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## File list (2)

# PHOTACs Enable Optical Control of Protein Degradation

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**PROTACs (proteolysis targeting chimeras) are bifunctional molecules that tag proteins for ubiquitylation by an E3 ligase complex and subsequent degradation by the proteasome. They have emerged as powerful tools to control the levels of specific cellular proteins and are on the verge of being clinically used. We now introduce photoswitchable PROTACs that can be activated with the temporal and spatial precision that light provides. These trifunctional molecules, which we named PHOTACs, consist of a ligand for an E3 ligase, a photoswitch, and a ligand for a protein of interest. We demonstrate this concept by using PHOTACs that target either BET family proteins (BRD2,3,4) or FKBP12. Our lead compounds display little or no activity in the dark but can be reversibly activated to varying degrees with different wavelengths of light. Our modular and generalizable approach provides a method for the optical control of protein levels with photopharmacology and could lead to new types of precision therapeutics that avoid undesired systemic toxicity.**

Cellular protein levels result from a tightly controlled balance between synthesis and degradation. A wide range of small molecules have been identified that interfere with these processes. Most of them do not address specific proteins as they broadly inhibit the machinery necessary for transcription, translation, trafficking, or degradation. In recent years, proteolysis targeting chimeras (PROTACs) have emerged as a new principle of pharmacology.[1–3] These bifunctional molecules combine a ligand for an E3 ubiquitin ligase with a second one that targets a protein of interest (POI), thereby promoting the physical interaction of the proteins, the polyubiquitylation of the POI, and its consequent proteasomal degradation. Both ends of the PROTAC are connected via a linker, the exact nature of which needs to be carefully chosen to ensure efficacy, cell permeability, and biodistribution.

First generation PROTACs used peptides to recruit POIs to E3 ligases,[4–6] but subsequent ones have relied on smaller and more cell-permeable synthetic ligands. These include

hydroxyproline derivatives and molecules derived from thalidomide, which bind two ubiquitin ligase substrate receptors, namely, von the Hippel–Lindau tumor suppressor (VHL)[7–9] and cereblon (CRBN)[10,11], respectively. Proteins that have been successfully targeted for degradation include the androgen and estrogen receptor,[5,12–15] the BET family epigenetic readers BRD2-4[10,11,16,17], and FKPB12 and its fusion proteins.[10,18–20] Soluble kinases, such as CDK9[21,22] and BCR-ABL,[23] as well as receptor tyrosine kinases, such as EGFR[24] and BTK,[25–29] have also been amenable to this approach. Covering a broad spectrum from membrane proteins to nuclear hormone receptors, PROTACs have proven to be a highly versatile approach.

PROTACs do not merely inhibit the activity of their targets, like conventional drugs, but rather influence the cellular levels of the targets by promoting their proteolysis. The transition from mere inhibition of proteins to their catalytic degradation enables the targeting of previously undruggable proteins. However, this mechanistic difference with conventional drugs also poses certain risks when applied systemically, since the POI is degraded and disappears with all of its functions. For instance, inhibition of BET bromodomains is tolerated, but a complete loss of BRD2 and BRD4 is lethal.[30–32] Therefore, it would be advantageous to locally activate PROTACs in tissues and cells where their effects (*e.g.,* cytotoxicity) are desirable, while avoiding deleterious effects elsewhere.

One method to localize the effect of drugs and achieve higher selectivity is to control their activity with light. In recent years, the usefulness of light to precisely regulate biological pathways has indeed become fully apparent. Optical control can be achieved in a variety of ways: with caged compounds,[33,34] genetically engineered photoreceptors (Optogenetics),[35,36] or with synthetic photoswitches whose the activity can be changed through a combination of photochemical isomerizations and thermal relaxation (Photopharmacology).[37–39]

Herein we report the application of photopharmacology to targeted protein degradation. By incorporating azobenzene photoswitches into PROTACs, we have designed photoswitchable versions that we named PHOTACs (PHOtochemically TArgeted Chimeras). These molecules show little or no activity as PROTACs in the dark, but can be activated with blue-violet light (380-440 nm). They can be used to degrade a variety of targets, including BRD2-4 and FKBP12, by binding to the $CRL4^{CRBN}$ E3 ligase complex and promoting polyubiquitylation in a light-dependent fashion. This translates to the optical control of protein levels, and in the case of BRD2-4, cell proliferation, survival, and viability.

## Results

### Design, synthesis, and photophysical characterization

The design of our PHOTACs was guided by a desire to render our molecules as diversifiable and modular as possible, whilst ensuring efficient synthetic access. To test the concept, we chose to target CRBN which, together with VHL, accounts for the majority of PROTAC platforms utilized to date. Accordingly, we focused on thalidomide derivatives, such as pomalidomide and lenalidomide, as CRBN ligands. As for the photoswitch, we decided to use azobenzenes, which are known for their fatigue resistance, large and predictable geometrical changes, and easily tunable photothermal properties. Azobenzenes are also among the smallest photoswitches and do not significantly increase the molecular weight of pharmaceuticals upon substitution. Ideal PHOTACs would be inactive in the dark and lead to efficient degradation of the POI upon irradiation. However, the impact of linker conformation on PROTAC activity is not fully understood. We therefore explored both regular azobenzenes (more stable in their *trans* form) and diazocines (more stable in *cis*).[40–43]

Several approaches are conceivable for the incorporation of these photoswitches into PHOTACs: (A) They could be part of the ligand for the E3 ligase and change the affinity

at this end of the chimera. (B) The photoswitches could mostly reside in the tether, changing the length and orientation of this segment. (C) The azobenzenes could be part of a POI ligand, controlling the affinity of the PHOTAC to the POI. It would be difficult, if not impossible, however, to define a strict boundary between ligands and linker, and combinations of all three modes are possible. As for our first POI ligand, we chose (+)-JQ1, a high-affinity inhibitor of BET proteins BRD2-4 and BRDT. PROTACs featuring this ligand, such as dBET1 (Fig. 1B), have proven to be particularly effective and have been developed for a variety of E3 ligases.

The small library of PHOTACs that resulted from these considerations is depicted in Fig. 2A. Amongst these, **PHOTAC-I-3**, emerged as one of the most effective. Its synthesis started with the diazotization of lenalidomide and coupling of the resulting diazonium ion **1** to 2,6-dimethoxyphenol, which yielded azobenzene **2** (Fig. 2B). Alkylation with *tert*-butyl bromoacetate and subsequent deprotection then afforded the key intermediate **3**. Amide coupling of this carboxylic acid with *N*-Boc-butane-1,4-diamine and deprotection then yielded **4**, which underwent another deprotection followed by peptide coupling with the free acid of (+)-JQ1 (**5**) to afford **PHOTAC-I-3**. HATU coupling of **3** to diaminoalkanes of different length provided easy access to library of PHOTACs with varying linker length (i.e. **PHOTAC-I-1,2,4,5**). **PHOTACs-I-6–8**, which lack two methoxy groups on the azobenzene core, and were synthesized analogously. **PHOTAC-I-9** bears a different substitution pattern and was prepared *via* Baeyer-Mills coupling (see Supporting Information. **PHOTACs-I-10–13**, which have the photoswitch more in the center of the molecule, were synthesized from 4-hydroxy thalidomide and azobenzene building blocks via alkylations and amide couplings (see Supporting Information).

The photoswitching and thermal relaxation properties of one of our lead compounds, **PHOTAC-I-3**, is shown in Fig. 3A-E. The optimal wavelength to switch to the *cis* isomer is 390 nm but similar photostationary states (PSS) can be obtained between 380 and 400 nm (Fig. 3C). At 390 nm a PSS of >90% *cis* could be obtained. Rapid *cis* to *trans* isomerization could be achieved by irradiation with wavelengths >450 nm, achieving PSS

of ca >70% *trans* (Fig. 3C). In the absence of light, *cis* **PHOTAC-I-3** slowly isomerized back to its *trans* form with a half-life of 8.8 h at 37 °C in DMSO (Fig. 3D). Multiple cycles of photochemical isomerizations are possible, in keeping with the fatigue-resistance of azobenzene photoswitches (Fig. 3E). Structurally related PHOTACS showed similar photophysical and thermal properties (see Supporting Information).

**Optical control of BRD-2-4 with PHOTACs**.

To assess the biological activity of our **PHOTACs**, we tested their effect on the viability of RS4;11 lymphoblast cells. Cells were treated in a 96-well plate with increasing concentrations of **PHOTACs** and were either irradiated with 390 nm light pulses (100 ms every 10s) for 72 h or incubated with the compound in the dark. Subsequently, we performed cell viability assays (Promega MTS), as previously described. **PHOTAC-I-3** showed a promising activity difference upon irradiation (Fig. 4A). The $EC_{50}$ was determined to be 88.5 nM when irradiated with 390 nm light and 631 nM in the dark, resulting in a 7.1-fold $EC_{50}$ difference. This indicates that cytotoxicity increases upon irradiation and that **PHOTAC-I-3** is less toxic in the dark. Similar trends were observed for **PHOTAC-I-1,2,4–8,10**, all of which were more active in viability assays following pulse irradiation (SI Fig. S4). By contrast, **PHOTACs-I-9,11–13** showed no light-dependent differences in activity (SI Fig. S4). In a control experiment, the BET inhibitor (+)-JQ1 showed no light dependent toxicity either (Fig. 4B).

Next, we analyzed the light-dependence of targeted protein degradation in RS4;11 cells by western blot analysis of BET proteins BRD2-4 (Fig 5). To this end, we treated cells with increasing concentrations of our lead compound **PROTAC-I-3** for 4 h and pulse-irradiated with 390 nm light (100 ms every 10 s). We observed a pronounced decrease of BRD4 levels in the presence of **PROTAC-I-3** (particularly between 100 nM to 3 μM) when irradiated with 390 nm light but not in the dark (Fig. 5A). At 10 μM, we observed less degradation, which is consistent with the “hook effect” commonly observed with PROTACs.[44,7,25] BRD3 levels were also significantly reduced upon exposure to concentrations in the range of 100 nM to 3 μM of **PHOTAC-I-3** when irradiated with violet

light, but not in the dark. In comparison, BRD2 was degraded to a lesser extent and within a narrower concentration range. Application of **PHOTAC-I-3** (1 μM) together with the CRL inhibitor MLN4924 (2.5 μM), which inhibits neddylation and, consequently, the activity of all cellular CRLs (including $CRL4^{CRBN}$), rescued BRD2-4 levels upon irradiation. As expected from photoactivatable degraders and inhibitors of BRD4, c-MYC levels were also affected.[45–47,17] Downregulation of this transcription factor, which is a notoriously difficult target for pharmacological intervention,[48,49] was more pronounced when cells were pulse-irradiated in the presence of low concentrations of PHOTAC-I-3 than in the dark.

The time-dependence of BRD degradation is shown in Fig. 5B. BRD2 and BRD3 are largely absent after 1.5 h exposure and irradiation, whereas BRD4 is degraded more slowly. We also observed sustained degradation and c-MYC downregulation over 24 hours. In the dark, **PHOTAC-I-3** had no effect on BRD levels and relatively little effect on c-MYC levels. The residual activity against c-MYC can be explained by inhibition of BRD4 with the (+)-JQ1 derivative **PHOTAC-I-3** in the absence of targeted degradation. Following sustained pulse irradiation, we also observed increasing cleavage of PARP-1 (Fig. 5B). This indicator of apoptosis[50] correlates to the cell viability assay shown in Fig. 4A.

One of the principal advantages of photoswitches over caged compounds is their reversibility. Azobenzene photoswitches can thermally relax to an inactive form or be isomerized back photochemically. We demonstrated the faster photochemical reversibility with a rescue experiment wherein PHOTAC-I-3 was continuously irradiated for 1 min with the activating wavelength (390 nm) and then pulse irradiated with the deactivating wavelength (525 nm). Under these conditions, BRD2 levels in RS4;11 cells initially decreased but recovered faster than when left in the dark after 1 min of irradiation with 390 nm (Fig. 5C).

Another characteristic feature of photopharmacology is “color-dosing” (*i.e.*, the ability to control the concentration of the active species with the color of the incident light).[51–53] The photostationary state (*i.e.,* the ratio between the two photoisomers) is a function of the wavelength. Figures 3A and 5D show that this principle can also be applied to PHOTACs. Cell viability assays gave left-shifted curves as the color gradually approached 390 nm (Fig. 4A). Western blots showed maximum degradation at 390 nm and gradually increasing BRD4 levels as the wavelength of the incident light increased (Fig. 5D). At 370 nm we also observed slightly increased protein levels. This is in accordance with the photostationary states measured at different wavelengths, which are maximized toward the active *cis* isomer at the slightly longer wavelength of 390 nm (Fig. 3C).

The effect of **PHOTAC-I-10**, which is derived from thalidomide and has the photoswitch positioned deeper in the linker on BRD4 levels, is shown in Fig. 6. We found robust photodegradation even with 10 nM **PHOTAC-I-10**. A clear hook-effect was observed (Fig. 6A). Once again, the thermally less stable *cis* azobenzene promoted ubiquitylation and degradation.

**Optical control of FKBP12 with PHOTACs**.

To demonstrate that the PHOTAC approach is generalizable, we next turned to the prolyl *cis-trans* isomerase FKBP12. The structures of the corresponding **PHOTACs** are shown in Fig. 2C. **PHOTACs** of this series consist of a CRBN-targeting glutarimide, an azobenzene photoswitch in different positions, a linker, and the ligand SLF that binds to native FKBP12.[54–56,10,18] The synthesis of **PHOTACs-II-1–6** is detailed in Supporting Information. The photophysical and thermal characterization of **PHOTACs-II-5** and **PHOTACs-II-6** is shown in Fig. 3 F–H and in Fig. 3 I–K, respectively.

Amongst the molecules tested, **PHOTAC-II-5** and **PHOTAC-II-6** turned out to be the most useful and our biological investigations have been focused on these molecules (for **PHOTACs-II-1–4**, see Fig. S7,S8). **PHOTAC-II-5**, which has the azobenzene switch in the same position as **PHOTACs-I-1–8**, had a pronounced effect on FKBP12 levels upon

pulse irradiation (Fig. 7A). The degradation was slower than in the case of BET proteins, but between 6 and 12 hours the protein was largely absent from our cell lysates (Fig. 7B). Again, no degradation could be observed in the dark. **PHOTAC-II-6**, wherein the photoswitch was moved further into the linker region, also elicited light-dependent degradation (Fig. 7C). In this case, however, we also observed slight dark activity. The time course of FKBP12 degradation by **PHOTAC-II-6** was similar to the one observed with **PHOTAC-II-5** (Fig. 7D). Both PHOTACs showed a pronounced "hook effect" and were inactive in the presence of MLN-4924.

## Discussion

By incorporating photoswitches into PROTACs, we have delineated a general strategy to control targeted protein degradation with the temporal and spatial precision that light affords. As such, we have applied the concept of photopharmacology to an important new target class, *i.e.,* E3 ligases, and have added a highly useful functional feature to existing PROTACs.

As a proof of principle, we developed **PHOTACs** that combine CRBN ligands with azobenzene photoswitches and ligands for either BET proteins (BRD2,3,4) or FKBP12. The modularity of our approach should enable the straightforward development of PHOTACs that target many other classes of proteins. For instance, existing PROTACs that target CDK4/6,[57–59] CDK8,[60] CDK9,[21,22] BTK,[25–29] ABL,[23,61] Sirt2,[62] FLT3,[63] ALK,[64–66] MET,[24] MDM2,[67] and Tau[68] could be adapted to become light activatable. Our PHOTACs for BRD2,3,4 may enable new insights into epigenetic pathways and potentially serve as precision tools in medicine. Color-dosing and reversibility could be particularity useful in this regard.

Our **PHOTACs** for FKBP12 could not only enable the optical degradation of wildtype prolyl *cis-trans* isomerases, but also of proteins that are tagged with this domain. This would significantly increase the utility of dTAGs, which have emerged as a broadly

applicable technology to influence the homeostasis of fusion proteins.[18–20] In addition, **PROTACs** that link thalidomide derivatives to an alkyl halide could be modified to optically degrade HALO-tagged proteins.[9,69] It should also be noted that some of our photoswitchable thalidomide derivatives, such as synthetic intermediates **2**-**4**, and derivatives thereof, could function as dimerizers that recruit Ikaros (IKZF1) and Aiolos (IKZF3) to CRBN in a light-dependent fashion.[70–75]

The **PHOTACs** introduced herein have several features that make them useful for biological studies: They are inactive as degraders in the dark and become active upon irradiation. Following activation, they gradually lose their activity through thermal relaxation. Alternatively, they can be quickly inactivated photochemically. In any scenario, their inactivation is much less dependent on dilution, clearance, or metabolism. In the case of compounds of the **PHOTAC-I** series, they still function as inhibitors of BET proteins, which explains the cytotoxicity observed in the dark. The concentrations needed for maximum photoeffect are low (nanomolar range) avoiding off-target effects. The light needed for photoactivation is not cytotoxic, given the low intensities needed for photoisomerization and the pulse protocol used.[52,76,39]

In principle, light activation of PROTACS could also be achieved with a caging strategy. Although this has not yet been reported, to the best of our knowledge, several caged protein dimerizes are known.[77–79] Hence, caged PROTACs are likely to emerge in future years, complementing **PHOTACs**. The advantage of the latter lies in their reversibility, the low intensities of light needed to trigger the photochemistry, the ease with which the active concentration can be tuned, and the avoidance of potentially toxic byproducts. Fast relaxing **PHOTACs** should also have advantages in terms of localization and temporal control.

Genetically encoded degrons fused to a photosensitive LOV2 domain have been reported as an optogenetic approach to protein degradation.[80–83] Although this approach works well *in vivo* and provides a powerful method to study biological pathways, it requires

transfection with a gene of interest, which limits its therapeutic applicability and can potentially create unphysiological protein levels and distributions within a cell. **PHOTACs,** by contrast, operate like drugs in native tissues.

An important question to address is how exactly PHOTACs work and why many of our present compounds are more active in their *cis* form. Although an effect of photoswitching on pharmacokinetics cannot be ruled out entirely, the increased activity as a degrader seems to be primarily driven by pharmacodynamics. We consistently observed a "hook effect" and found no light-dependent activity in the presence of the neddylation inhibitor MLN4924, which indicates that **PHOTACs** function as true PROTACs. Whether the photoswitch primarily affects binding to CRBN or the relative positioning of the E3 ligase and the POI remains to be determined. The importance of the exact nature, length, and orientation of the linker in PROTACs has been noted ("linkerology").[84–86] In keeping with this, we also observed a pronounced effect of the diamine spacers on our systems. In the series targeting BET proteins, **PHOTAC-I-3**, which bears a 1,4-diamino butane spacer, showed the largest difference between light and dark activity in MTS assays. Related compounds that feature shorter or longer spacers, showed smaller or no differences (see Supporting Information, Fig. S4). **PHOTAC-I-10**, wherein the photoswitch is positioned more centrally, also showed a light-dependent behavior, whereas analogs with different linker lengths did not. Amongst compounds targeting FKBP12, the lenalidomide derivative **PHOTAC-II-5** and the thalidomide derivative **PHOTAC-II-6**, which feature different photoswitch incorporation modes, showed the largest light activation. In the case of derivative **PHOTAC-II-5**, longer linkers were detrimental to optical control (Fig. S7, S8). A satisfying explanation of these observations will require detailed biophysical and structural investigations, which are beyond the scope of this study.

The future development of **PHOTACs** can be taken into many different directions. The incorporation of red-shifted and faster relaxing photoswitches could further improve the temporal and spatial precision of dark-inactive compounds.[87–90] Our synthetic approaches are well suited to increase photoswitch diversity. Different ligands for E3 ligases should be explored, for instance compounds that bind to VHL or MDM2. The optimal position of

the photoswitches will depend on the exact nature of the system. Here, we have explored two different modes of photoswitch incorporation but the third variety (photoswitch in the POI ligand), and combinations thereof, should also be considered. The POI ligand should ideally bind to its target without interfering with its function to cleanly distinguish between degradation and inhibition, and avoid unwanted toxicity.

We anticipate that **PHOTACs** will be useful tools in cell biology but we believe that their clinical potential is also worthy of consideration. Since PROTACs operate in a catalytic fashion, their toxicity is a major concern. **PHOTACs** of the type described herein could be activated with light within a tissue or before administration. They would then lose their activity with a given rate, which can be determined through engineering of the switch, or they could be actively turned off with a second wavelength. Moreover, **PHOTACs** locally activated by light would also lose efficacy by dilution diffusing away from the point of irradiation. The usefulness of light in medicine is well established and the combination of light and molecules has been studied for decades, *e.g.* in Photodynamic Therapy (PDT).[91] PDT has been applied with encouraging results to treat non-small cell lung cancer, dermatological cancers, and premalignant lesions of the upper digestive tract, and is currently in clinical trials for the treatment of a large variety of other malignancies, including prostate, brain, and breast cancers.[92,93] In the past, we have used PDT both in cultured cells and in mouse models to induce death of prostate cancer cells in a calcium-dependent manner.[94] However, from a molecular point of view, conventional PDT, while effective, is unspecific. **PHOTACs**, by contrast, can be reversibly activated with the temporal and spatial precision that light affords and target specific proteins whose elimination would promote cell death. Therefore, we believe that **PHOTACs** may provide a promising new direction in photomedicine.

