3-[2-(2-aminoethoxy)ethoxy]-1-oxopropyl]-3-methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-, (4R)- HCl O=C(NC(C(N1CC(O)CC1C(NCC2=CC=C(C=C2)C3=C(N=CS3)C)=O)=O)C(C)(C)CCOCCOCCN.Cl	
<u>936596</u>	1<i>H</i>-Isoindole-1,3(2<i>H</i>)-dione, 4-[(4-aminobutyl)amino]-2-(2,6-dioxo-3-piperidinyl)-, hydrochloride Cl.O=C1NC(=O)C(N2C(=O)C=3C=CC=C(NCCCCN)C3C2=O)CC1	

QuicTPD Amine Plate Map

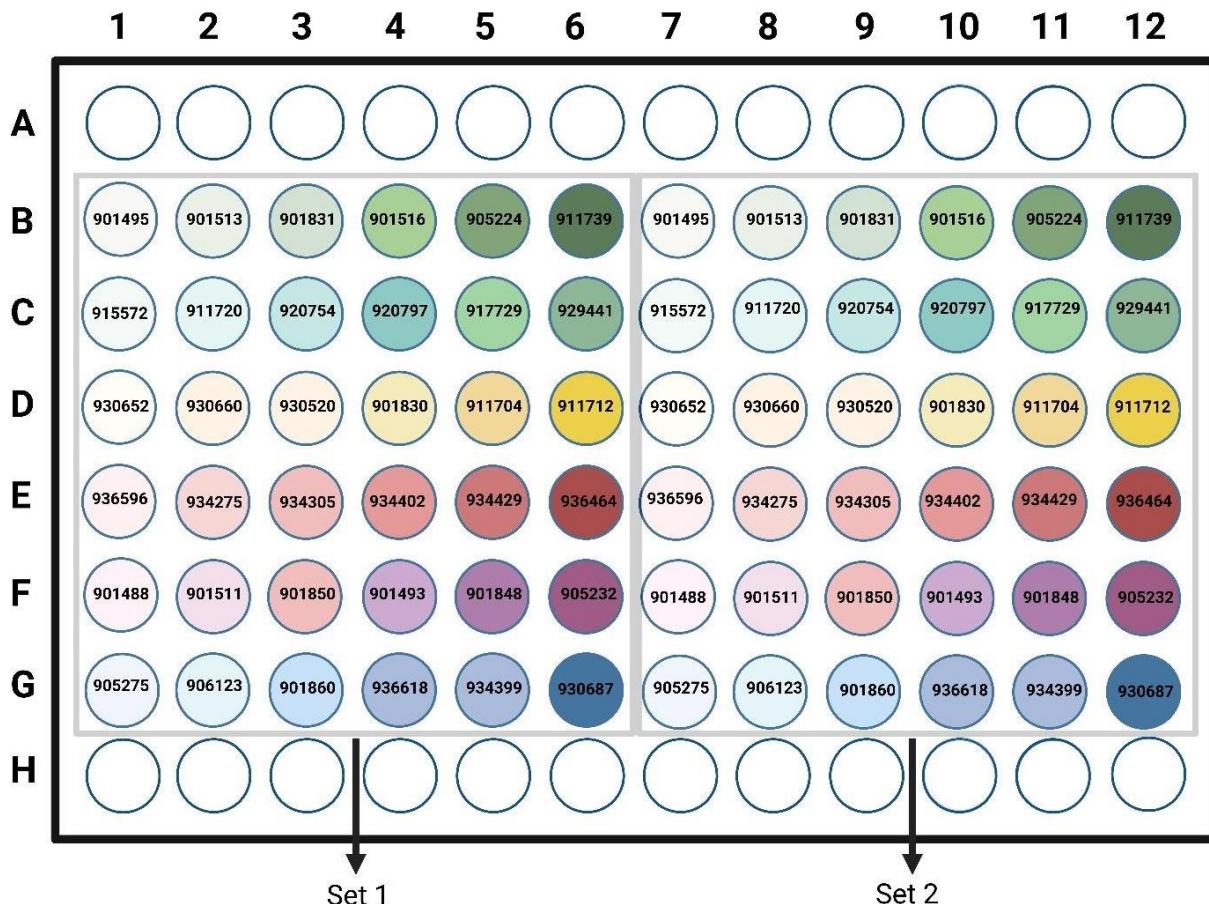


Figure 3: Plate Map for QuicTPD Amine Screening Set. Each Shell Vial houses 1.5 micromoles or 5 micromoles based on SKU size.