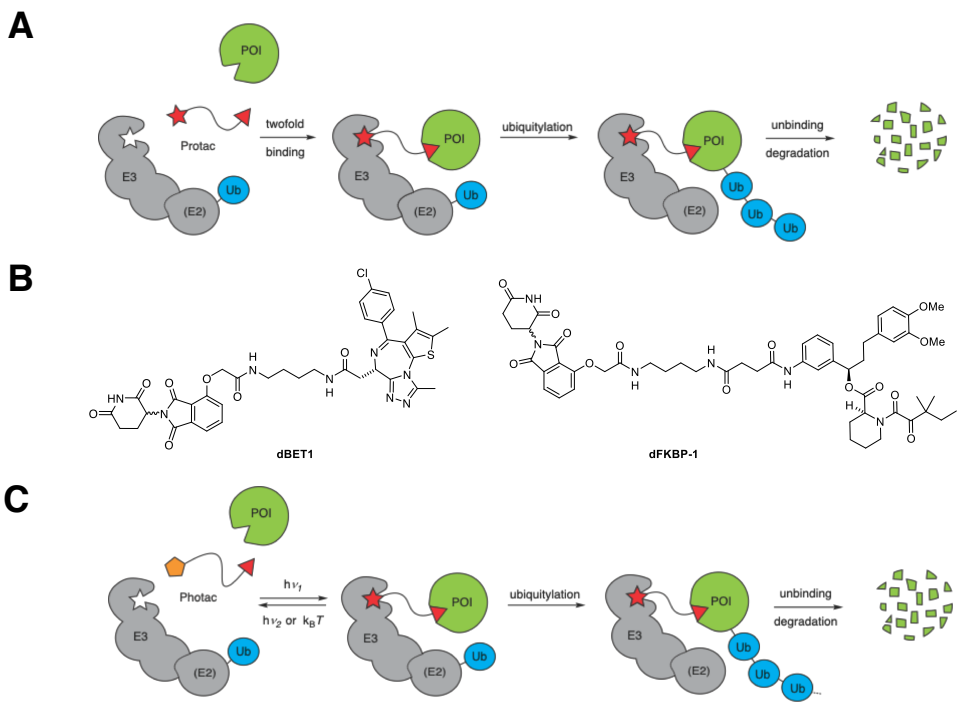


**Figure 1**. PROTACS and **Photacs**. A) Schematic depiction of a PROTAC. Formation of a ternary complex between an E3-ligase, a PROTAC and a protein of interest (POI) leads to degradation of the POI. B) Chemical structures of PROTACs dBET1 and dFKBP-1. C) Schematic depiction of a **PHOTAC**. The molecules toggles between an inactive form (yellow pentagon) and an active form (red star) upon irradiation.

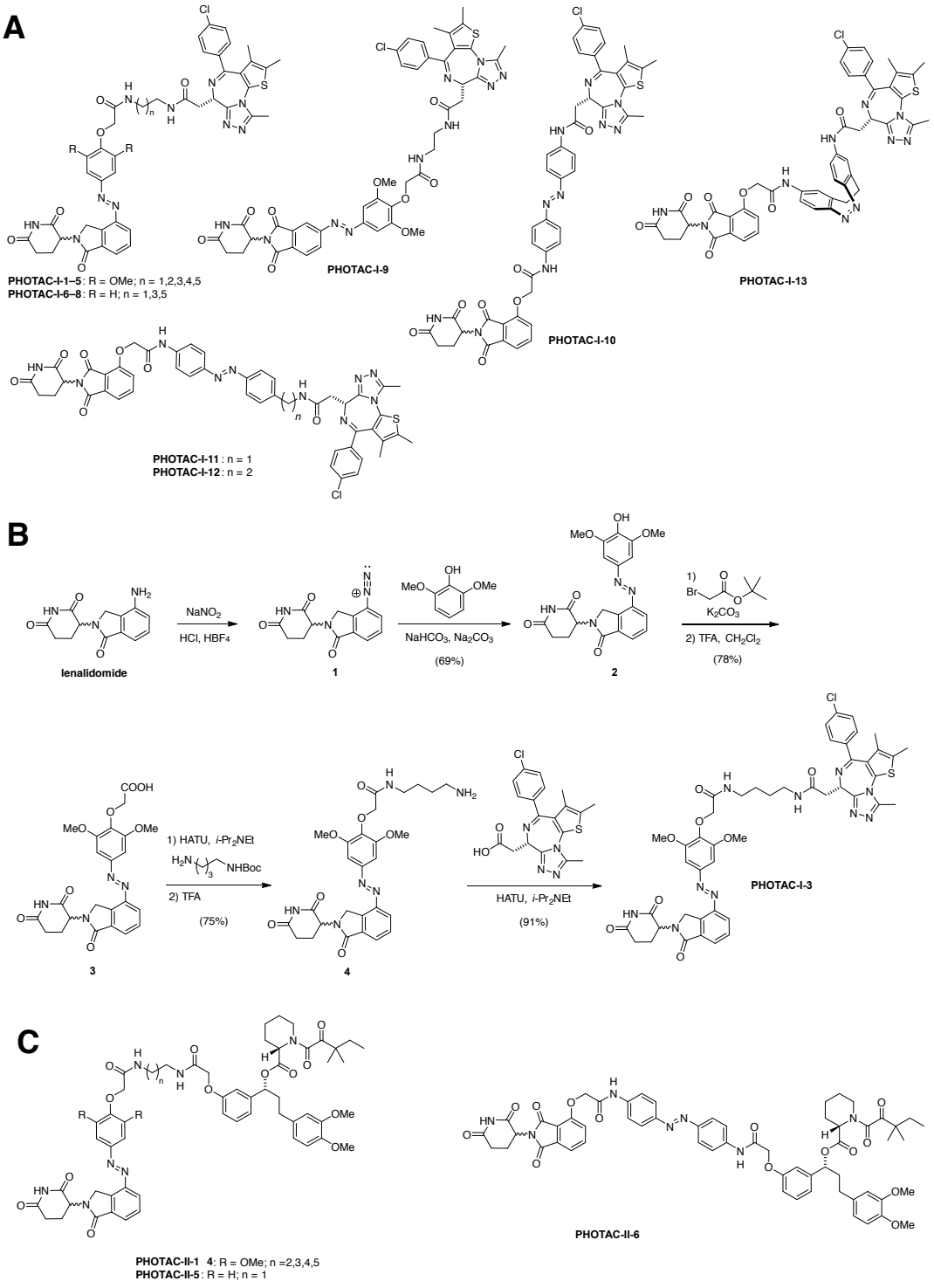


**Figure 2**. Structure and synthesis of **PHOTACs**. A) Members of the **PHOTAC-I** series targeting BRDs. B) Synthesis of **PHOTAC-I-3** starting from lenalidomide. C) Members of the **PHOTAC-II** series targeting FKBP12.

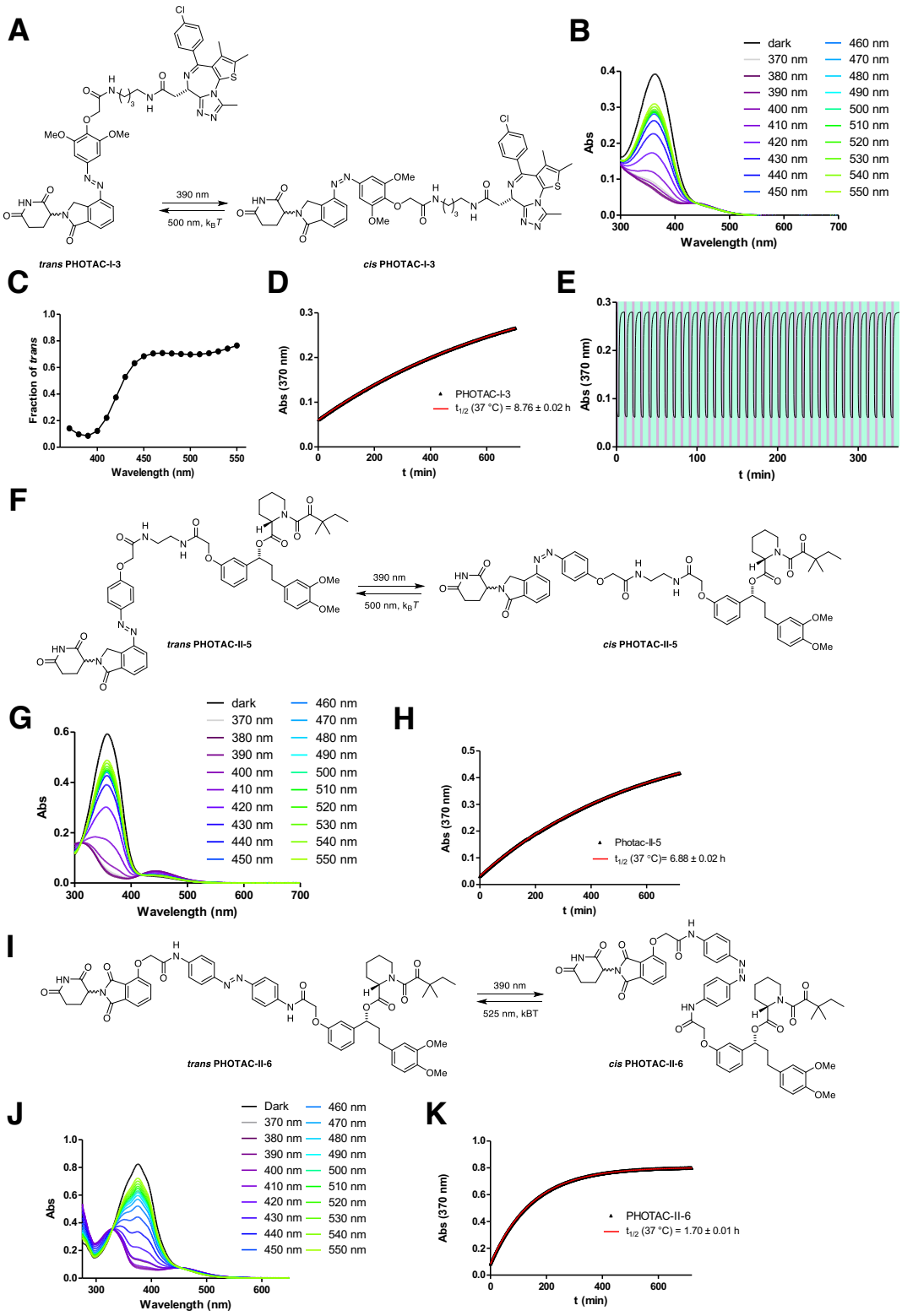


**Figure 3**. Photophysical properties, switching, and bistability of **Photacs**. A) Switching of **PHOTAC-I-3** between the *trans* isomer (left) and *cis* isomer (right). B) UV-VIS spectra

**PHOTAC-I-3** following irradiation with different wavelengths for 5 min. C) Fraction of *trans* **PHOTAC-I-3** in the PSS. D) Thermal relaxation of *cis* **PHOTAC-I-3** at 37 °C in DMSO . E) Reversible switching and photochemical stability of **PHOTAC-I-3**. F) Switching of **PHOTAC-II-5** between the *trans* isomer (left) and *cis* isomer (right). G) UV-VIS spectra **PHOTAC-II-5** following irradiation with different wavelengths for 5 min. H) Thermal relaxation of *cis* **PHOTAC-II-5** at 37 °C in DMSO. I) Switching of **PHOTAC-II-6** between the *trans* isomer (left) and *cis* isomer (right). J) UV-VIS spectra **PHOTAC-II-6** following irradiation with different wavelengths for 5 min. K) Thermal relaxation of *cis* **PHOTAC-II-6** at 37 °C in DMSO.

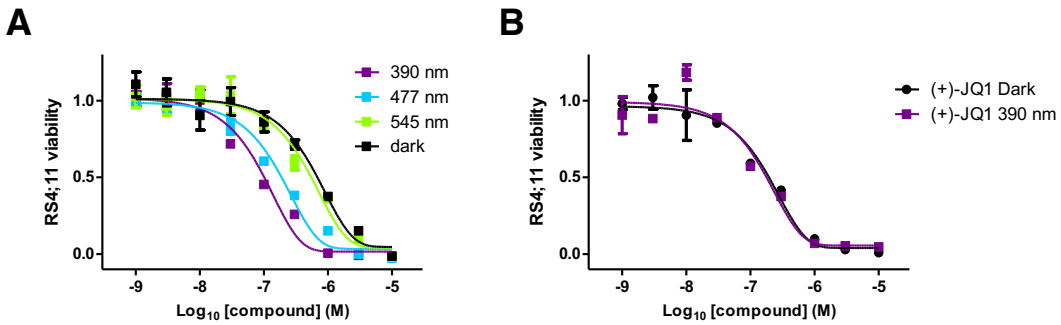


**Figure 4**. A) Viability of RS4;11 acute lymphoblastic leukemia cells after treatment with **PHOTAC-I-3** for 72 h in the dark or under pulsed (100 ms every 10 s) 390 nm, 477 nm or 545 nm irradiation. B) RS4;11 Viability after (+)-JQ1 treatment for 72 h in the dark or under pulsed (100 ms every 10 s) 390 nm irradiation.

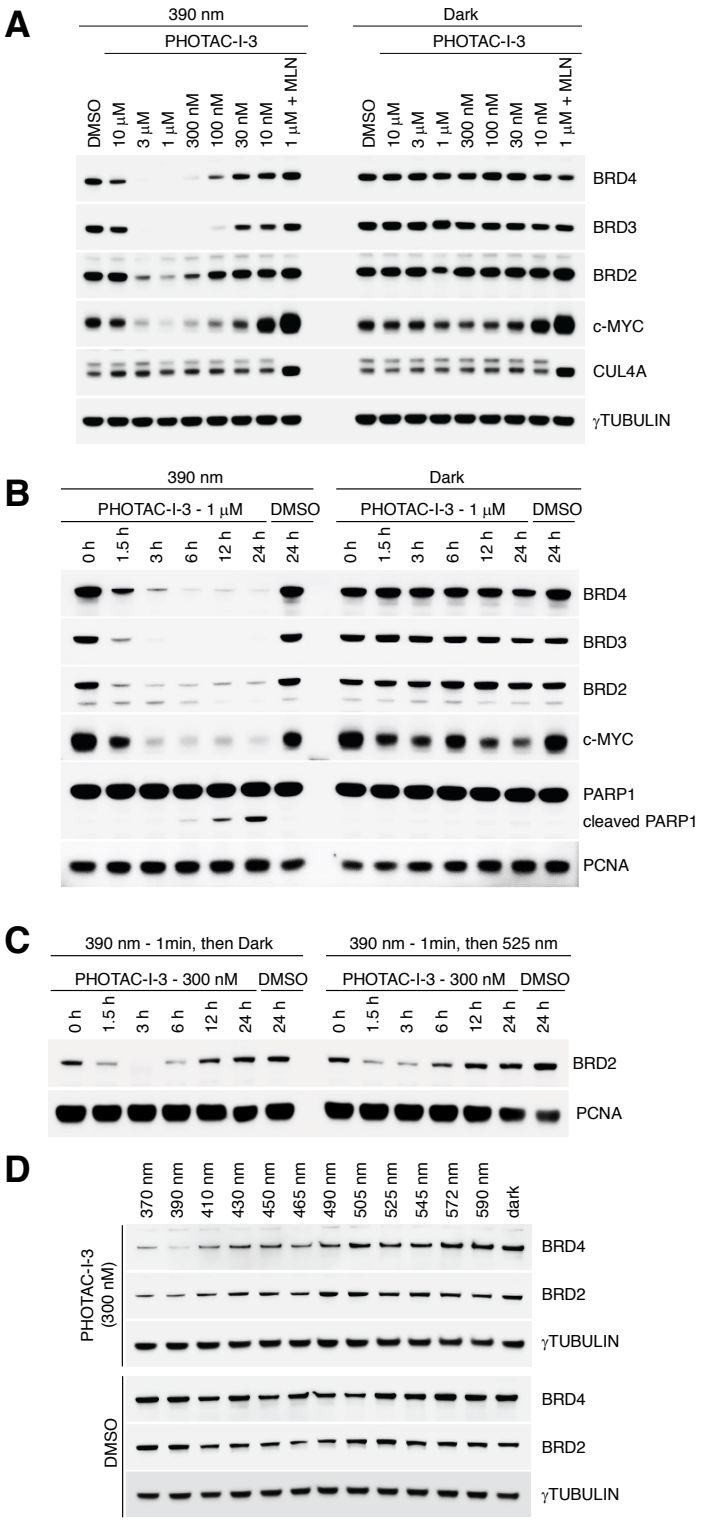


**Figure 5**. Optical control of BRD2-4 levels. A) Immunoblot analysis after treatment of RS4;11 cells with **PHOTAC-I-3** for 4 h at different concentrations. Cells were either

irradiated with 100 ms pulses of 390 nm light every 10 s (left) or kept in the dark (right). B) Time course of BRD2-4 degradation, c-MYC levels and PARP1 cleavage assayed by immunoblotting. RS4;11 cells were treated with **PHOTAC-I-3** (1 $\mu$M) and collected at the indicated time points. **PHOTAC-I-3** has no effect on BRD2-4 levels in the dark over several hours. C) Immunoblot of a rescue experiment demonstrating the reversibility of degradation promoted by **PHOTAC-I-3** through thermal relaxation (left) or optical inactivation by 525 nm pulsed irradiation (right, 100 ms every 10 s). D) Color-dosing: Wavelength dependence of BRD2/4 degradation promoted by 300 nM **PHOTAC-I-3**.

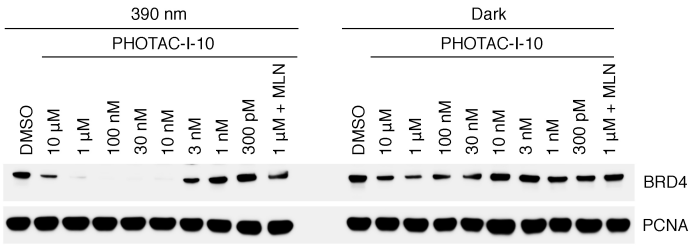


**Figure 6**. Optical degradation of BRD4 with the thalidomide derivative **PHOTAC-I-10**. Immunoblot analysis of RS4;11 cells after treatment with **PHOTAC-I-10** for 4 h at different concentrations which were either irradiated with 100 ms pulses of 390 nm light every 10 s (left) or kept in the dark (right).

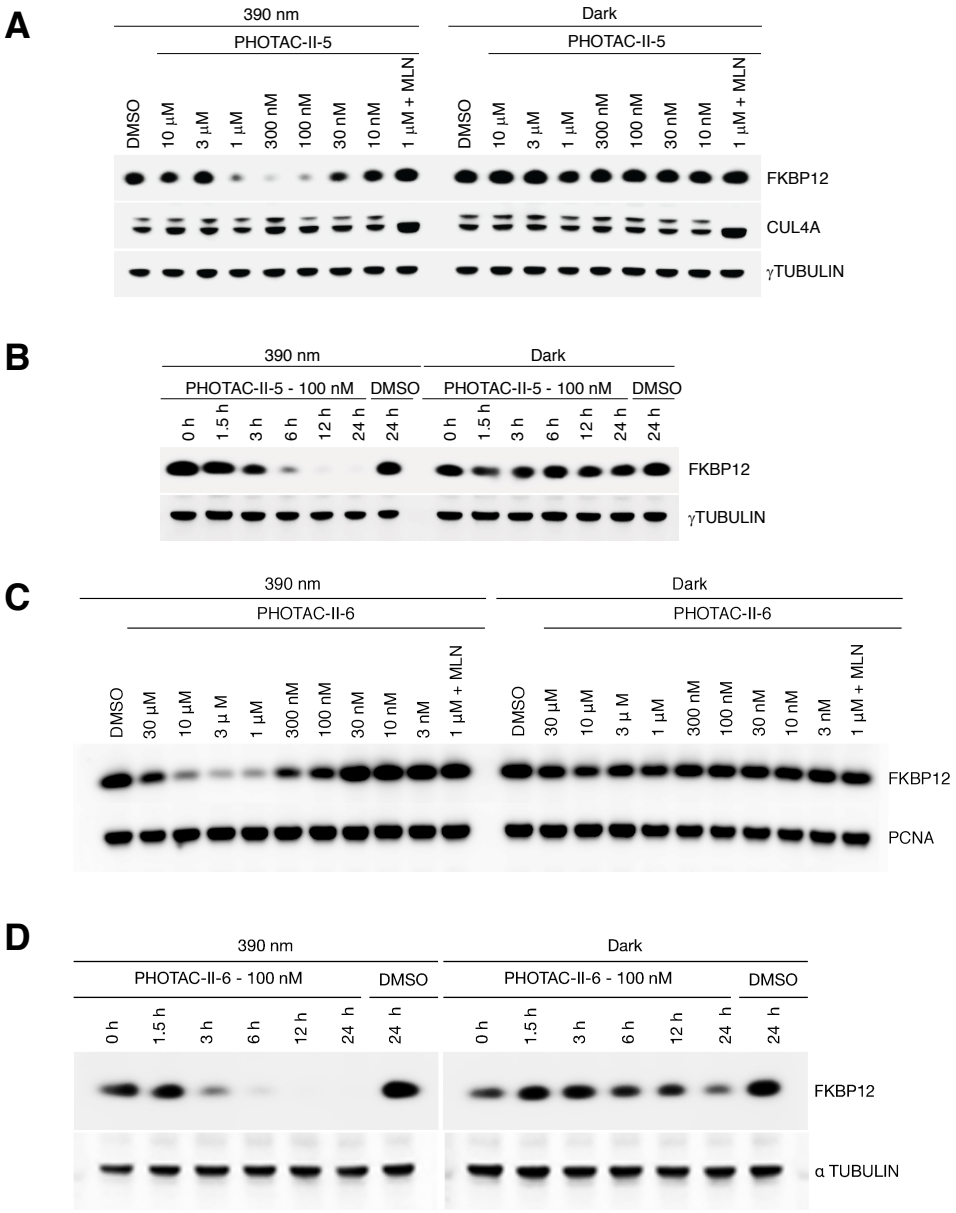


**Figure 7**. Optical control FKBP12 degradation. A) Immunoblot analysis of FKBP12 after treatment of RS4;11 cells with **PHOTAC-II-5** for 4 h at different concentrations. Cells were either irradiated with pulses of 390 nm light (left, 100 ms every 10 s) or kept in the dark (right). B) Time course of FKBP12 degradation visualized by immunoblotting. RS4;11 cells were treated with **PHOTAC-II-5** (100 nM) and collected at the indicated time points. C) Immunoblot analysis of FKBP12 after treatment of RS4;11 cells with **PHOTAC-II-6** for 4 h at different concentrations. Cells were either irradiated with pulses of 390 nm light

(left, 100 ms every 10 s) or kept in the dark (right). D) Time course of FKBP12 degradation visualized by immunoblotting. RS4;11 cells were treated with **PHOTAC-II-6** (100 nM) and collected at the indicated time points.

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**Conflict of interest**

M.R., B.M., M.B., D.T. are inventors on a patent application on PHOTACs. M.P. is a member of the scientific advisory boards of CullGen Inc. and Kymera Therapeutics and a consultant for BeyondSpring Pharmaceutical.

**Author contributions**

D.T., M.R., and B. M. conceived the study. M.R., B.M., M.B., D.S, and A.M. designed experiments and analyzed the data. D.T. and M.P. supervised the experiments. D.T., M.R. and B.M. wrote the paper with input from all authors.

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# Supplementary Information

for

# PHOTACs Enable Optical Control of Protein Degradation

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## Supplementary Figures

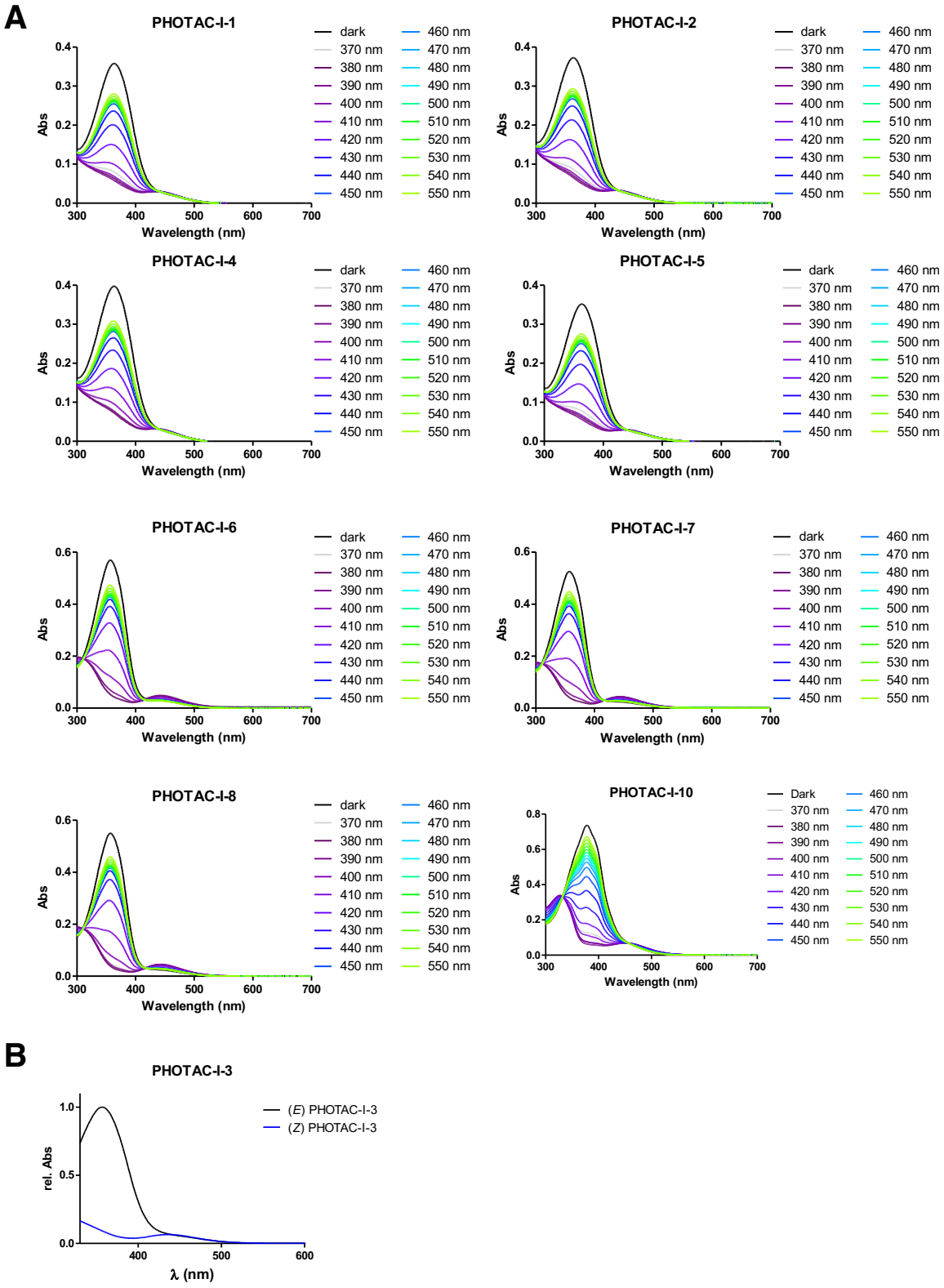



**Fig. S1**. A) UV-VIS spectra **PHOTACs-I** following irradiation with the indicated wavelengths for 5 min. B) Separated UV-VIS spectra of **(*E*)-** and **(*Z*)-PHOTAC-I-3** as obtained from the LCMS and normalized at the isosbestic point.

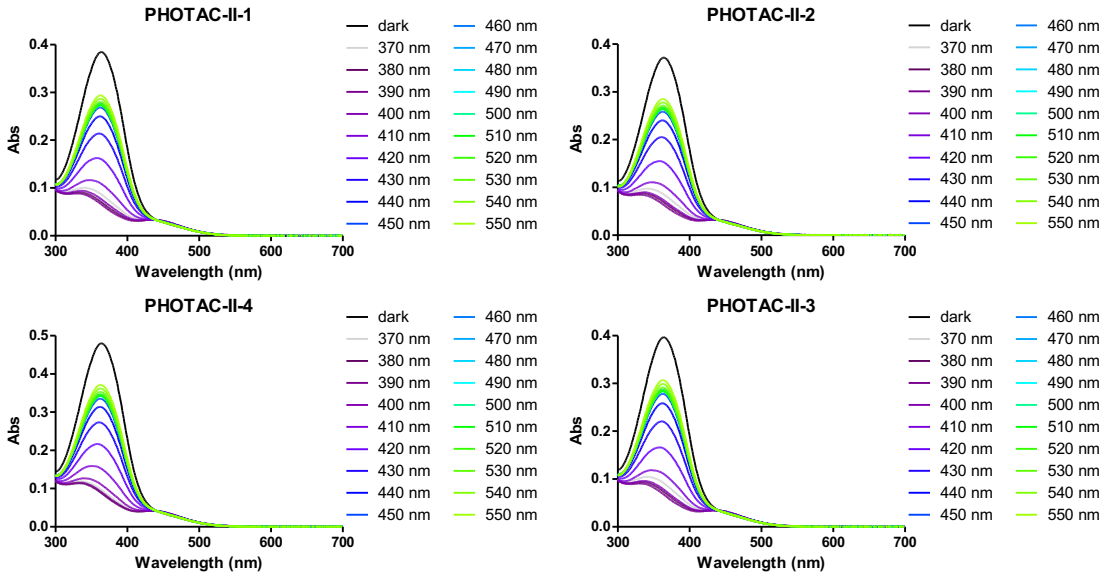



**Fig. S2**. UV-VIS spectra **PHOTACs-II** following irradiation with different wavelengths for 5 min.

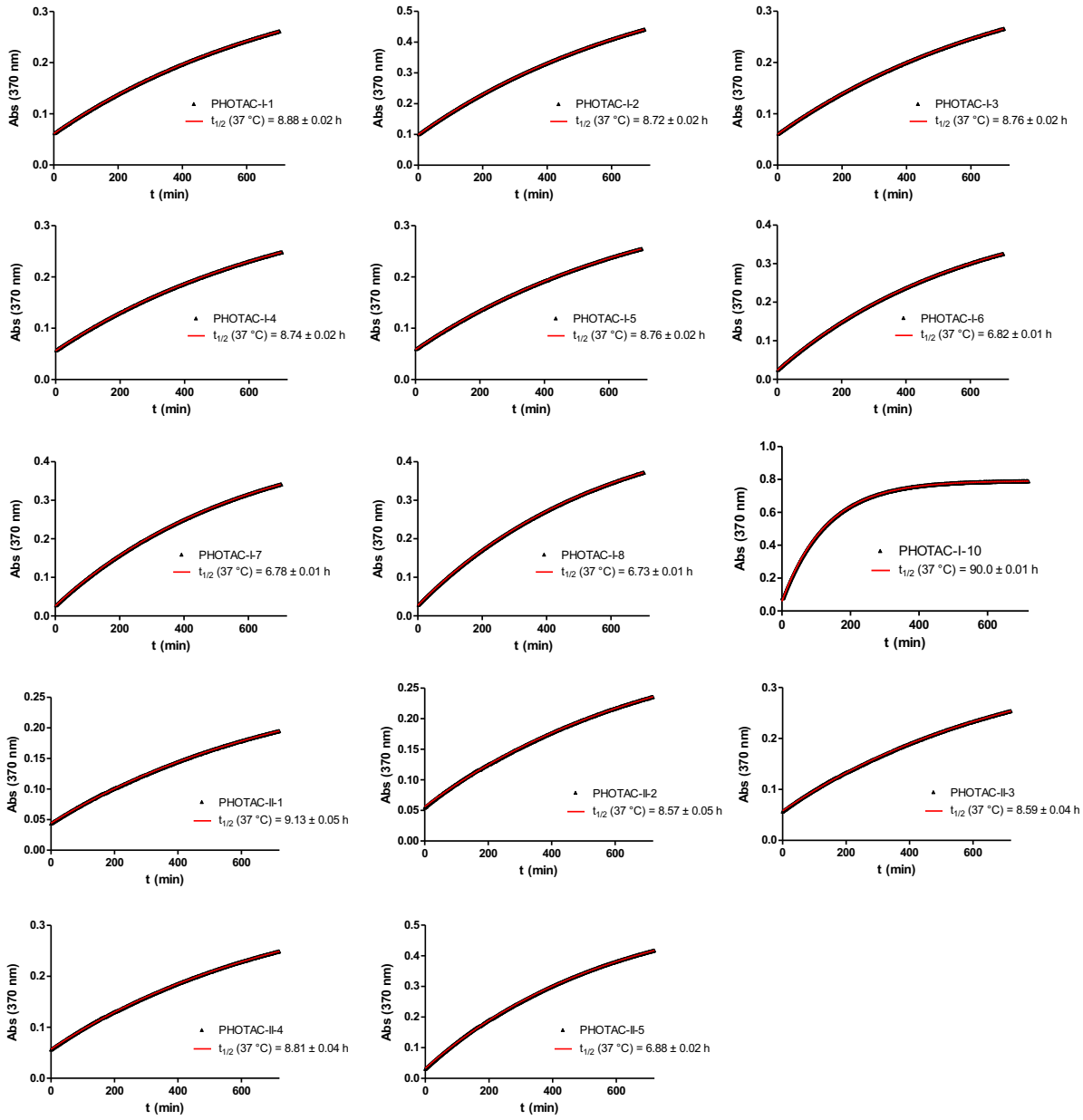
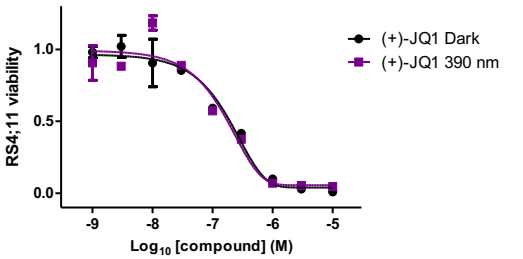



**Fig. S3**. Thermal relaxation of **(*Z*)-PHOTACs** in DMSO.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Log10 [compound] (M)
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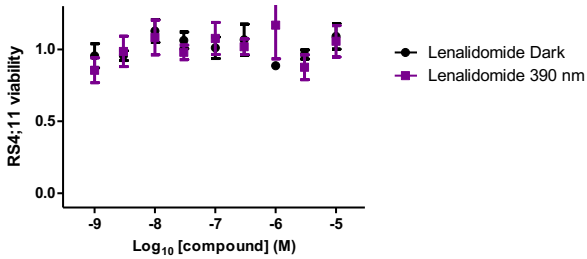
(+)-JQ1 390 nm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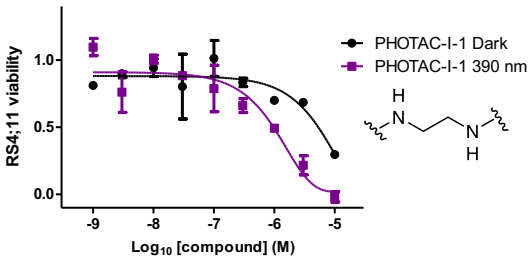
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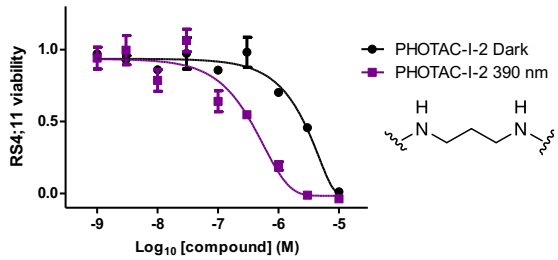
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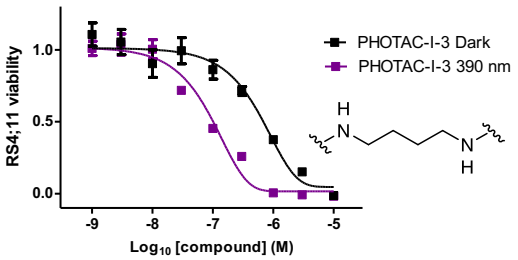
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PHOTAC-I-3 390 nm
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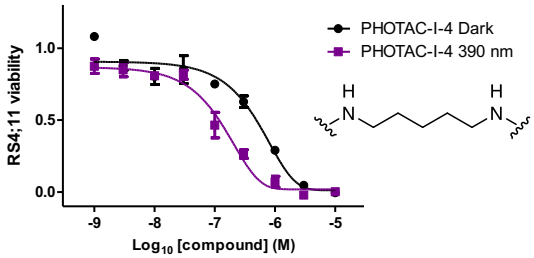
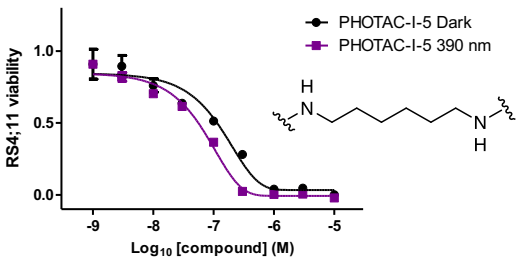
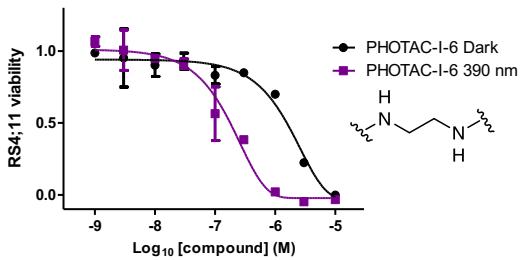
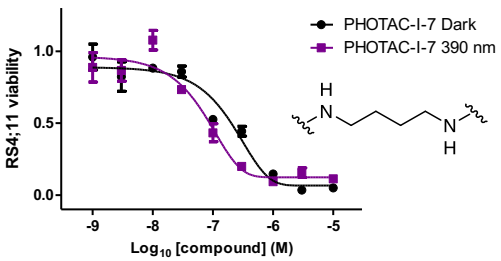
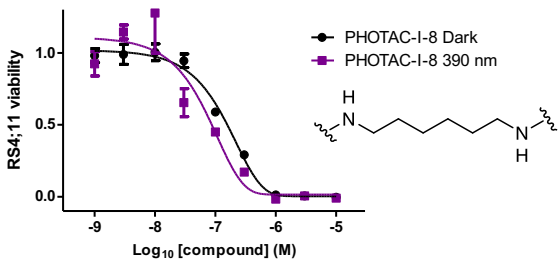
N
H


RS4;11 viability
1.0
0.5
0.0
-9
-8
-7
-6
-5
Log10 [compound] (M)
PHOTAC-I-4 Dark
PHOTAC-I-4 390 nm
H
N
H
N


RS4;11 viability
1.0
0.5
0.0
-9
-8
-7
-6
-5
Log10 [compound] (M)
PHOTAC-I-5 Dark
PHOTAC-I-5 390 nm
H
N
N
H


RS4;11 viability
1.0
0.5
0.0
-9
-8
-7
-6
-5
Log10 [compound] (M)
PHOTAC-I-6 Dark
PHOTAC-I-6 390 nm
H
N
N
H


RS4;11 viability
1.0
0.5
0.0
-9
-8
-7
-6
-5
Log10 [compound] (M)
PHOTAC-I-7 Dark
PHOTAC-I-7 390 nm
H
N
N
H


RS4;11 viability
1.0
0.5
0.0
-9
-8
-7
-6
-5
Log10 [compound] (M)
PHOTAC-I-8 Dark
PHOTAC-I-8 390 nm
H
N
N
H

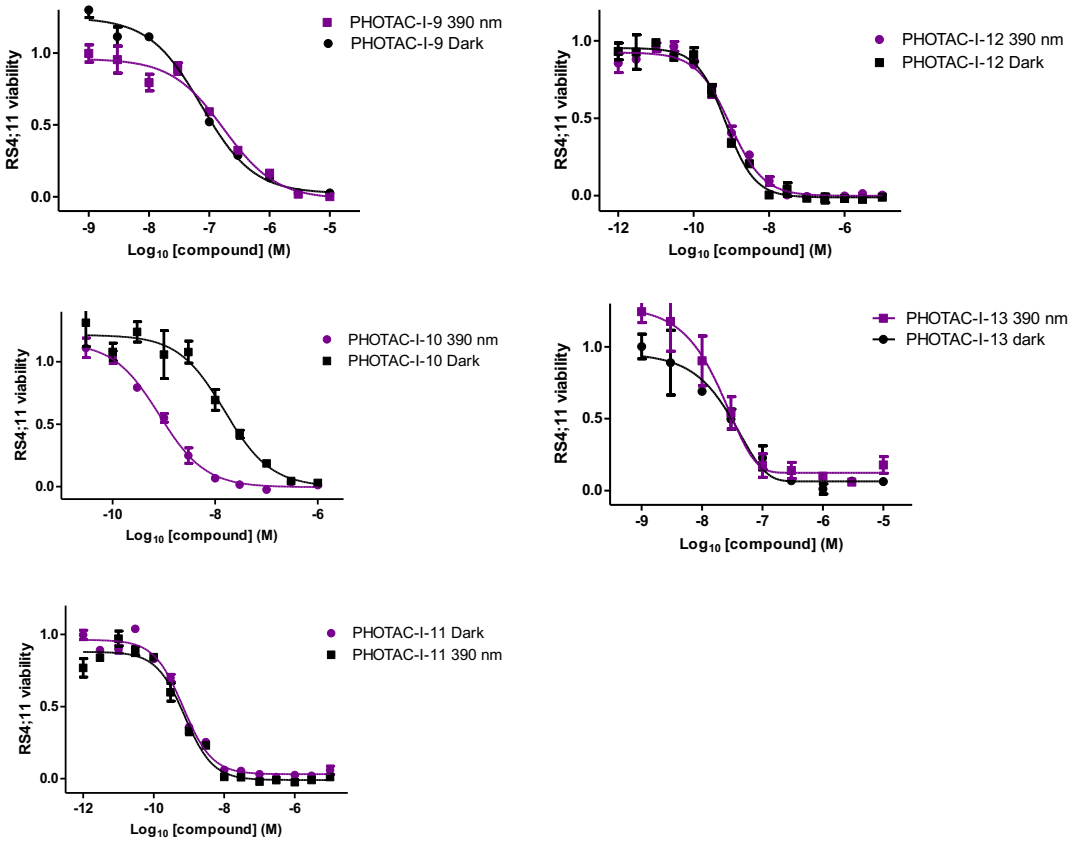



**Fig. S4**. Viability of RS4;11 acute lymphoblastic leukemia cells after treatment with **PHOTACs-I** for 72 h in the dark or under pulsed (100 ms every 10 s) 390 nm irradiation.