High Throughput Synthesis Optimization using QuicTPD Amine Optimization Set

The QuicTPD Amine Optimization set is designed to facilitate high throughput synthesis optimization for researchers working with PROTACs. This set includes 4 representative partial PROTACs, each provided at 5 micromole quantity. Two of these PROTACs are tailored to target the CRBN E3 ligase, while the other two are directed towards VHL, incorporating both hydrophobic and hydrophilic linkers.

By utilizing this optimization set, customers can explore a variety of reaction conditions—up to 24 or more—before proceeding to the complete screening set. This exploration includes testing different coupling reagents, solvents, and bases, allowing for a comprehensive assessment of the synthesis parameters that influence the efficiency and yield of the desired products.

It is highly advisable for researchers to purchase and utilize the QuicTPD Amine Optimization (QAMINESET-1KT) set prior to engaging with the complete screening set. This preliminary step will not only enhance the understanding of the reaction conditions but also significantly improve the chances of successful outcomes in subsequent experiments. By optimizing the synthesis process early on, researchers can streamline their workflows and achieve more reliable results in their PROTAC development efforts.

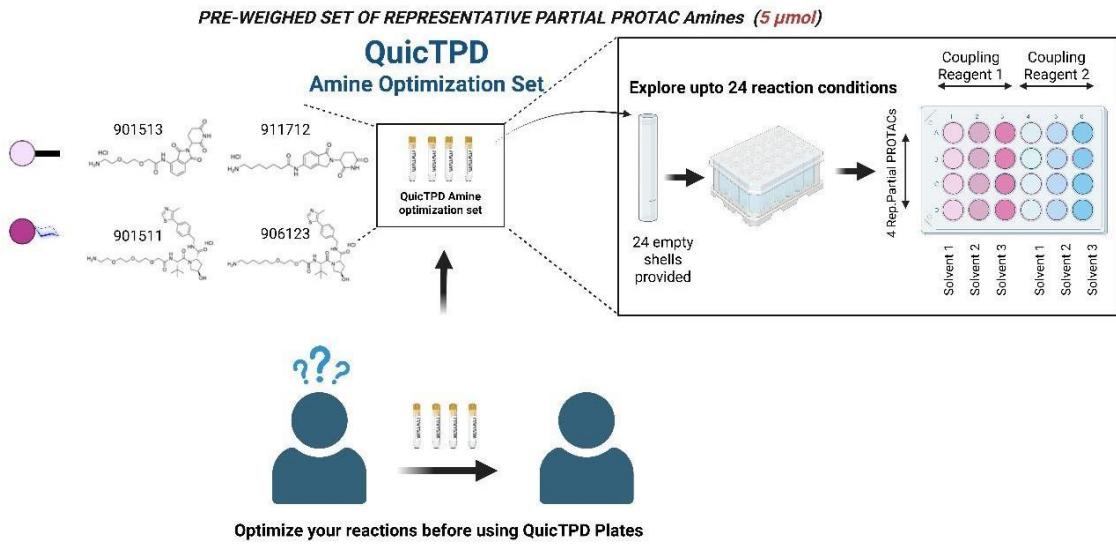


Figure 4: Structures of compounds provided in QuicTPD Amine Optimization set and possible usage.

PROTAC Library Generation with QuicTPD Amine Screening Set

The following general protocol is recommended for constructing PROTAC libraries through peptide coupling reactions. This involves the reaction between warheads targeting specific proteins with pendant acid/amine-reactive terminals, which can effectively couple with the amine or acid partial PROTACs available in the QuicTPD Screening Sets.