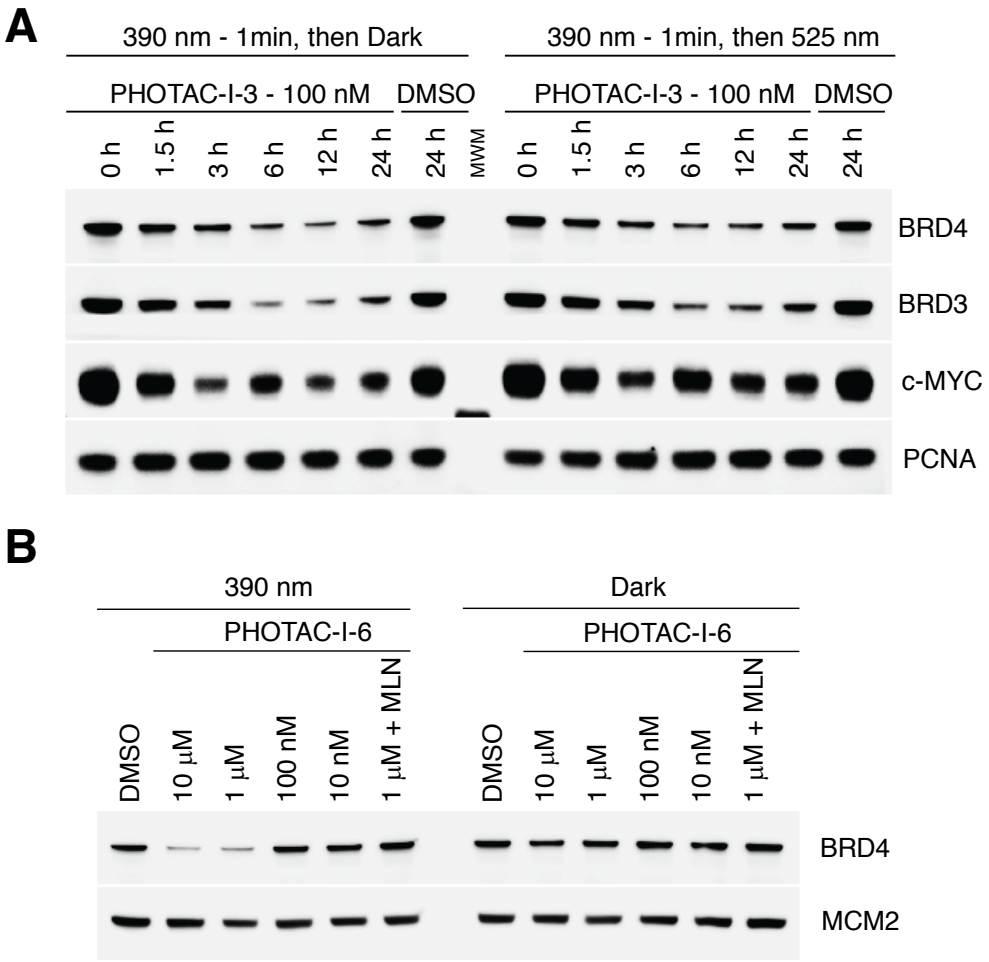



**Fig. S5**. A) Immunoblot of a rescue experiment demonstrating the reversibility of BRD3 and BRD4 degradation promoted by **PHOTAC-I-3** (100 nM) through thermal relaxation (left) or optical inactivation by 525 nm pulsed irradiation (right, 100 ms every 10 s). (MWM, molecular weight marker). B) Immunoblot analysis after treatment of RS4;11 cells with **PHOTAC-I-6** for 4 h at different concentrations. Cells were either irradiated with 100 ms pulses of 390 nm light every 10 s (left) or kept the dark (right).

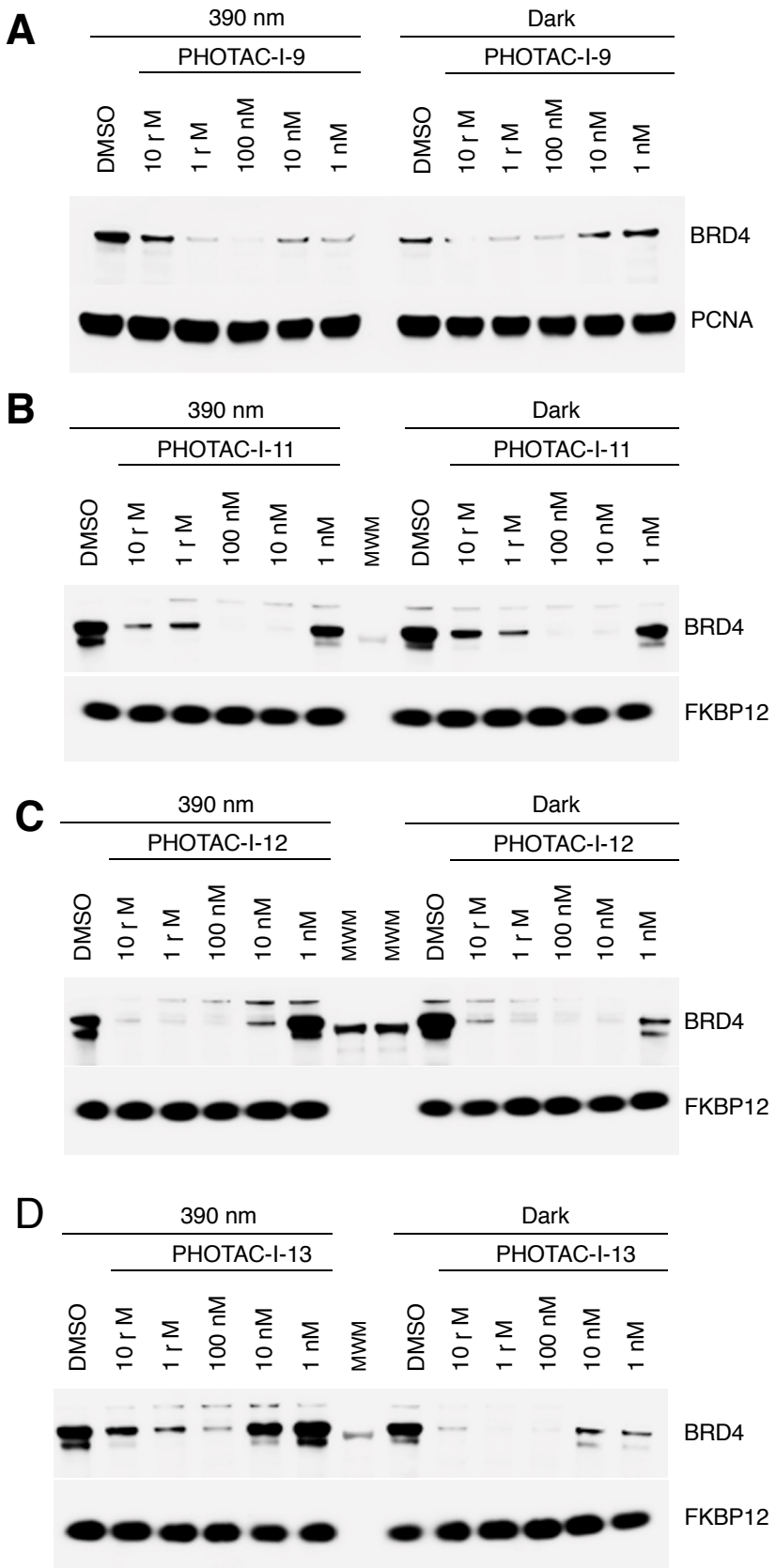



**Fig. S6**. Immunoblot analysis of BRD4 after treatment of RS4;11 cells with A) **PHOTAC-I-9**, B) **PHOTAC-I-11**, C) **PHOTAC-I-12** or D) **PHOTAC-I-13** for 4 h at different concentrations. Cells were either irradiated with 100 ms pulses of 390 nm light every 10 s (left) or kept the dark (right). (MWM, molecular weight marker).

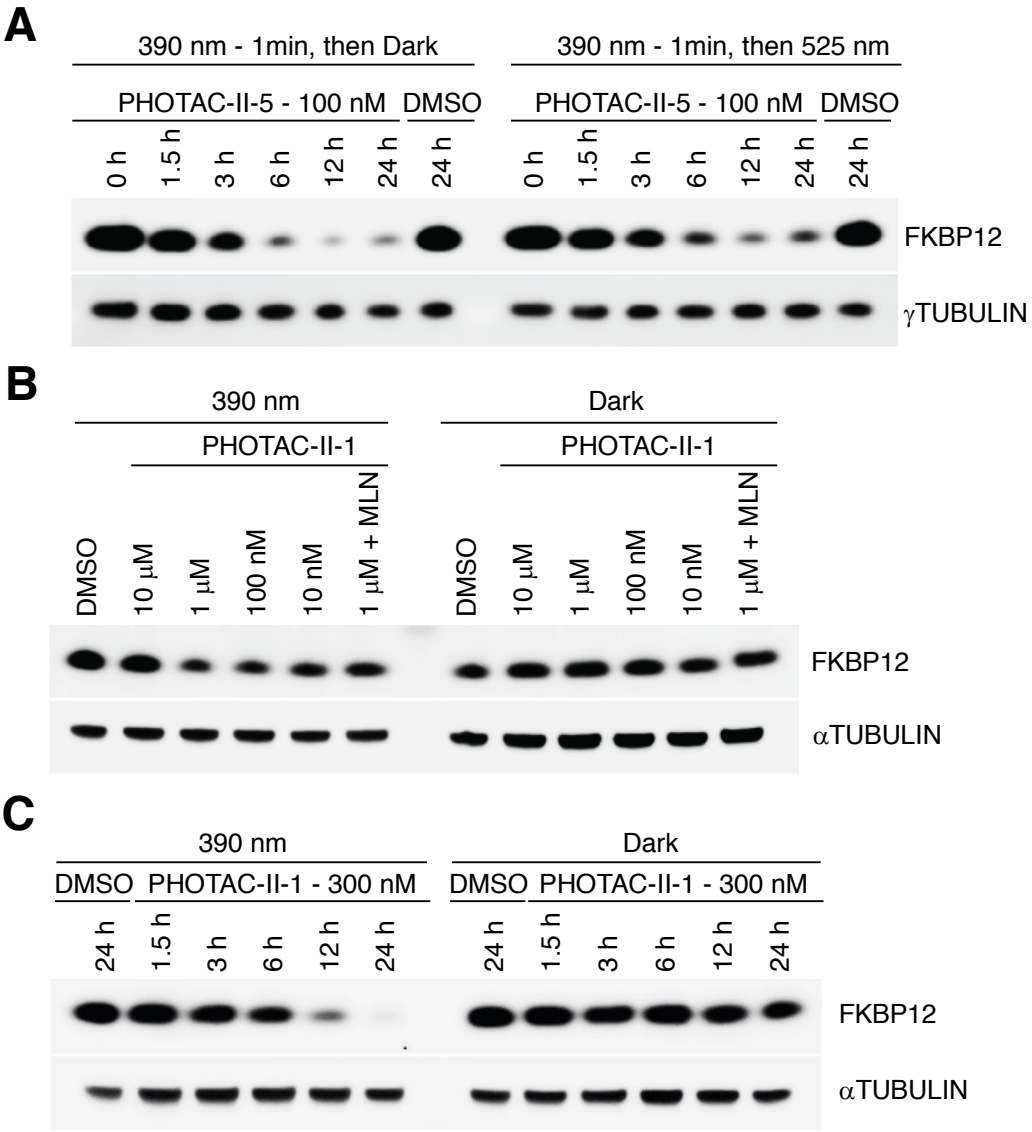



**Fig. S7**. A) Immunoblot of a rescue experiment demonstrating the reversibility of FKBP12 degradation promoted by **PHOTAC-II-5** through thermal relaxation (left) or optical inactivation by 525 nm pulsed irradiation (right, 100 ms every 10 s). B) Degradation of FKBP12. Immunoblot analysis of FKBP12 after treatment of RS4;11 cells with **PHOTAC-II-1** for 4 h at different concentrations. Cells were either irradiated with 100 ms pulses of 390 nm light every 10 s (left) or kept the dark (right). C) Time course of FKBP12 degradation visualized by immunoblotting. RS4;11 cells were treated with **PHOTAC-II-1** (300 nM) and collected at the indicated time points, showing

slow, but sustained FKBP12 degradation over time when irradiated with 390 nm light (left, 100 ms every 10 s), but not when kept in the dark (right).

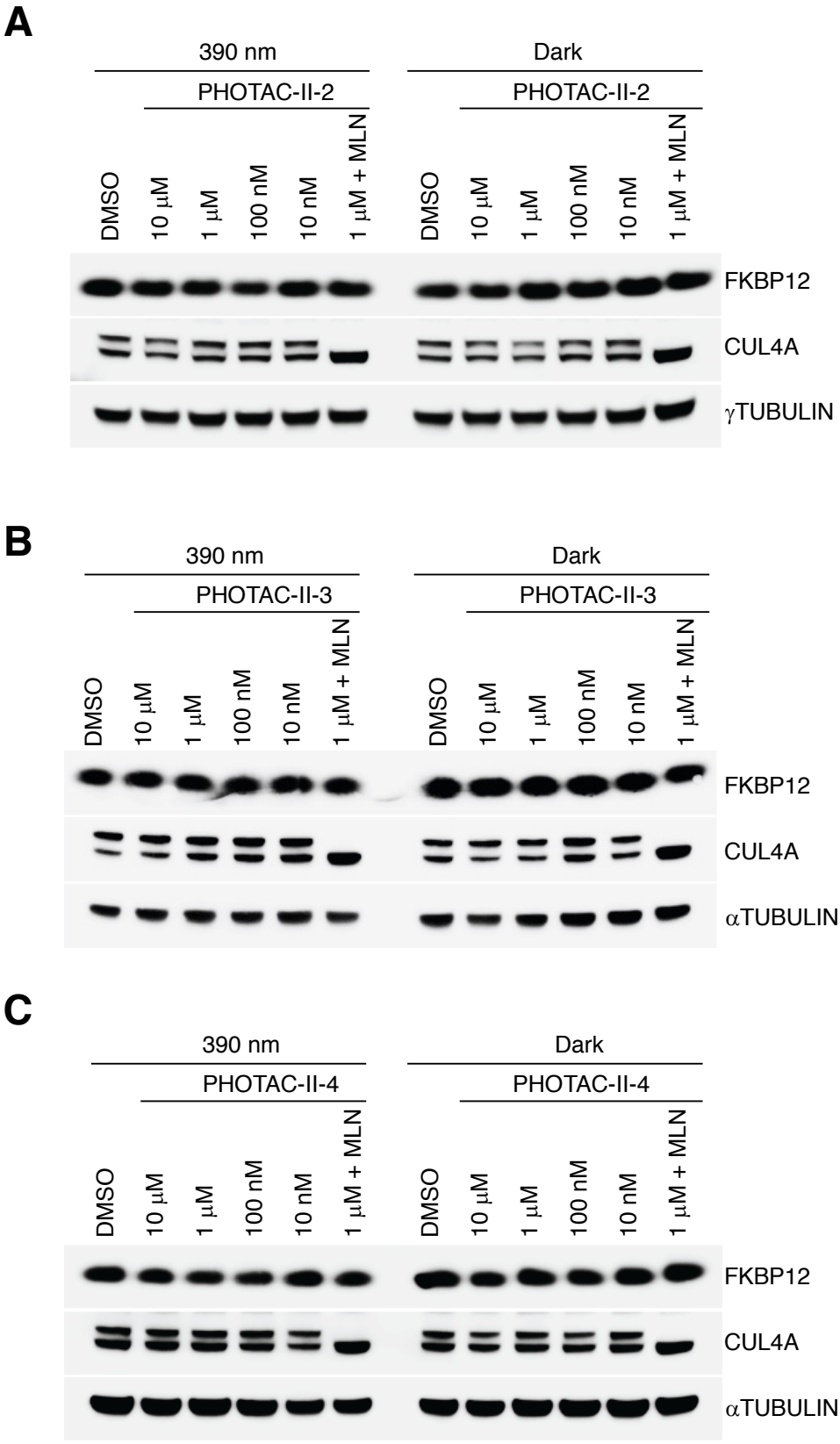



**Fig. S8**. Immunoblot analysis of FKBP12 after treatment of RS4;11 cells with A) **PHOTAC-II-2**, B) **PHOTAC-II-3** or C) **PHOTAC-II-4** for 4 h at different concentrations.

Cells were either irradiated with pulses of 390 nm light (right, 100 ms every 10 s) or kept the dark (left).

## General methods

The reagents and solvents used in this study were bought from the following chemical suppliers: ABCR, Acros Organics, Alfa Aesar, Ark Pharm, Combi-Blocks, Oakwood, OxChem, Sigma-Aldrich, Strem, Toronto Research Chemicals and were used as purchased.

Dry solvents used in reactions performed under inert atmosphere were obtained by passing the degassed solvents through activated alumina columns.

Column chromatography was carried out on silica gel (60 Å pore size, 40–63 μm, Merck KGaA) using a Teledyne Isco Combiflash EZprep flash purification system.

Thin-layer chromatography (TLC) was performed on glass plates precoated with silica gel (0.25 mm,60-Å pore size, Merck). TLC plates were visualized by exposure to UV light (254 and 366 nm).

NMR spectra were obtained on a Bruker Avance III HD 400 MHz spectrometer equipped with a CryoProbe$^{TM}$ operating at 400 MHz for $^{1}$H and 100 MHz for $^{13}$C spectra or on a Bruker AVIII-600 High Performance Digital NMR Spectrometer (600 MHz for $^{1}$H and 150 MHz for $^{13}$C spectra) with CPTCI-cryoprobehead.

Integration results and multiplets are reported as observed and denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), h (hextet), and m (multiplet) and as combinations thereof.

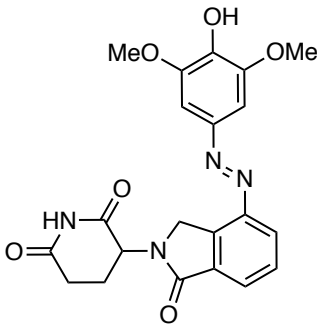
High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies 6224 Accurate-Mass time-of-flight spectrometer with either atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) ionization sources.

LCMS were measured on an Agilent Technologies 1260 II Infinity connected to an Agilent Technologies 6120 Quadrupole mass spectrometer with ESI ionization source. Elution was performed using a gradient from 5:95% to 100:0% MeCN:$H_2O$ with 0.1% formic acid over 5 min, if not indicated otherwise. Separated isomer spectra of azobenzenes were obtained by irradiation of the LCMS sample prior to injection.

UVVis spectrometry was performed on a Varian Cary 60 UV-Visible Spectrophotometer using disposable BRAND UV-Cuvette Disposable Spectrophotometer/Photometer Ultra-Micro Cuvettes, BrandTech (10 mm light path), an Agilent Technologies PCB 1500 Water Peltier system for temperature control and samples were irradiated with a Cairn Research Optoscan Monochromator with Optosource High Intensity Arc Lamp equipped with a 75 W UXL-S50A lamp from USHIO Inc. Japan and set to 15 nm full width at half maximum.

## Synthesis

### (*E*)-3-(4-((4-hydroxy-3,5-dimethoxyphenyl)diazenyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (2)




Lenalidomide (500 mg, 1.93 mmol, 1.0 eq.) was dissolved in 1 M HCl (50 mL). Concentrated aqueous $HBF_4$ (2 mL, 48 wt.%) was added to the mixture. After completely dissolving of the starting material, 2 M $NaNO_2$ (1.06 mL) was added to the solution at 0 °C. After stirring for 1 h the solution was added dropwise into a mixture of 2,6-Dimethoxyphenol (357 mg, 2.32 mmol, 1.2 eq.) in $H_2O$ (50 mL), MeOH (20 mL), $NaHCO_3$ (4.000 g, 47.62 mmol, 24.7 eq.) and $Na_2CO_3$ (5.000 g, 47.18 mmol, 24.5 eq.). Upon addition the solution turned from violet to strong red and was stirred for 1 additional hour at 0 °C. The reaction was extracted with EtOAc (7x 100 mL) and washed once with brine (1x 100 mL). The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0 → 10% MeOH) gave **2** (562.0 mg, 1.324 mmol, 69%) as a yellow solid.

**$R_f$** = 0.33 ($CH_2Cl_2$:MeOH, 19:1).

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.03 (s, 1H), 9.48 (s, 1H), 8.16 (d, *J* = 7.7 Hz, 1H), 7.87 (d, *J* = 7.4 Hz, 1H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.34 (s, 2H), 5.16 (dd, *J* = 13.1, 5.0 Hz, 1H), 4.82 (d, *J* = 19.0 Hz, 1H), 4.69 (d, *J* = 19.0 Hz, 1H), 3.90 (s, 6H), 2.95 (ddd, *J* = 17.5, 13.4, 5.2 Hz, 1H), 2.63 (d, *J* = 18.8 Hz, 1H), 2.55 (dd, *J* = 13.1, 4.5 Hz, 1H), 2.11 – 2.03 (m, 1H) ppm.

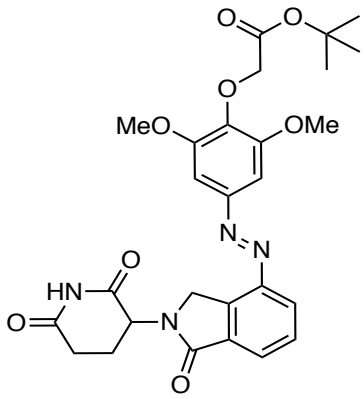
**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 173.39, 171.52, 167.76, 148.68, 147.05, 144.80, 140.86, 134.77, 134.21, 129.98, 128.37, 125.04, 101.69, 56.68, 52.30, 48.76, 31.76, 22.7 ppm.

**HRMS** (ESI):calcd. for $C_{21}H_{21}N_4O_6^+$: 425.1456 m/z $[M+H]^+$

found: 425.1458 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.90 min. 425 m/z $[M+H]^+$.

## *Tert*-butyl (*E*)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetate (S1)



To tert-butyl bromoacetate (234 mg, 1.20 mmol, 1 eq.) was added dry DMF (10 mL), **2** (509 mg, 1.20 mmol, 1 eq.) and $K_2CO_3$ (215 mg, 1.56 mmol, 1.3 eq.) at room temperature. After stirring for 2.5 hours, the mixture was diluted with EtOAc (100 mL), separated against $NaHCO_3$ (50 mL), extracted with EtOAc (3x 50 mL) and washed with 10% LiCl (3x 50 mL) and brine (2x 50 mL). The reaction was concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography (Hx/Ea gradient, 20 → 100% Ea, the product was eluted at 75%.) gave **S1** (501 mg, 0.93 mmol, 78%) as a yellow solid.

**R**$_f$ = 0.64 [EtOAc].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.03 (s, 1H), 8.22 (d, $J$ = 7.7 Hz, 1H), 7.92 (d, $J$ = 7.4 Hz, 1H), 7.80 (t, $J$ = 7.7 Hz, 1H), 7.34 (s, 2H), 5.16 (dd, $J$ = 13.2, 5.0 Hz, 1H), 4.83 (d, $J$ = 19.1 Hz, 1H), 4.70 (d, $J$ = 19.1 Hz, 1H), 4.60 (s, 2H), 3.90 (s, 6H), 2.93 (d, $J$ = 12.7 Hz, 1H), 2.63 (d, $J$ = 19.6 Hz, 1H), 2.58 – 2.52 (m, 1H), 2.11 – 2.03 (m, 1H), 1.43 (s, 9H) ppm.

**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 173.38, 171.48, 168.16, 167.64, 152.82, 148.14, 146.86, 139.71, 134.96, 134.28, 130.08, 128.97, 125.78, 101.26, 81.47, 69.69, 56.77, 52.31, 48.78 31.75, 28.20, 22.77 ppm.

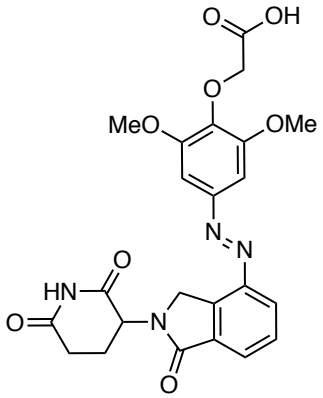
**HRMS** (ESI): calcd. for $C_{27}H_{31}N_4O_8^+$: 539.2137 m/z $[M+H]^+$

found: 539.2172 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.42 (*Z*). 539 m/z $[M+H]^+$.

$t_{ret}$ = 3.93 (*E*) min. 539 m/z $[M+H]^+$.

## (*E*)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetic acid (3)




**S2** (475 mg, 0.88 mmol, 1 eq.) was dissolved in $CH_2Cl_2$:TFA (1:1; 5 mL each). Upon TFA addition (5 mL) the solution turned from yellow to dark red. After 2 hours the reaction was concentrated under reduced pressure, turning from red to an orange solid. The reaction was dried under high vacuum for 48 h. **3** (557 mg, 0.878 mmol, 99%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

$\mathbf{R}_f$ = 0.5 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.03 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.80 (t, *J* = 7.7 Hz, 1H), 7.34 (s, 2H), 5.16 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.83 (d, *J* = 19.1 Hz, 1H), 4.70 (d, *J* = 19.1 Hz, 2H), 4.60 (s, 2H), 3.90 (s, 6H), 2.95 (ddd, *J* = 17.7, 13.6, 5.3 Hz, 1H), 2.68 – 2.53 (m, 2H), 2.11 – 2.03 (m, 1H). ppm.

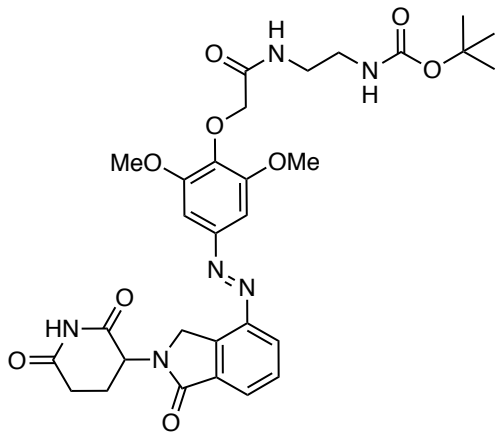
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.91, 171.01, 169.95, 167.17, 152.58, 147.80, 146.41, 139.24, 134.50, 133.82, 129.63, 128.52, 125.35, 100.87, 68.67, 56.33, 51.85, 48.30, 31.28, 22.30 ppm.

**HRMS** (ESI): calcd. for $C_{23}H_{23}N_4O_8^+$: 483.1510 m/z $[M+H]^+$

found: 483.1549 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.93 min. 483.1 m/z $[M+H]^+$.

## *tert*-butyl (*E*)-(2-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)ethyl)carbamate (S4)



**3** (19.0 mg, 0.039 mmol, 1.0 eq.) and HATU (26.9 mg, 0.083 mmol, 2.1 eq.) were dissolved in dry DMF (1 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,2-ethylendiamine (33.2 mg, 0.207 mmol, 5.3 eq.) and *i*-$Pr_2NEt$ (26.8 mg, 0.207 mmol, 5.3 eq., 36 $\mu$L) were added to the mixture and stirred for additional 12 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against $H_2O$ (20 mL), extracted with EtOAc (3x 20 mL) and washed with 10% LiCl (2x 20 mL) and brine (3x 30 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) gave **S4** (15.3 mg, 0.024 mmol, 62%) as a yellow solid.

**$R_f$** = 0.32 [Hx:EA, 2:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.21 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.99 (s, 1H), 7.89 (t, *J* = 5.6 Hz, 1H, br), 7.72 (t, *J* = 7.7 Hz, 1H), 7.22 (s, 2H), 5.27 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.86 (d, *J* = 17.9 Hz, 1H), 4.75 (s, 1H), 4.62 (s, 2H), 4.01 (s, 6H), 3.49 (q, *J* = 6.0 Hz, 2H), 3.32 (d, *J* = 5.7 Hz, 2H), 3.00 – 2.81 (m, 2H), 2.47 (dd, *J* = 13.1, 5.0 Hz, 1H), 2.27 (ddd, *J* = 10.3, 5.1, 2.6 Hz, 1H), 1.44 (d, *J* = 4.7 Hz, 9H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ =170.98, 170.37, 169.51, 168.57, 156.18, 152.79, 149.09, 146.93, 139.86, 134.12, 133.45, 130.05, 129.66, 126.44, 100.65, 79.66, 72.84, 56.60, 52.15, 48.29, 39.17, 31.76, 28.54, 23.62, 19.18 ppm.

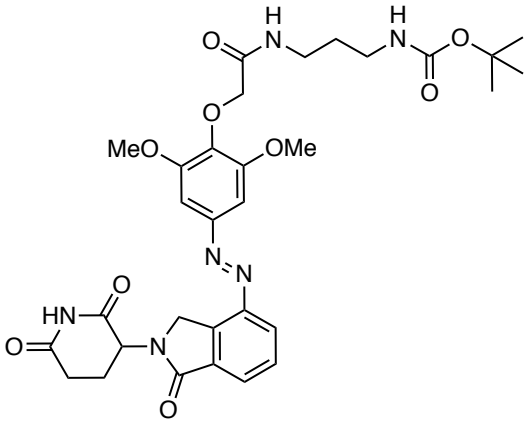
**HRMS** (ESI): calcd. for $C_{30}H_{37}N_6O_9^+$: 265.2617 m/z $[M+H]^+$

found: 265.2629 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.11 (*Z*) min. 623 m/z $[M–H]^–$.

$t_{ret}$ = 3.44 (*E*) min. 623 m/z $[M–H]^–$.

## *tert*-butyl (*E*)-(3-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)propyl)carbamate (S5)



**3** (60.0 mg, 0.095 mmol, 1.0 eq.) and HATU (46.0 mg, 0.142 mmol, 1.5 eq.) were dissolved in dry DMF (5 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,3-diaminopropane (65.9 mg, 0.378 mmol, 4.0 eq. 70 $\mu L$) and *i*-$Pr_2NEt$ (48.9 mg, 0.378 mmol, 4.0 eq., 66 $\mu L$) were added to the mixture and stirred for additional 12 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against $H_2O$/10% LiCl (1:1, 10 mL:10 mL), extracted with EtOAc (2x 20 mL) and washed with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) gave **S5** (51.5 mg, 0.081 mmol, 86%) as a yellow solid.

**$R_f$** = 0.19 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.21 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.99 (s, 1H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.70 (s, 1H), 7.22 (s, 2H), 5.27 (dd, *J* = 13.3, 5.1 Hz, 1H), 5.01 (s, 1H), 4.86 (d, *J* = 17.9 Hz, 1H), 4.72 (d, *J* = 17.9 Hz, 1H), 4.60 (s, 2H), 4.00 (s, 6H), 3.43 (q, *J* = 6.5 Hz, 2H), 3.18 (d, *J* = 6.3 Hz, 2H), 2.99 – 2.81 (m, 2H), 2.47 (dd, *J* = 13.1, 5.0 Hz, 1H), 2.27 (dtd, *J* = 12.8, 5.1, 2.5 Hz, 1H), 1.74 (p, *J* = 6.6 Hz, 1H), 1.43 (s, 9H) ppm.

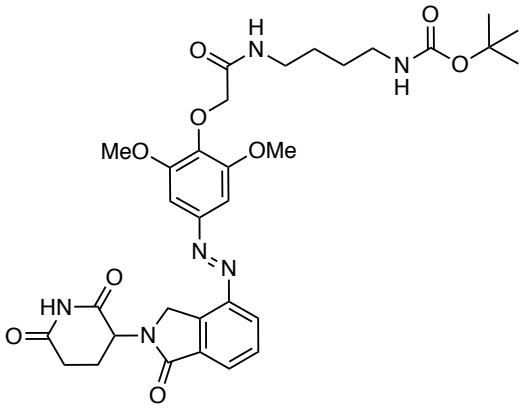
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 170.99, 170.05, 169.51, 168.57, 156.22, 152.82, 149.07, 146.93, 139.88, 134.11, 133.45, 130.06, 129.66, 126.42, 100.61, 79.22, 72.83, 56.54, 52.15, 48.29, 37.68, 36.24, 31.76, 30.30, 28.57, 23.63 ppm.

**HRMS** (ESI): calcd. for $C_{31}H_{39}N_6O_9^+$: 639.2773 m/z $[M+H]^+$

found: 639.2785 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.23 min (*Z*). 639.2 m/z $[M+H]^+$.

## *Tert*-butyl (*E*)-(4-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)butyl)carbamate (S3)



**3** (70.0 mg, 0.11 mmol, 1.0 eq.) and HATU (62.9 mg, 0.165 mmol, 1.5 eq.) were dissolved in dry DMF (1 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,4-diaminobutane (83.1 mg, 0.441 mmol, 4 eq.) and *i*-$Pr_2NEt$ (57 mg, 0.44 mmol, 4 eq., 74 $\mu$L) were added to the mixture and stirred for additional 14 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (3x 20 mL) and washed with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) gave **S3** (53.7 mg, 0.082 mmol, 75%) as a yellow solid.

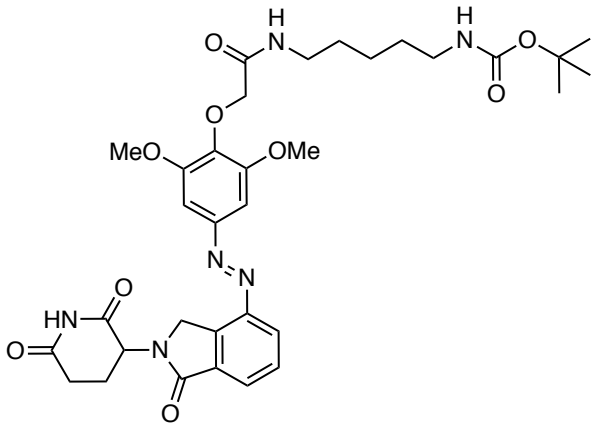
**$R_f$** = 0.42 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.21 (d, *J* = 7.7 Hz, 1H), 8.10 (s, 1H), 8.01 (d, *J* = 7.4 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 7.69 (s, 1H), 7.22 (s, 2H), 5.26 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.86 (d, *J* = 17.9 Hz, 1H), 4.72 (d, *J* = 17.9 Hz, 1H), 4.60 (s, 2H), 3.99 (s, 6H), 3.37 (q, *J* = 6.5 Hz, 2H), 3.17 (d, *J* = 5.9 Hz, 2H), 2.99 – 2.81 (m, 2H), 2.47 (dd, *J* = 13.1, 5.1 Hz, 1H), 2.27 (dtd, *J* = 12.8, 7.6, 6.3, 3.7 Hz, 1H), 1.66-1.53 (m, 4H), 1.43 (s, 9H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.04, 169.58, 169.55, 168.57, 156.14, 152.76, 149.02, 146.92, 139.91, 134.12, 133.45, 130.03, 129.64, 126.41, 100.60, 79.36, 72.89, 56.52, 52.15, 48.30, 40.39, 38.80, 31.76, 28.56, 27.73, 27.13, 23.62 ppm.

**HRMS** (APCI): calcd. for $C_{31}H_{41}N_6O_9^+$: 653.2929 m/z $[M+H]^+$
found: 653.2928.9606 m/z $[M+H]^+$.
**LCMS** (ESI): $t_{ret}$ = 3.31 min (*Z*) 675 m/z $[M+Na]^+$.
$t_{ret}$ = 3.63 min (*E*) 675 m/z $[M+Na]^+$.

## *Tert*-butyl (*E*)-(5-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)pentyl)carbamate (S6)



**3** (60.0 mg, 0.095 mmol, 1.0 eq.) and HATU (46.0 mg, 0.142 mmol, 1.5 eq.) were dissolved in dry DMF (5 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,4-diaminopentane (76.9 mg, 0.380 mmol, 4 eq.) and *i*-$Pr_2NEt$ (49.1 mg, 0.380 mmol, 4 eq., 66 $\mu L$) were added to the mixture and stirred for additional 12 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (3x 20 mL) and washed with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) yielded **S6** (54.2 mg, 0.081 mmol, 85%) as a yellow solid.

**$R_f$** = 0.42 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.21 (d, *J* = 7.8 Hz, 1H), 8.14 (d, *J* = 13.9 Hz, 1H), 8.01 (d, *J* = 7.5 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 7.66 (s, 1H), 7.22 (s, 2H), 5.26 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.86 (d, *J* = 17.9 Hz, 1H), 4.73 (d, *J* = 17.9 Hz, 1H), 4.61 (s, 2H), 3.99 (s, 6H), 3.35 (q, *J* = 6.8 Hz, 2H), 3.12 (d, *J* = 5.7 Hz, 3H), 3.00 – 2.81 (m, 2H), 2.47 (qd, *J* = 13.1, 5.0 Hz, 1H), 2.30 – 2.21 (m, 1H), 1.67-1.48 (m, 6H), 1.43 (s, 9H) ppm.

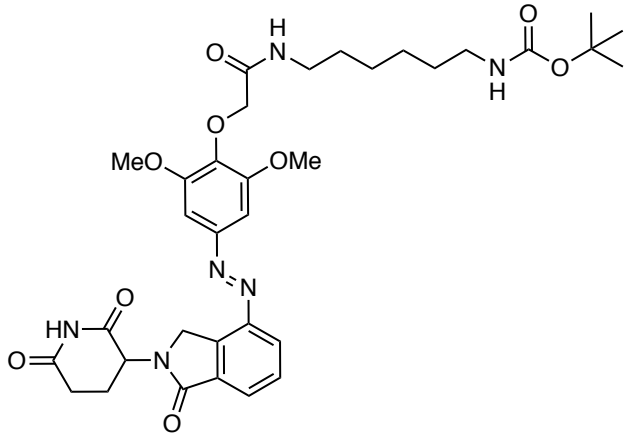
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.11, 171.04, 169.54, 168.57, 156.14, 152.77, 149.02, 146.93, 139.93, 134.10, 133.44, 130.07, 129.64, 126.39, 100.60, 79.29, 72.90, 56.51, 52.15, 48.31, 40.52, 38.96, 31.76, 29.92, 29.45, 28.56, 24.23, 23.62 ppm.

**HRMS** (ESI): calcd. for $C_{33}H_{42}N_6NaO_9^+$: 689.2905 m/z $[M+Na]^+$

found: 689.2935 m/z $[M+Na]^+$

**LCMS** (ESI): $t_{ret}$ = 3.43 min (*Z*) 665 m/z $[M–H]^–$.

## *Tert*-butyl (*E*)-(6-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)hexyl)carbamate (S7)



**3** (60.0 mg, 0.095 mmol, 1.0 eq.) and HATU (46.0 mg, 0.142 mmol, 1.5 eq.) were dissolved in dry DMF (5 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,4-diaminohexane (81.8 mg, 0.378 mmol, 4 eq., 0.09 mL) and *i*-$Pr_2NEt$ (48.9 mg, 0.378 mmol, 4 eq., 66 $\mu L$) were added to the mixture and stirred for additional 13 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against 5 % LiCl (20 mL), extracted with EtOAc (3x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) gave **S7** (56.7 mg, 0.083 mmol, 88%) as a yellow solid.

$\mathbf{R}_f$ = 0.25 [$CH_2Cl_2$: MeOH, 19:1].

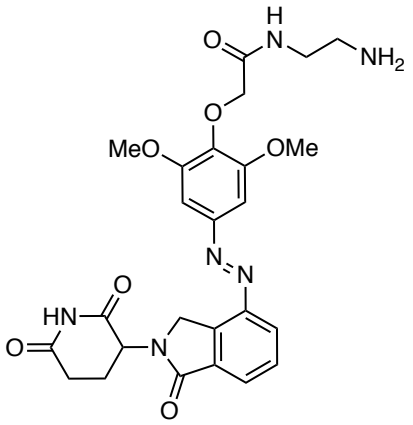
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.20 (d, *J* = 7.8 Hz, 2H), 8.01 (d, *J* = 7.5 Hz, 1H), 7.71 (t, *J* = 7.7 Hz, 1H), 7.64 (t, *J* = 5.3 Hz, 1H), 7.21 (s, 2H), 5.26 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.86 (d, *J* = 17.9 Hz, 1H), 4.72 (d, *J* = 17.9 Hz, 1H), 4.61 (s, 2H), 3.99 (s, 6H), 3.34 (q, *J* = 6.6 Hz, 2H), 3.10 (d, *J* = 6.1 Hz, 2H), 2.99 – 2.80 (m, 2H), 2.46 (qd, *J* = 13.1, 5.0 Hz, 1H), 2.26 (dtd, *J* = 12.8, 5.1, 2.6 Hz, 1H), 1.58 (p, *J* = 7.1 Hz, 2H), 1.52 – 1.46 (m, 2H), 1.43 (s, 9H), 1.40 – 1.34 (m, 4H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.16, 169.64, 169.48, 168.56, 156.14, 152.76, 148.99, 146.92, 139.93, 134.08, 133.44, 130.08, 129.63, 126.38, 100.59, 79.25, 72.89, 56.50, 52.13, 48.29, 40.64, 39.02, 31.76, 30.17, 29.67, 28.57, 26.74, 26.63, 23.63 ppm.