Required Reagents and Set-up Essentials.

Warheads: It is advisable to use at least 2 warheads to generate 72 PROTAC degraders through combinatorial synthesis with the 36 pre-plated partial protacs at given SKU size of 3 and 10 micromoles. Direct-to-biology reactions can very well be carried out at nanomole scale; hence the contents of the plates can be dissolved and aliquoted into multiple plates to react with desired number of warheads with complimentary reactive termini. Ensure that the warheads are generally inert in downstream screening assays, especially if a direct-to-biology approach is employed.

Coupling Agents: The following commonly used reagents are essential for typical peptide coupling reactions. These are not included with the screening set and should be purchased separately

- EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide [39391](#))
- NMM (N-Methylmorpholine) as a base [M56557](#)
- Oxyma Pure as an additive [8.51086](#)

Solvents: Dimethyl sulfoxide (DMSO) is typically used in peptide coupling reactions. This solvent is not provided with the screening set and should be acquired separately.

Temperature, Time, and Mixing Parameters: Conduct the reaction at room temperature (25°C) for 12-24 hours on a horizontal shaker using standard settings. For more challenging peptide coupling reactions, the reaction vials can be transferred to aluminum heating block reactors.

Scale of Reaction: The reaction scale typically varies from nanomole to micromole.

Final Concentration of PROTAC: The desired final concentration typically ranges from 1 to 10 mM.

Final Volume of Reaction: 50-500 microliters. It is recommended to keep low reaction volumes to ensure enough headspace in reaction vials to prevent cross contamination between reactions.

Concentration Limits of Coupling Agents: It is recommended to keep EDCI, Oxyma Pure and NMM concentrations in reaction at or below 175mM, 500 mM and 240 mM respectively for direct to biology screening. Refer to the papers listed in the References section for further reading on reaction conditions.

Detailed Protocol

1. **Stoichiometric calculations:** For assistance with stoichiometric calculations, please refer to the Excel file online.
2. **Weighing Reagents:** Accurately weigh the required amounts of warheads, acid activator, additive, and base into glass vials.
3. **Preparation of Stock Solutions:** Prepare stock solutions of the acid activator, additive, base, and warheads in your solvent of choice, preferably anhydrous DMSO.
4. **Prepping Up the Reaction Plate:** Add the reaction solvent in the necessary quantities to the glass shell vials containing the partial protacs. Mix the contents by aspiration and shaking for 30 mins to ensure proper dissolution.
5. **Aliquoting for Smaller Scales:** If the user is conducting reactions below SKU size, aliquot the required volume into daughter plates for small-scale reactions.
6. **Addition of Warheads and Coupling Reagents:** It is recommended to pre-activate the acid warheads with the coupling reagent. However, you can also sequentially add the required quantities of warheads, followed by the acid activator, additives, and base to achieve the desired final concentration and reaction volume. Generally, the ratios should be, warhead: Partial Protac: Coupling Reagent: Additive: Base = 1: 1.2-1.5: 1.5: 2: 8.0.
7. **Setting Up Controls:** Use empty glass shell vials to set up negative controls to assess whether the reaction components themselves affect downstream assays. Examples of negative controls that can be included in technical replicates are warheads only, warheads and coupling agents, coupling agents only, and solvent only. These controls are recommended if a direct to biology approach is employed.
8. **Reaction Time, Temperature, and Mixing:** Peptide coupling reactions are typically conducted for 12 to 24 hours at room temperature. For optimal results, place the reaction plates securely on a horizontal shaker overnight at low to medium speed. To minimize exposure of the formed PROTACs to moisture and light, ensure that the silicone mat is properly secured on each glass shell vial to prevent cross-contamination.

9. **Post-Reaction Procedures:** After the reaction is complete, transfer an aliquot of the contents from the plate to a separate 96-well plate for analysis of warhead conversion. Warhead consumption can be assessed using HPLC/MS to evaluate reaction efficiency. The remaining contents of the plate, including the PROTACs formed in situ, can be stored at -20°C for further downstream analysis. Ensure that the silicone mat is secured on each glass shell vial to limit exposure to moisture and light. Align the A1 corner of the vial holder with the A1 mark on the silicon mat. Proper alignment is required to ensure the probe maps correctly to each vial and to avoid cross-contamination.

Handling and Safety

The partial PROTACs are contained within glass shell vials, which are securely held in a vial holder as specified in the product specifications. When transferring these vials between locations, it is recommended to first install the TPU downloader, followed by placing the silicone mat on top of the glass shell vials. This setup ensures that all vials are held tightly in place, preventing any drops during transfer. The silicone mat features markings A-H and 1-12 to help easily locate each vial's position. Avoid excessive exposure of the TPU Downloader to solvents, as this could weaken the product. This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data sheet for individual components for information regarding hazards and safe handling practices.

Troubleshooting

When utilizing our plated products for Direct 2 Biology applications, particularly with partial PROTACs featuring acid or amine terminals for peptide coupling reactions, it's essential to ensure optimal performance and address any potential issues that may arise during experimentation. Below are common troubleshooting tips and considerations to facilitate a smooth workflow:

Suboptimal Coupling Efficiency:

Issue: Low yields of the small molecule degrader.

Solution: Verify the molar ratios of reagents used in the coupling reaction. Ensure that the concentration of the partial PROTAC is adequate to facilitate effective coupling. Additionally, consider optimizing the reaction conditions, such as temperature and reaction time, to enhance coupling efficiency.

Incomplete Reaction:

Issue: Unreacted starting materials remain after the coupling process.

Solution: Check the purity of the starting materials and ensure they are free from contaminants. If the reaction is incomplete, extending the reaction time or increasing the temperature may help drive the reaction to completion. Utilizing other coupling reagents such as HATU (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate) Product number: [445460](#) may also improve results.

Formation of Side Products:

Issue: The presence of unexpected by-products in the reaction mixture.

Solution: Analyze the reaction conditions, including the choice of solvents and coupling agents, as these can influence side reactions. Implementing protective groups on functional groups that are not participating in the reaction may also help minimize side product formation.

Difficulties in Purification in case a D2B workflow is not followed

Issue: Challenges in isolating the desired product from the reaction mixture.

Solution: Optimize purification techniques, such as column chromatography or HPLC, tailored to the properties of the desired product. Adjusting the solvent system or using different stationary phases may enhance separation efficiency.

Stability of Plated Products:

Issue: Degradation or instability of plated products during storage or handling.

Solution: Store plated products under recommended conditions, avoiding exposure to light, moisture, or extreme temperatures. If degradation is suspected, consider using fresh products or verifying the stability of stored compounds before use.

Inconsistent Biological Activity:

Issue: Variability in the biological response observed in assays.

Solution: Ensure that the biological assay conditions are consistent across experiments. Factors such as cell line, incubation time, and assay readouts can influence results. Conducting control experiments with known active compounds can help validate assay performance.

Other Products of Interest.

QuicTPD VHL and CRBN Sets (Product Nos. QVHLSET-1KT and QCRBNSET-1KT), specifically designed for customers who already have warheads ligated to linkers targeting their proteins of interest. With these sets, customers can utilize the E3 recruiters provided in the QVHLSET and QCRBNSET to construct a diverse library of PROTACs tailored to their research needs.

Once a customer identifies a small molecule degrader of interest, they can further enhance their studies using the QuicTPD Negative Control Set (Catalog no: QNEGCONSET-1KT). This set includes CRBN and VHL recruiters with slightly varied structures, allowing for the construction of PROTACs that are not recognized by E3 ligases. These negative controls are essential for confirming the mechanism of action of the identified degraders.

Additionally, to validate that degradation occurs via the ubiquitin-proteasome pathway, customers can utilize MG132 (M8699), a potent proteasome inhibitor, and MLN4924 (5.05477), a neddylation inhibitor. Together, these tools will enable researchers to comprehensively explore and confirm their findings in targeted protein degradation.

References

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