**HRMS** (APCI):calcd. for $C_{34}H_{45}N_6O_9^+$: 681.3243 m/z $[M+H]^+$

found: 681.3236 m/z $[M+H]^+$.

## (*E*)-(2-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)ethyl)carbamic acid (S8)



**S4** (15.3 mg, 0.024 mmol, 1 eq.) was dissolved in TFA:$CH_2Cl_2$ (1 mL:0.5 mL) and stirred at room temperature. After 2 h, the mixture was diluted with $CH_2Cl_2$, concentrated under reduced pressure and dried on high vacuum overnight. **S8** (15.6 mg, 0.024 mmol, >99%.) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

$\mathbf{R}_f$ = 0.18 [$CH_2Cl_2$:MeOH, 7:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.04 (s, 1H), 8.23 (dd, *J* = 9.3, 7.1 Hz, 2H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 3H), 7.37 (s, 2H), 5.17 (dd, *J* = 13.3, 5.0 Hz, 1H), 4.82 (d, *J* = 19.1 Hz, 1H), 4.69 (d, *J* = 19.1 Hz, 1H), 4.44 (s, 2H), 3.94 (s, 6H), 3.45 (q, *J* = 6.3 Hz, 2H), 3.02 – 2.88 (m, 4H), 2.69 – 2.54 (m, 2H) ppm.

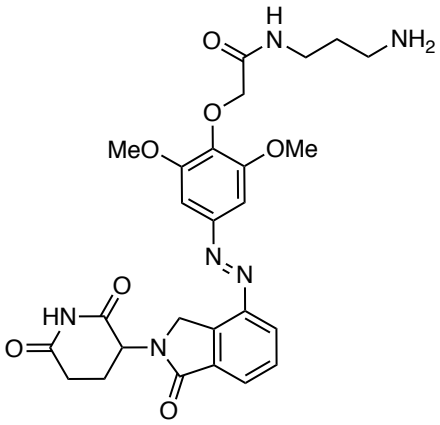
**$^{13}$C NMR** (100 MHz, DMSO-$d_6$) δ = 172.92, 171.02, 169.13, 167.14, 152.77, 148.38, 146.35, 139.08, 134.49, 133.84, 129.68, 128.67, 125.54, 100.57, 71.67, 56.36, 51.83, 48.26, 38.80, 36.23, 31.28, 30.70 ppm.

**HRMS** (ESI): calcd. for $C_{25}H_{29}N_6O_7^+$: 525.2092 m/z $[M+H]^+$

found: 525.2096 m/z $[M+H]^+$

**LCMS** (ESI): $t_{ret}$ = 2.39 min. 525 m/z $[M+H]^+$.

## (*E*)-*N*-(3-aminopropyl)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamide (S9)



**S5** (41.5 mg, 0.061 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1, 1 mL:1 mL) and stirred for 2 h at room temperature. The reaction was diluted with MeOH and concentrated under reduced pressure. The mixture was triturated with $CH_2Cl_2$ and dried on high vacuum overnight. **S9** (40 mg, 0.061 mmol, >99%) was obtained as a yellow solid with traces of residual TFA.

**$R_f$** = 0.19 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.04 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 8.16 (t, *J* = 5.9 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.73 (s, 2H), 7.36 (s, 2H), 5.17 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.82 (d, *J* = 19.1 Hz, 1H), 4.69 (d, *J* = 19.1 Hz, 1H), 4.43 (s, 2H), 3.94 (s, 6H), 3.27 (q, *J* = 6.5 Hz, 2H), 2.95 (ddd, *J* = 17.9, 13.8, 5.2 Hz, 1H), 2.82 (dt, *J* = 12.9, 6.2 Hz, 2H), 2.69 – 2.53 (m, 2H), 2.11 – 2.03 (m, 1H), 1.77 (p, *J* = 6.9 Hz, 2H) ppm.

**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.92, 171.02, 168.55, 167.15, 152.72, 148.34, 146.35, 139.15, 134.48, 133.84, 129.68, 128.70, 125.54, 100.60, 71.72, 56.39, 51.83, 48.27, 36.75, 35.36, 31.28, 27.39, 22.34 ppm.

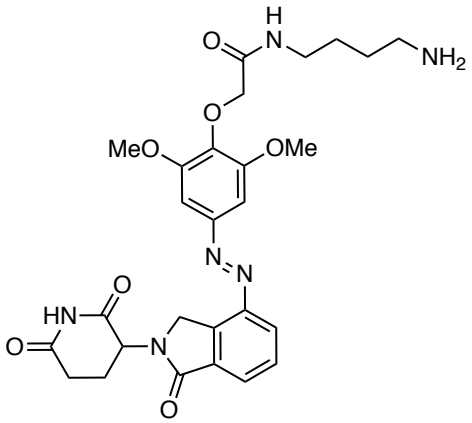
**HRMS** (ESI): calcd. for $C_{26}H_{31}N_6O_7^+$: 539.2249 m/z $[M+H]^+$

found: 539.2312 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.34 min (*Z*) 539 m/z $[M+H]^+$.

$t_{ret}$ = 2.45 min (*E*) 539 m/z $[M+H]^+$.

## (*E*)-*N*-(4-aminobutyl)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamide (4)




**S3** (39.5 mg, 0.057 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1; 1mL:1mL) and stirred for 2 h at room temperature. The reaction was diluted with $CH_2Cl_2$ and concentrated under reduced pressure. The reaction was triturated with $Et_2O$ and dried on high vacuum overnight. **4** (38 mg, 0.057 mmol, >99%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

**R**$_f$ = 0.4 [$CH_2Cl_2$:MeOH, 8:2].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.04 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 8.02 (t, *J* = 5.8 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.70 (s, 2H), 7.37 (s, 2H), 5.17 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.82 (d, *J* = 19.1 Hz, 1H), 4.69 (d, *J* = 19.1 Hz, 1H), 4.42 (s, 2H), 3.95 (s, 6H), 3.25 – 3.19 (m, 2H), 2.95 (ddd, *J* = 17.9, 13.7, 5.3 Hz, 1H), 2.86 – 2.79 (m, 2H), 2.67 – 2.53 (m, 2H), 2.11 – 2.03 (m, 1H), 1.58 – 1.51 (m, 4H) ppm.

**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.92, 171.02, 168.11, 167.15, 152.66, 148.34, 146.35, 139.20, 134.48, 133.84, 129.68, 128.70, 125.54, 100.59, 71.83, 56.39, 51.83, 48.28, 38.58, 37.58, 31.28, 26.13, 24.46, 22.33 ppm.

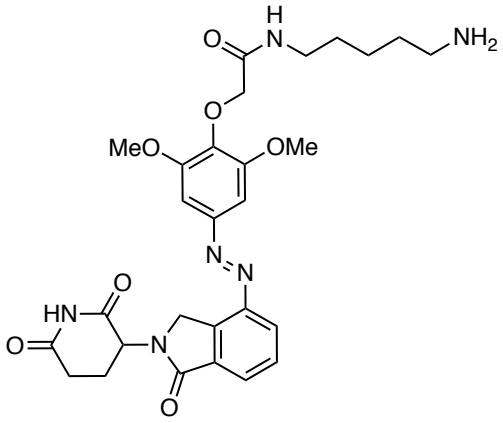
**HRMS** (APCI): calcd. for $C_{27}H_{33}N_6O_9^+$: 553.2405 m/z $[M+H]^+$

found: 553.2384 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ =2.23 min (*Z*). 553 m/z $[M+H]^+$.

$t_{ret}$ =2.49 min (*E*). 553 m/z $[M+H]^+$.

## (*E*)-*N*-(5-aminopentyl)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamide (S10)




**S6** (40.5 mg, 0.057 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1; 1mL:1mL) and stirred for 2 h at room temperature. The reaction was diluted with $CH_2Cl_2$ and concentrated under reduced pressure. The reaction was triturated with $Et_2O$ and dried on high vacuum overnight. **S10** (38.8 mg, 0.057 mmol, >99%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

**$R_f$** = 0.03 [$CH_2Cl_2$:MeOH, 8:2].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.03 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.97 (d, *J* = 5.7 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.68 (s, 2H), 7.36 (s, 2H), 5.17 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.82 (d, *J* = 19.1 Hz, 1H), 4.69 (d, *J* = 19.1 Hz, 1H), 4.42 (s, 2H), 3.95 (s, 6H), 3.19 (q, *J* = 6.7 Hz, 2H), 2.95 (ddd, *J* = 17.9, 13.7, 5.3 Hz, 1H), 2.79 (h, *J* = 5.8 Hz, 2H), 2.68 – 2.53 (m, 2H), 2.11 – 2.03 (m, 1H), 1.53 (dp, *J* = 22.1, 7.5 Hz, 4H), 1.33 (p, *J* = 7.5, 6.9 Hz, 2H) ppm.

**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.92, 171.02, 168.00, 167.15, 152.64, 148.31, 146.35, 139.24, 134.49, 133.84, 129.67, 128.68, 125.53, 100.61, 71.85, 56.38, 51.83, 48.28, 38.76, 37.92, 31.29, 28.59, 26.67, 23.12, 22.33 ppm.

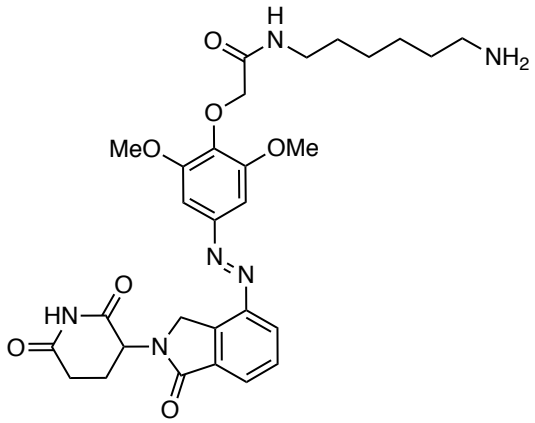
**HRMS** (ESI): calcd. for $C_{28}H_{35}N_6O_7^+$: 567.2562 m/z $[M+H]^+$

found: 567.2656 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.27 min (*Z*) 567 m/z $[M+H]^+$.

$t_{ret}$ = 2.53 min (*E*) 567 m/z $[M+H]^+$.

## (*E*)-*N*-(6-aminohexyl)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamide (S11)



**S7** (51.3 mg, 0.071 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1; 1mL:1mL) and stirred for 2 h at room temperature. The reaction was diluted with $CH_2Cl_2$ and concentrated under reduced pressure. The reaction was triturated with $Et_2O$ and dried on high vacuum overnight. **S11** (49.3 mg, 0.071 mmol, >99%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

**$R_f$** = 0.19 [$CH_2Cl_2$: MeOH, 9:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.03 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.94 (dd, *J* = 11.5, 6.5 Hz, 2H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.66 (s, 2H), 7.36 (s, 2H), 5.16 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.82 (d, *J* = 19.1 Hz, 1H), 4.69 (d, *J* = 19.1 Hz, 1H), 4.42 (s, 2H), 3.94 (s, 6H), 3.19 (q, *J* = 6.6 Hz, 2H), 2.95 (ddd, *J* = 17.9, 13.8, 5.3 Hz, 1H), 2.78 (h, *J* = 5.8 Hz, 2H), 2.63 (d, *J* = 18.1 Hz, 1H), 2.56 (dd, *J* = 13.1, 4.4 Hz, 1H), 2.06 (d, *J* = 5.4 Hz, 1H), 1.51 (dp, *J* = 13.7, 6.9 Hz, 4H), 1.37 – 1.25 (m, 4H) ppm.

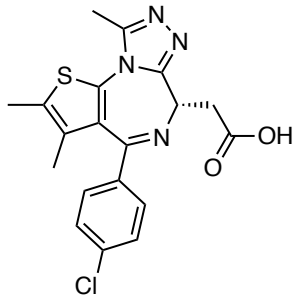
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.95, 171.04, 167.99, 167.18, 152.64, 148.32, 146.37, 139.28, 134.51, 133.85, 129.70, 128.71, 125.56, 100.63, 71.89, 56.40, 51.86, 48.31, 38.82, 38.09, 31.30, 28.95, 26.98, 25.84, 25.48, 22.35 ppm.

**HRMS** (APCI): calcd. for $C_{29}H_{37}N_6O_7^+$: 581.2718 m/z $[M+H]^+$

found: 581.2717 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.66 min. 581 m/z $[M+H]^+$.

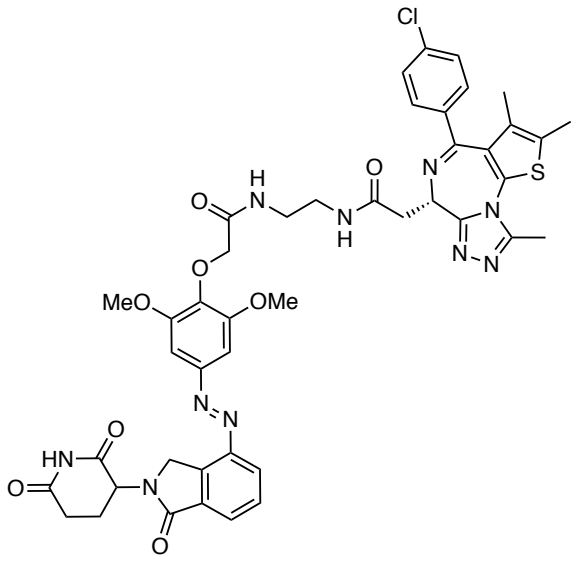
## (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetic acid ((+)-JQ1 free acid, 5)




As previously described,[1] (+)-JQ1 (9 mg, 0.02 mmol, 1 eq.) was dissolved in formic acid (0.5 mL) and stirred for 3 days at room temperature. The reaction was concentrated under reduced pressure and was dried on high vacuum overnight. **(+)-JQ1 free acid**, **5** (8.0 mg, 0.02 mmol, >99%) was obtained as yellow solid and used without further purification.

**LCMS** (ESI): $t_{ret}$ = 3.35 min. 401, 402 m/z $[M+H]^+$.

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(2-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)ethyl)acetamide (PHOTAC-I-1)**




**S8** (15.3 mg, 0.024 mmol, 1 eq.) and HATU (11.7 mg, 0.036 mmol, 1.5 eq.) were added to a round bottom flask under nitrogen. **(+)-JQ1 free acid** was taken up in dry DMF (1 mL) and added to the mixture. After addition of *i*-$Pr_2NEt$ (0.025 mL, 0.144 mmol, 6 eq.) the reaction was stirred for 15 h at room temperature. Then the mixture was diluted with EtOAc (20 mL), separated against $H_2O$ (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-1** (8.6 mg, 0.009 mmol, 38%) as a yellow solid.

$\mathbf{R}_f$ = 0.19 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Methanol-$d_4$) δ = 8.20 (d, *J* = 7.8 Hz, 1H), 7.90 (dd, *J* = 7.5, 4.1 Hz, 1H), 7.74 (td, *J* = 7.7, 2.8 Hz, 1H), 7.40 (dd, *J* = 8.7, 3.0 Hz, 2H), 7.36 – 7.28 (m, 4H), 5.23 – 5.09 (m, 1H), 4.83 (s, 2H), 4.60 – 4.47 (m, 3H), 3.96 (d, *J* = 2.8 Hz, 6H), 3.58 – 3.33 (m, 5H), 3.30 – 3.19 (m, 1H), 2.92 (ddt, *J* = 17.9, 13.4, 4.8 Hz, 1H), 2.79 (ddt, *J* = 17.8, 5.1, 2.8 Hz, 1H), 2.70 – 2.61 (m, 1H), 2.58 (d, *J* = 19.6 Hz, 3H), 2.40 (d, *J* = 2.2 Hz, 3H), 2.25 – 2.14 (m, 1H), 1.66 (s, 3H).

**$^{13}$C NMR** (100 MHz, MeOD) δ = 174.68, 173.16, 172.58, 172.18, 170.35, 166.19, 156.89, 154.11, 152.07, 150.18, 148.12, 141.02, 138.01, 137.92, 135.56, 134.82, 133.42, 133.14, 132.07, 131.92, 131.57, 131.30, 130.69, 129.74, 126.55, 101.71, 73.23, 56.97, 54.99, 53.91, 50.68, 40.04, 39.91, 38.72, 32.42, 24.03, 14.39, 12.93, 11.58 ppm.

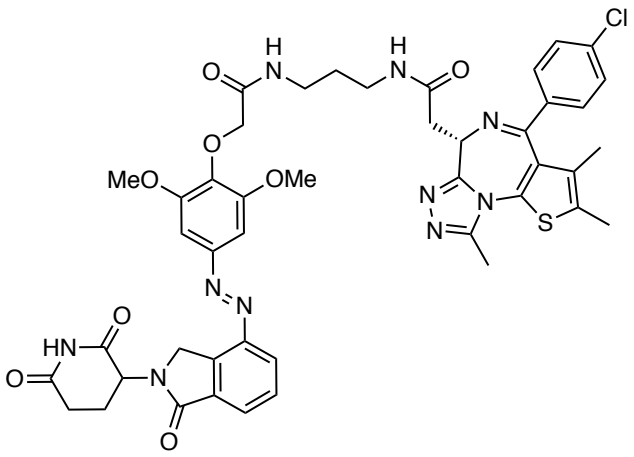
**HRMS** (ESI): calcd. for $C_{44}H_{44}ClN_{10}O_8S^+$: 907.2747 m/z $[M+H]^+$

found: 907.2790 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.51 min (*Z*). 907 m/z $[M+H]^+$.

$t_{ret}$ = 3.67 min (*E*). 907 m/z $[M+H]^+$.

## 2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(3-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)propyl)acetamide (PHOTAC-I-2)



Into a round bottom flask with dry **(+)-JQ1 free acid** (7.2 mg, 0.018 mmol, 1 eq.) were added **S9** (23.5 mg, 0.036 mmol, 2 eq.) and HATU (11.7 mg, 0.036 mmol, 1.5 eq.) under nitrogen. The reaction was dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (17 mg, 0.13 mmol, 7.2 eq., 0.023 mL) the reaction was stirred for 14 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-2** (15.1 mg, 0.016 mmol, 91%) as a yellow solid.

**R**$_f$ = 0.31 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.52 (s, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 7.5 Hz, 1H), 7.80 (t, *J* = 5.8 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 2.9 Hz, 2H), 7.07 (s, 1H), 5.28 – 5.19 (m, 1H), 4.85 (d, *J* = 18.0 Hz, 1H), 4.71 (dd, *J* = 18.1, 3.2 Hz, 1H), 4.63 (d, *J* = 6.8 Hz, 1H), 4.60 (s, 2H), 3.97 (s, 6H), 3.55 (dd, *J* = 14.4, 7.6 Hz, 1H), 3.42 (q, *J* = 6.4 Hz, 2H), 3.35 (q, *J* = 7.6, 6.9 Hz, 3H), 2.95 – 2.77 (m, 2H), 2.64 (s, 3H), 2.46 (dt, *J* = 12.9, 4.5 Hz, 1H), 2.38 (s, 3H), 2.28 – 2.18 (m, 1H), 1.79 (p, *J* = 6.4 Hz, 2H), 1.66 (s, 3H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.37, 170.84, 169.95, 169.72, 168.57, 164.10, 155.77, 152.78, 150.07, 148.99, 146.90, 139.86, 136.92, 136.69, 134.15, 133.46, 132.26, 131.02,

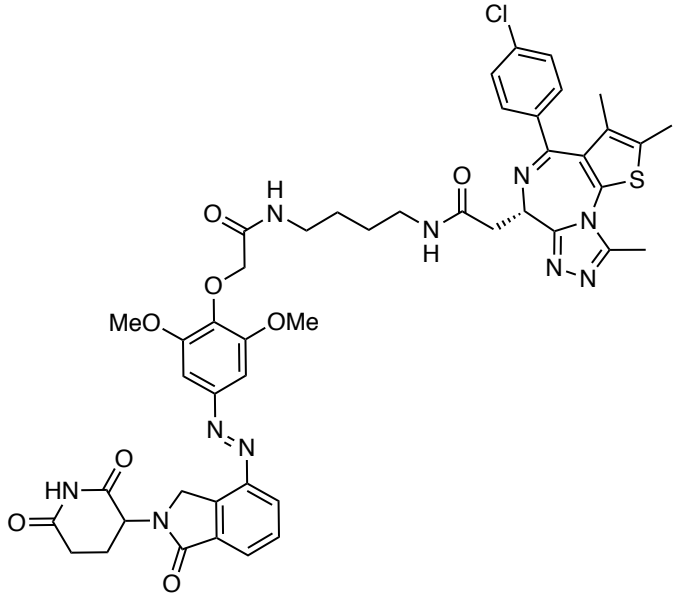
130.98, 130.58, 130.02, 129.96, 129.57, 128.83, 126.29, 100.64, 72.79, 56.55, 54.55, 52.12, 48.37, 39.39, 36.91, 36.45, 31.73, 29.76, 23.56, 14.52, 13.22, 11.94 ppm.

**HRMS** (ESI): calcd. for $C_{45}H_{45}ClN_{10}NaO_8S^+$: 943.2723 m/z $[M+Na]^+$

found: 943.2738 m/z $[M+Na]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.71 min. 920 m/z $[M–H]^–$.

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)butyl)acetamide (PHOTAC-I-3)**




Into a round bottom flask with dry **(+)-JQ1 free acid** (7.2 mg, 0.018 mmol, 1 eq.) were added **4** (26.6 mg, 0.04 mmol, 2 eq.) and HATU (11.7 mg, 0.036 mmol, 1.8 eq.) under nitrogen atmosphere. The solids were dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (18.6 mg, 0.144 mmol, 7.2 eq., 0.025 mL) the reaction was stirred for 16 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL), washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-3** (15.6 mg, 0.017 mmol, 85%) as a yellow solid.

**$R_f$** = 0.30 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.80 (d, *J* = 67.2 Hz, 1H), 8.15 (t, *J* = 8.7 Hz, 1H), 8.00 – 7.92 (m, 1H), 7.75 (d, *J* = 4.9 Hz, 1H), 7.67 (q, *J* = 7.2 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 6.85 (dt, *J* = 10.4, 5.2 Hz, 1H), 5.21 (dd, *J* = 9.2, 3.9 Hz, 1H), 4.84 (d, *J* = 18.0 Hz, 1H), 4.69 (d, *J* = 18.0 Hz, 1H), 4.62 (d, *J* = 6.8 Hz, 3H), 3.97 (d, *J* = 2.5 Hz, 6H), 3.53 (dd, *J* = 12.5, 7.5 Hz, 1H), 3.42 – 3.23 (m, 5H), 2.90 – 2.72 (m, 2H),

2.64 (d, *J* = 5.4 Hz, 3H), 2.50 – 2.41 (m, 1H), 2.38 (s, 3H), 2.24 – 2.13 (m, 1H), 1.65 (s, 4H), 1.61 (s, 3H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.44, 170.62, 169.90, 169.64, 168.53, 164.21, 155.74, 152.69, 150.12, 148.91, 146.83, 139.88, 136.96, 136.68, 134.18, 133.45, 132.24, 131.04, 131.01, 130.58, 130.01, 129.94, 129.52, 128.85, 126.20, 100.64, 72.85, 56.54, 54.59, 52.10, 48.35, 39.46, 39.37, 38.78, 31.71, 27.17, 27.04, 23.54, 14.51, 13.21, 11.91 ppm.

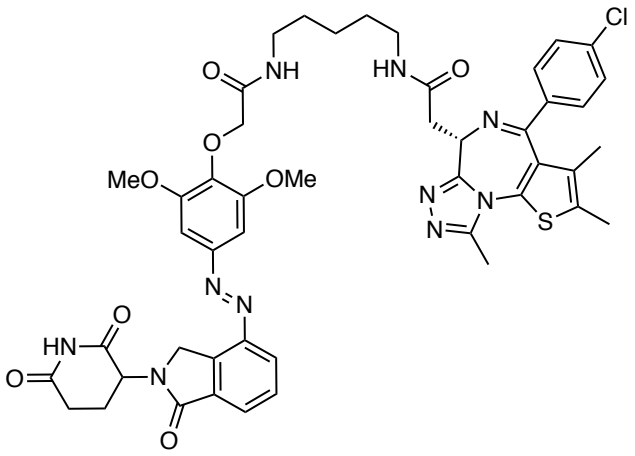
**HRMS** (ESI): calcd. for $C_{46}H_{48}ClN_{10}O_8S^+$: 935.3060 m/z $[M+H]^+$

found: 973.2597 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.55 min (*Z*). 935 m/z $[M+H]^+$.

$t_{ret}$ = 3.74 min (*E*). 935 m/z $[M+H]^+$.

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(5-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)pentyl)acetamide (PHOTAC-I-4)**




Into a round bottom flask with dry **(+)-JQ1 free acid** (7.2 mg, 0.018 mmol, 1 eq.) were added **S10** (24.4 mg, 0.036 mmol, 2 eq.) and HATU (10.5 mg, 0.032 mmol, 1.8 eq.) under nitrogen atmosphere. The solids were dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (16.7 mg, 0.129 mmol, 7.2 eq., 0.023 mL) the reaction was stirred for 15 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL), washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-4** (15.4 mg, 0.016 mmol, 89%) as a yellow solid.

**R**$_f$ = 0.31 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.66 (d, *J* = 14.5 Hz, 1H), 8.18 (dd, *J* = 7.6, 3.5 Hz, 1H), 7.99 (d, *J* = 7.5 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 7.7 Hz, 2H), 7.22 (d, *J* = 5.8 Hz, 2H), 6.79 – 6.68 (m, 1H), 5.23 (dt, *J* = 12.2, 5.2 Hz, 1H), 4.87 (s, 1H), 4.74 (s, 1H), 4.60 (d, *J* = 11.6 Hz, 3H), 3.98 (s, 6H), 3.55 (dt, *J* = 10.9, 5.4 Hz, 1H), 3.31 (dt, *J* = 14.7, 5.9 Hz, 5H), 2.95 – 2.77 (m, 2H), 2.65 (s, 3H), 2.46 (d, *J* = 12.7 Hz, 1H), 2.39 (s, 3H), 2.31 – 2.14 (m, 1H), 1.66 (s, 3H), 1.57 (q, *J* = 6.9 Hz, 4H), 1.48 – 1.34 (m, 2H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.39, 170.62, 169.82, 169.59, 168.57, 164.15, 155.73, 152.71, 150.09, 148.96, 146.90, 139.89, 136.97, 136.67, 134.17, 133.45, 132.23, 131.05,

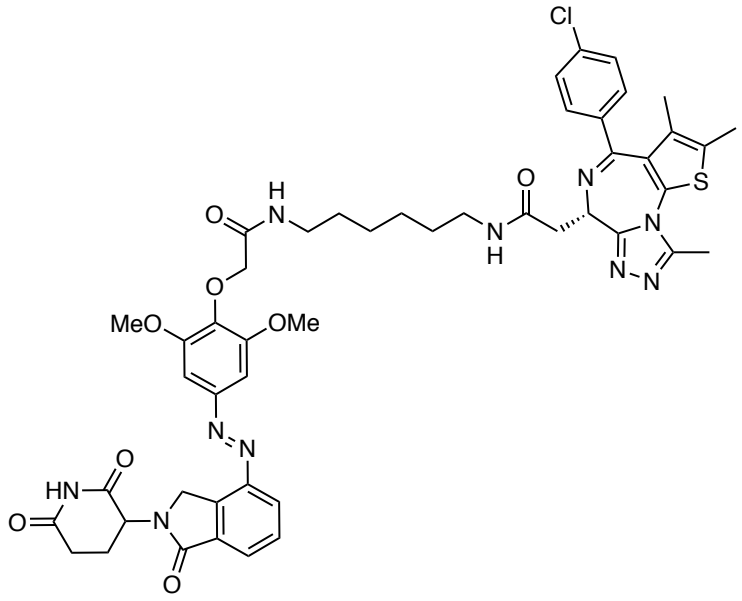
131.04, 130.59, 130.09, 129.94, 129.56, 128.86, 126.29, 100.65, 72.86, 56.53, 54.65, 52.11, 48.35, 39.58, 39.51, 38.92, 31.73, 29.37, 29.26, 24.30, 23.57, 14.51, 13.23, 11.92 ppm.

**HRMS** (ESI): calcd. for $C_{47}H_{49}ClKN_{10}O_8S^+$: 987.2776 m/z $[M+K]^+$

found: 987.2755 m/z $[M+K]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.83 min 949 m/z $[M+H]^+$.

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(6-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)hexyl)acetamide (PHOTAC-I-5)**




Into a round bottom flask with dry **(+)-JQ1 free acid** (7.6 mg, 0.019 mmol, 1 eq.) were added **S11** (26.3 mg, 0.038 mmol, 2 eq.) and HATU (11.1 mg, 0.034 mmol, 1.8 eq.) under nitrogen atmosphere. The solids were dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (17.6 mg, 0.137 mmol, 7.2 eq., 0.024 mL) the reaction was stirred for 16 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL), washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-5** (16.4 mg, 0.017 mmol, 90%) as a yellow solid.

**R**$_f$ = 0.31 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.78 (d, *J* = 29.2 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 1H), 7.69 (t, *J* = 7.5 Hz, 2H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.26 (s, 1H), 7.22 (s, 1H), 6.72 – 6.60 (m, 1H), 5.22 (td, *J* = 13.8, 4.9 Hz, 1H), 4.93 – 4.79 (m, 1H), 4.70 (dd, *J* = 18.0, 5.8 Hz, 1H), 4.62 (s, 3H), 3.98 (d, *J* = 3.6 Hz, 6H), 3.54 (td, *J* = 7.7, 3.8 Hz, 1H), 3.38 – 3.21 (m, 5H), 2.96 – 2.74 (m, 2H), 2.64 (d, *J* = 10.7 Hz, 3H), 2.52 – 2.42 (m, 1H), 2.38 (s, 3H), 2.22 (s, 1H), 1.65 (s, 3H), 1.60 – 1.48 (m, 4H), 1.44 – 1.31 (m, 4H) ppm.

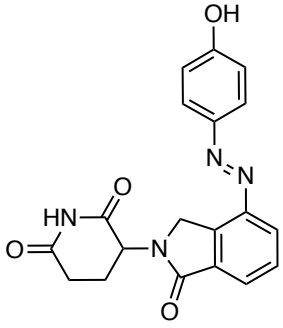
**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.42, 170.53, 169.87, 169.53, 168.56, 164.29, 155.70, 152.71, 150.10, 148.94, 146.88, 139.91, 136.98, 136.67, 134.29, 133.46, 132.27, 131.04, 131.02, 130.58, 129.96, 129.75, 129.56, 128.85, 126.29, 100.68, 72.91, 56.51, 54.65, 52.03, 48.24, 39.64, 39.51, 38.98, 31.74, 29.57, 29.55, 26.74, 26.70, 23.58, 14.50, 13.22, 11.88 ppm.

**HRMS** (ESI): calcd. for $C_{48}H_{51}ClN_{10}NaO_8S^+$: 985.3193 m/z $[M+Na]^+$

found: 985.3234 m/z $[M+Na]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.93 min. 963 m/z $[M+H]^+$.

## (*E*)-3-(4-((4-hydroxyphenyl)diazenyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (S12)




Lenalidomide (500 mg, 1.93 mmol, 1.0 eq.) was dissolved in 1 M HCl (50 mL). Concentrated aqueous $HBF_4$ (1 mL) was added to the mixture. After complete dissolving of the starting material, 2 M $NaNO_2$ (1.06 mL) was added to the solution at 0 °C. After stirring for 1 h the solution was added dropwise into a mixture of Phenol (217.9 mg, 2.315 mmol, 1.2 eq., 0.204 mL) in $H_2O$ (50 mL), MeOH (20 mL), $NaHCO_3$ (4.000 g, 47.62 mmol, 24.7 eq.) and $Na_2CO_3$ (5.000 g, 47.18 mmol, 24.5 eq.) at 0°C. Upon addition the solution turned from white to orange and stirred for additional 1 h at 0°C. The reaction was extracted with EtOAc (7x 100 mL) and washed once with brine (1x 100 mL). The organic phase was dried over $Na_2SO_4$ and then concentrated under reduced pressure. **S12** (605.2 mg, 1.661 mmol, 86%) was obtained as a yellow solid.

**$R_f$** = 0.26 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.01 (s, 1H), 10.42 (s, 1H), 8.13 (d, $J$ = 7.5 Hz, 1H), 7.97 – 7.68 (m, 4H), 6.96 (d, $J$ = 8.1 Hz, 2H), 5.23 – 5.07 (m, 1H), 4.84 – 4.57 (m, 2H), 2.94 (t, $J$ = 12.9 Hz, 1H), 2.67 – 2.54 (m, 2H), 2.10 – 1.95 (m, 1H) ppm.

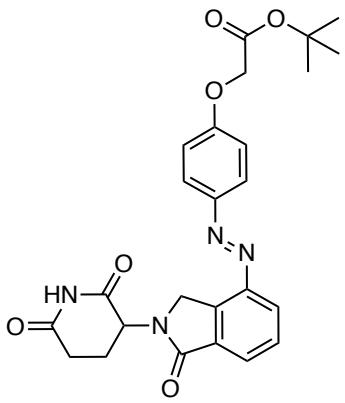
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.92, 171.02, 167.28, 161.61, 146.70, 145.40, 134.16, 133.69, 129.50, 128.11, 125.16, 124.52, 115.97, 51.65, 48.21, 31.25, 22.34 ppm.

**HRMS** (ESI): calcd. for $C_{19}H_{17}N_4O_4^+$: 365.1244 m/z $[M+H]^+$

found: 365.1257 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.94 min. 365 m/z $[M+H]^+$.

## (*E*)-3-(4-((4-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)diazenyl)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-ium (S13)



To a solution of tert-butyl bromoacetate (267.6 mg, 1.372 mmol, 1 eq., 0.20 mL) was added **S12** (500 mg, 1.372 mmol, 1 eq.) dissolved in dry DMF (13 mL). After addition of $K_2CO_3$ (246.6 mg, 1.784 mmol, 1.3 eq.) the reaction was stirred for 6.5 h at room temperature. Upon addition the solution turned from orange to dark red. After 6.5 hours of stirring, the mixture was diluted with EtOAc (100 mL), separated against $NaHCO_3$ (50 mL), extracted with EtOAc (3x 50 mL) and washed with brine (4x 50 mL). The reaction was concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography (Hx/Ea gradient, 20 → 100% Ea) gave **S13** (397.7 mg, 0831 mmol, 61%) as a yellow solid.

**$R_f$** = 0.44 [Hx:EA,1:4].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.01 (s, 1H), 8.18 (d, *J* = 7.7 Hz, 1H), 8.03 – 7.94 (m, 2H), 7.89 (d, *J* = 7.4 Hz, 1H), 7.78 (t, *J* = 7.7 Hz, 1H), 7.17 – 7.08 (m, 2H), 5.17 (dd, *J* = 13.5, 5.0 Hz, 1H), 4.79 (m, 3H), 4.67 (d, *J* = 18.9 Hz, 1H), 2.92 (d, *J* = 12.7 Hz, 1H), 2.58 (dd, *J* = 23.6, 14.7 Hz, 2H), 2.02 (dd, *J* = 16.0, 9.6 Hz, 1H), 1.44 (s, 9H) ppm.

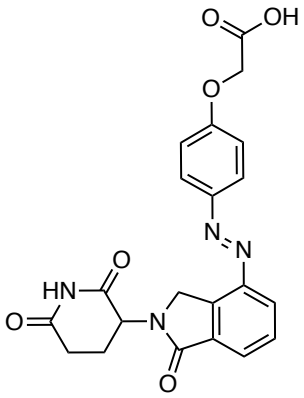
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.92, 171.01, 167.42, 167.22, 160.89, 146.64, 146.60, 134.26, 133.75, 129.58, 128.58, 124.99, 124.73, 115.18, 81.70, 65.20, 51.66, 48.26, 31.25, 27.70, 22.32 ppm.

**HRMS** (APCI):calcd. for $C_{25}H_{27}N_4O_6^+$: 479.1925 m/z $[M+H]^+$

found: 479.1928 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 4.45 min. 479 m/z $[M+H]^+$.

## (*E*)-3-(4-((4-(carboxymethoxy)phenyl)diazenyl)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-ium (S14)



**S13** (331.1 mg, 0.692 mmol, 1 eq.) was dissolved in $CH_2Cl_2$:TFA (1:1; 4 mL each). Upon TFA addition (4 mL) the solution turned from yellow to dark red. After 4 hours the reaction was concentrated under reduced pressure, turning from red to an orange solid. The reaction was triturated with $Et_2O$ and dried under high vacuum for 24 h. **S14** (340.5 mg, 0.635 mmol, 92%) was obtained in form of a yellow solid.

$\mathbf{R}_f$ = 0.10 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.01 (s, 1H), 8.22 – 8.13 (m, 1H), 8.02 – 7.94 (m, 2H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.78 (t, *J* = 7.8 Hz, 1H), 7.20 – 7.09 (m, 2H), 5.16 (dd, *J* = 13.4, 5.0 Hz, 1H), 4.80 (m, 3H), 4.67 (d, *J* = 19.2 Hz, 1H), 2.94 (ddd, *J* = 18.4, 13.9, 5.3 Hz, 1H), 2.69 – 2.54 (m, 2H), 2.08 – 1.98 (m, 1H) ppm.

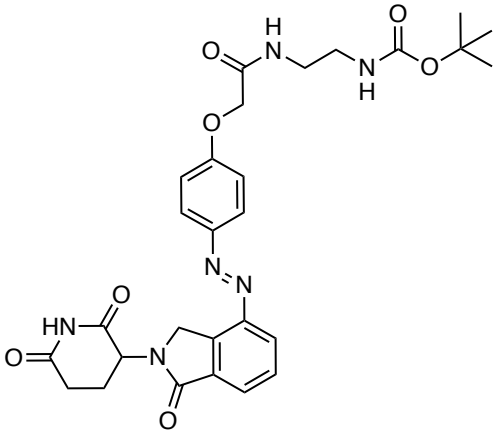
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.93, 171.01, 169.76, 167.23, 161.01, 146.62, 146.62, 134.32, 133.75, 129.58, 128.43, 124.97, 124.74, 115.19, 64.76, 51.68, 48.24, 31.25, 22.32 ppm.

**HRMS** (APCI): calcd. for $C_{21}H_{19}N_4O_6^+$: 423.1299 m/z $[M+H]^+$

found: 423.1284 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.01 min. 423 m/z $[M+H]^+$.

## *Tert*-butyl (*E*)-(2-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)ethyl)carbamate (S15)



**S14** (50.0 mg, 0.093 mmol, 1.0 eq.) and HATU (45.3 mg, 0.140 mmol, 1.5 eq.) were dissolved in dry DMF (4.5 mL) at room temperature under nitrogen atmosphere. After 5 minutes of stirring *N*-Boc-1,4-diaminoethane (80.7 mg, 0.373 mmol, 4 eq.) and *i*-$Pr_2NEt$ (48.2 mg, 0.373 mmol, 4 eq., 66 *µL*) were added to the mixture and stirred for further 12 hours. The reaction was diluted with EtOAc (20 mL), separated against $H_2O$: sat. NaCl (20 mL), extracted with EtOAc (3x 20 mL) and washed with brine (4x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. **S15** (57.2 mg, 0.092 mmol, 99%) was obtained as an orange solid.

**$R_f$** = 0.38 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 10.99 (s, 1H), 8.20 (dd, *J* = 16.2, 6.6 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 7.4 Hz, 1H), 7.78 (t, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 2H), 6.87 (q, *J* = 5.7, 4.7 Hz, 1H), 5.17 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.80 (d, *J* = 19.1 Hz, 1H), 4.67 (d, *J* = 19.3 Hz, 1H), 4.61 (s, 2H), 3.18 (q, *J* = 6.3 Hz, 2H), 3.04 (q, *J* = 6.3 Hz, 2H), 2.96 – 2.88 (m, 1H), 2.60 (dd, *J* = 16.2, 12.6 Hz, 2H), 2.09 – 1.98 (m, 1H), 1.37 (s, 9H) ppm.

**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.91, 171.00, 167.26, 167.21, 160.88, 155.74, 146.68, 146.59, 134.27, 133.74, 129.59, 128.52, 124.99, 124.75, 115.40, 77.73, 67.11, 51.66, 48.24, 38.72, 31.25, 28.22, 22.34, 22.30 ppm.

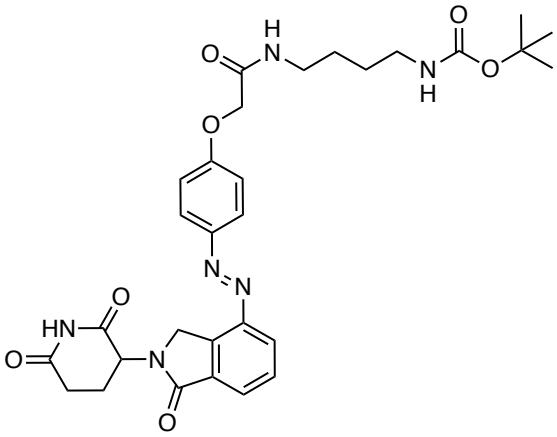
**HRMS** (APCI): calcd. for $C_{28}H_{33}N_6O_7^+$: 565.2405 m/z $[M+H]^+$

found: 565.2381 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 2.96 min (*Z*). 563 m/z $[M–H]^–$.

$t_{ret}$ = 3.38 min (*E*). 563 m/z $[M–H]^-$.

## *Tert*-butyl (*E*)-(4-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)butyl)carbamate (S16)



**S14** (50.0 mg, 0.093 mmol, 1.0 eq.) and HATU (46.0 mg, 0.142 mmol, 1.5 eq.) were dissolved in dry DMF (4.5 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,4-diaminobutane (70.2 mg, 0.373 mmol, 4 eq.) and *i*-$Pr_2NEt$ (48.2 mg, 0.373 mmol, 4 eq., 66 $\mu L$) were added to the mixture and stirred for additional 12 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (3x 20 mL) and washed with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) gave **S16** (50.9 mg, 0.086 mmol, 92%) as an orange solid.

**$R_f$** = 0.38 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.18 (d, *J* = 7.9 Hz, 1H), 8.12 (s, 1H), 7.99 (d, *J* = 7.5 Hz, 1H), 7.95 – 7.90 (m, 2H), 7.70 (t, *J* = 7.7 Hz, 1H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.66 (s, 1H), 5.26 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.85 (d, *J* = 17.9 Hz, 1H), 4.74 (d, *J* = 18.0 Hz, 1H), 4.57 (s, 2H), 3.39 (q, *J* = 6.6 Hz, 2H), 3.14 (q, *J* = 6.5 Hz, 2H), 3.01 – 2.79 (m, 2H), 2.46 (qd, *J* = 13.1, 5.0 Hz, 1H), 2.26 (dtd, *J* = 12.9, 5.0, 2.5 Hz, 1H), 1.62 – 1.48 (m, 4H), 1.43 (s, 9H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.08, 169.55, 168.67, 167.59, 160.02, 156.19, 147.95, 147.09, 134.17, 133.37, 129.70, 129.57, 126.02, 125.12, 115.24, 79.43, 67.62, 52.11, 48.35, 40.26, 38.94, 31.74, 28.56, 27.69, 26.93, 23.61 ppm.

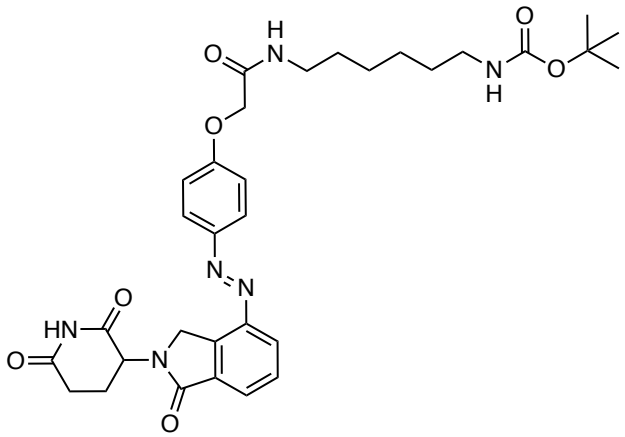
**HRMS** (APCI): calcd. for $C_{30}H_{37}N_6O_7^+$: 593.2718 m/z $[M+H]^+$

found: 593.2700 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.17 min (*Z*). 591 m/z $[M–H]^-$.

$t_{ret}$ = 3.53 min (*E*). 591 m/z $[M–H]^-$.

## *Tert*-butyl (*E*)-(6-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)hexyl)carbamate (S17)



**S14** (50.0 mg, 0.093 mmol, 1.0 eq.) and HATU (45.3 mg, 0.140 mmol, 1.5 eq.) were dissolved in dry DMF (4.5 mL) at room temperature. After 5 minutes of stirring *N*-Boc-1,4-diaminohexane (80.7 mg, 0.373 mmol, 4 eq.) and *i*-$Pr_2NEt$ (48.2 mg, 0.373 mmol, 4 eq., 66 $\mu L$) were added to the mixture and stirred for additional 12 h at room temperature. The reaction was diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (3x 20 mL) and washed with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0-20% MeOH) gave **S17** (57.2 mg, 0.092 mmol, 99%) as an orange solid.

$\mathbf{R}_f$ = 0.34 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.19 (d, *J* = 7.8 Hz, 1H), 8.04 (s, 1H), 7.99 (d, *J* = 7.5 Hz, 1H), 7.94 (d, *J* = 8.9 Hz, 2H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.9 Hz, 2H), 6.59 (s, 1H), 5.26 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.86 (d, *J* = 17.9 Hz, 1H), 4.74 (d, *J* = 18.0 Hz, 1H), 4.59 (s, 2H), 3.36 (q, *J* = 6.8 Hz, 2H), 3.09 (s, 2H), 3.00 – 2.79 (m, 2H), 2.46 (dd, *J* = 13.1, 5.0 Hz, 1H), 2.27 (ddd, *J* = 13.9, 7.0, 3.6 Hz, 1H), 1.55 (d, *J* = 7.0 Hz, 2H), 1.50 – 1.43 (m, 2H), 1.44 (s, 9H), 1.34 (d, *J* = 6.0 Hz, 4H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 170.90, 169.91, 169.40, 168.53, 167.38, 159.95, 147.82, 146.98, 134.03, 133.25, 129.63, 129.45, 125.90, 125.00, 115.11, 79.16, 67.54, 51.96, 48.19, 40.29, 38.93, 31.61, 30.00, 29.47, 28.44, 26.34, 26.23, 23.50 ppm.

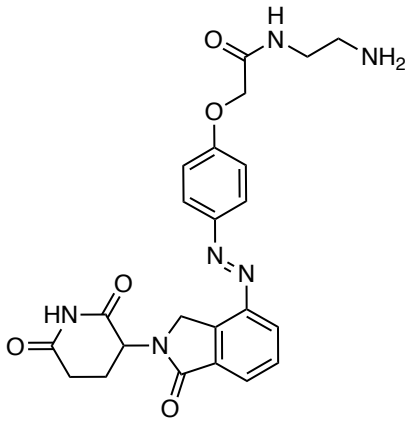
**HRMS** (ESI): calcd. for $C_{32}H_{41}N_6O_7^+$: 621.3031 m/z $[M+H]^+$

found: 621.3017 m/z $[M+H]^+$.

**LCMS** (APCI): $t_{ret}$ = 3.44 min. (*Z*) 619 m/z $[M–H]^–$.

$t_{ret}$ = 3.80 min. (*E*) 619 m/z $[M–H]^–$.

## (*E*)-*N*-(2-aminoethyl)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamide (S18)



**S15** (35.0 mg, 0.062 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1; 1mL:1mL) and stirred for 2 h at room temperature. The reaction was diluted with $CH_2Cl_2$ and concentrated under reduced pressure. The reaction was triturated with $Et_2O$ and dried on high vacuum overnight. **S18** (34.5 mg, 0.06 mmol, 96%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

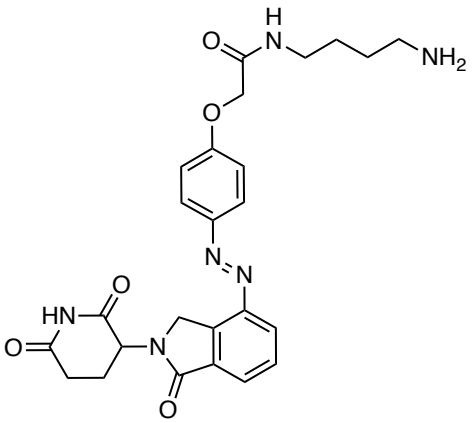
**R**$_f$ = 0.13 [$CH_2Cl_2$+1%TEA:MeOH, 5:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.02 (s, 1H), 8.36 (t, *J* = 6.0 Hz, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 2H), 7.90 (d, *J* = 7.4 Hz, 1H), 7.83 – 7.71 (m, 4H), 7.21 (d, *J* = 8.4 Hz, 2H), 5.19 (d, *J* = 13.4 Hz, 1H), 4.80 (d, *J* = 19.1 Hz, 1H), 4.67 (d, *J* = 16.9 Hz, 3H), 2.99 – 2.87 (m, 3H), 2.69 – 2.51 (m, 1H), 2.11 – 1.98 (m, 1H) ppm.

**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.93, 171.02, 168.11, 167.21, 160.73, 146.76, 146.58, 134.27, 133.76, 129.62, 128.57, 125.07, 124.80, 115.44, 67.14, 51.65, 48.22, 38.72, 36.20, 31.25, 22.35 ppm.

**HRMS** (APCI): calcd. for $C_{23}H_{25}N_6O_5^+$: 465.1881 m/z $[M+H]^+$
found: 465.1887 m/z $[M+H]^+$.
**LCMS** (ESI): $t_{ret}$ = 2.00 min (*Z*). 465 m/z $[M+H]^+$.
$t_{ret}$ = 2.32 min (*E*). 465 m/z $[M+H]^+$.

## *N*-(4-aminobutyl)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamide (S19)

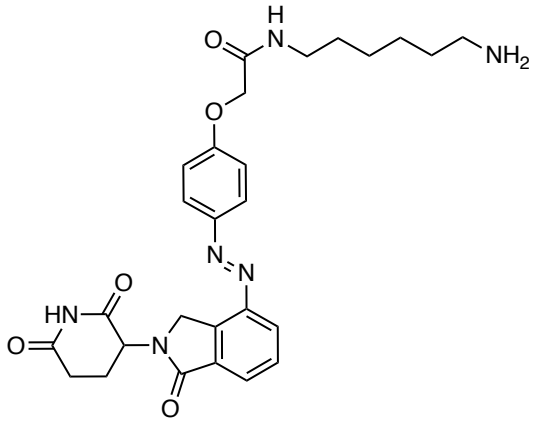


**S16** (40.0 mg, 0.067 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1; 1mL:1mL) and stirred for 2 h at room temperature. The reaction was diluted with $CH_2Cl_2$ and concentrated under reduced pressure. The reaction was triturated with $Et_2O$ and dried on high vacuum overnight. **S19** (40.5 mg, 0.067 mmol, 99%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

$\mathbf{R}_f$ = 0.13 [$CH_2Cl_2$+1%TEA:MeOH, 5:1].
**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.02 (s, 1H), 8.26 (t, *J* = 5.9 Hz, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.89 (d, *J* = 7.5 Hz, 1H), 7.78 (t, *J* = 7.7 Hz, 1H), 7.67 (s, 2H), 7.22 – 7.14 (m, 2H), 5.18 (dd, *J* = 13.1, 5.0 Hz, 1H), 4.80 (d, *J* = 19.1 Hz, 1H), 4.67 (d, *J* = 19.3 Hz, 1H), 4.62 (s, 2H), 3.17 (q, *J* = 6.2 Hz, 2H), 2.94 (dd, *J* = 10.9, 6.4 Hz, 1H), 2.80 (q, *J* = 6.4 Hz, 2H), 2.65 – 2.54 (m, 2H), 2.05 (d, *J* = 6.1 Hz, 1H), 1.51 (hept, *J* = 6.0, 5.4 Hz, 4H) ppm.
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.94, 171.03, 167.22, 167.09, 160.89, 146.71, 146.59, 134.28, 133.76, 129.62, 128.53, 125.04, 124.78, 115.41, 67.18, 51.65, 48.22, 38.60, 37.67, 31.25, 26.18, 24.48, 22.34 ppm.

**HRMS** (APCI): calcd. for $C_{25}H_{29}N_6O_5^+$: 493.2194 m/z $[M+H]^+$
found: 493.2177 m/z $[M+H]^+$.
**LCMS** (ESI): $t_{ret}$ = 2.21 min (*Z*) 493 m/z $[M+H]^+$.
$t_{ret}$ = 2.44 min (*E*) 493 m/z $[M+H]^+$.

## *Tert*-butyl-(6-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)-acetamido)hexyl)carbamate (S20)

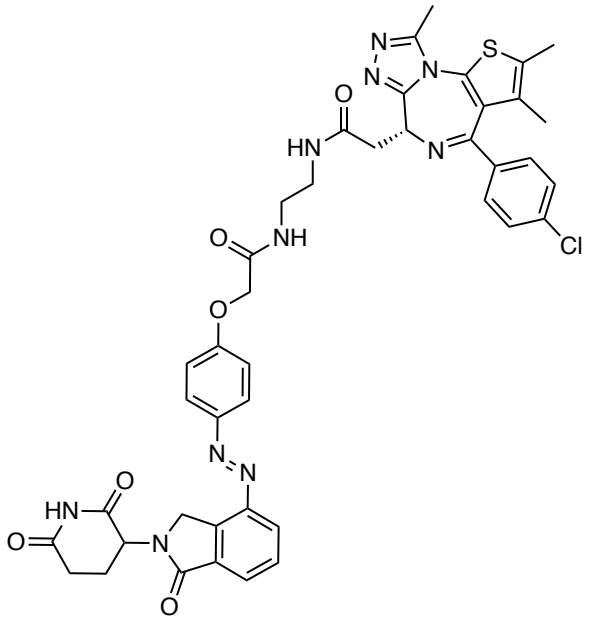


**S17** (44.5 mg, 0.072 mmol, 1 eq.) was dissolved in TFA/$CH_2Cl_2$ (1:1; 2mL:2mL) and stirred for 2 h at room temperature. The reaction was diluted with $CH_2Cl_2$ and concentrated under reduced pressure. The reaction was triturated with $Et_2O$ and dried on high vacuum overnight. **S20** (45.6 mg, 0.072 mmol, >99%) was obtained as trifluoroacetate in form of a yellow solid with traces of residual TFA.

$\mathbf{R}_f$ = 0.16 [$CH_2Cl_2$+1%TEA:MeOH, 5:1].
**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.02 (s, 1H), 8.24 – 8.14 (m, 2H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.89 (d, *J* = 7.4 Hz, 1H), 7.78 (t, *J* = 7.7 Hz, 1H), 7.66 (s, 2H), 7.17 (d, *J* = 7.6 Hz, 2H), 5.18 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.80 (d, *J* = 19.1 Hz, 1H), 4.67 (d, *J* = 19.4 Hz, 1H), 4.61 (s, 2H), 3.14 (q, *J* = 6.7 Hz, 2H), 2.94 (ddd, *J* = 21.8, 11.5, 4.6 Hz, 1H), 2.76 (h, *J* = 6.2 Hz, 2H), 2.67 – 2.53 (m, 2H), 2.04 (dt, *J* = 11.8, 4.5 Hz, 1H), 1.57 – 1.39 (m, 4H), 1.29 (tq, *J* = 11.9, 7.0 Hz, 4H) ppm.
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.94, 171.03, 167.22, 166.92, 160.95, 146.69, 146.59, 134.29, 133.76, 129.61, 128.51, 125.03, 124.75, 115.40, 67.19, 51.66, 48.23, 38.78, 38.19, 31.25, 28.93, 26.95, 25.84, 25.46, 22.34 ppm.

**HRMS** (APCI): calcd. for $C_{27}H_{33}N_6O_5^+$: 521.2507 m/z $[M+H]^+$
found: 521.2498 m/z $[M+H]^+$.
**LCMS** (ESI): $t_{ret}$ = 2.37 min (cis) 521 m/z $[M+H]^+$.
$t_{ret}$ = 2.80 min (trans) 521 m/z $[M+H]^+$.

## 2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(2-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)ethyl)acetamide (PHOTAC-I-6)



Into a round bottom flask with dry (+)-JQ1 free acid (6.0 mg, 0.015 mmol, 1 eq.) were added **S18** (17.3 mg, 0.030 mmol, 2 eq.) and HATU (8.7 mg, 0.027 mmol, 1.8 eq.) under nitrogen atmosphere. The solids were dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (17.6 mg, 0.137 mmol, 7.2 eq., 0.024 mL) the reaction was stirred for 15 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5%LiCl (30 mL), extracted with EtOAc (2x 20 mL), washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-6** (12.4 mg, 0.015 mmol, 98%) as a yellow solid.

**$R_f$** = 0.10 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.71 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.55 (s, 1H), 7.37 (d, *J* = 8.2 Hz, 3H), 7.29 (d, J = 8.3 Hz, 2H), 6.93 (d, *J* = 8.6 Hz, 2H), 5.19 (dt, *J* = 13.5, 4.6 Hz, 1H), 4.74 (d, *J* = 18.9 Hz, 1H), 4.64 (dt, *J* = 11.4, 5.9 Hz, 2H), 4.43 (s, 2H), 3.48 (dddd, *J* = 51.8, 22.1, 11.6, 5.5 Hz, 6H), 2.93 – 2.74 (m, 2H), 2.61 (s, 3H), 2.45 (s, 1H), 2.35 (s, 3H), 2.24 – 2.14 (m, 1H), 1.62 (s, 3H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.67, 171.50, 169.95, 168.67, 168.28, 164.30, 160.24, 155.76, 150.14, 147.54, 147.01, 137.04, 136.62, 134.10, 133.41, 132.22, 131.10, 130.50, 130.00, 129.73, 129.69, 129.47, 128.88, 125.67, 124.97, 115.18, 67.39, 54.46, 52.25, 48.67, 39.80, 39.33, 39.11, 31.65, 23.44, 14.49, 13.22, 11.91 ppm.

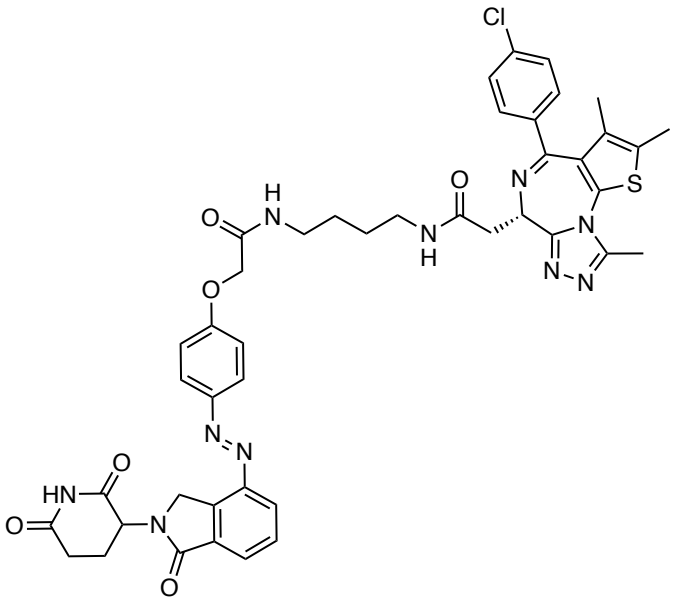
**HRMS** (ESI): calcd. for $C_{42}H_{39}ClN_{10}NaO_6S^+$: 869.2355 m/z $[M+Na]^+$

found: 869.2362 m/z $[M+Na]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.44 min (*Z*). 847 m/z $[M+H]^+$.

$t_{ret}$ = 3.74 min (*E*). 847 m/z $[M+H]^+$.

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)butyl)acetamide (PHOTAC-I-7)**



Into a round bottom flask with dry (+)-JQ1 free acid (6.0 mg, 0.015 mmol, 1 eq.) were added **S19** (18.2 mg, 0.030 mmol, 2 eq.) and HATU (8.7 mg, 0.027 mmol, 1.8 eq.) under nitrogen atmosphere. The solids were dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (13.9 mg, 0.108 mmol, 7.2 eq., 0.020 mL) the reaction was stirred for 16 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (30 mL), extracted with EtOAc (2x 20 mL), washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-7** (12.7 mg, 0.015 mmol, 97%) as a yellow solid.

**R**$_f$ = 0.09 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.57 (s, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.92 (t, *J* = 7.1 Hz, 1H), 6.82 (s, 1H), 5.23 (dt, *J* = 13.4, 4.3 Hz, 1H), 4.80 (dd, *J* = 18.1, 3.2 Hz, 1H), 4.71 (dd, *J* = 18.1, 4.5 Hz, 1H), 4.62 (t, *J* = 7.1 Hz, 1H), 4.51 (s, 2H), 3.56 (dd, *J* = 14.3, 8.2 Hz, 1H), 3.34 (qd, *J* = 17.8, 15.6, 6.3 Hz, 5H),

2.94 – 2.75 (m, 2H), 2.64 (s, 3H), 2.51 – 2.43 (m, 1H), 2.37 (s, 3H), 2.27 – 2.18 (m, 1H), 1.65 (s, 3H), 1.57 (s, 4H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.33, 170.67, 169.81, 168.65, 167.67, 164.26, 160.15, 155.72, 150.14, 147.78, 147.05, 137.03, 136.63, 134.15, 133.40, 132.22, 131.11, 130.58, 129.99, 129.75, 129.69, 129.52, 128.88, 125.86, 125.09, 115.25, 67.60, 54.63, 52.09, 48.45, 39.52, 39.12, 38.85, 31.70, 26.89, 26.79, 23.53, 14.51, 13.22, 11.93 ppm.

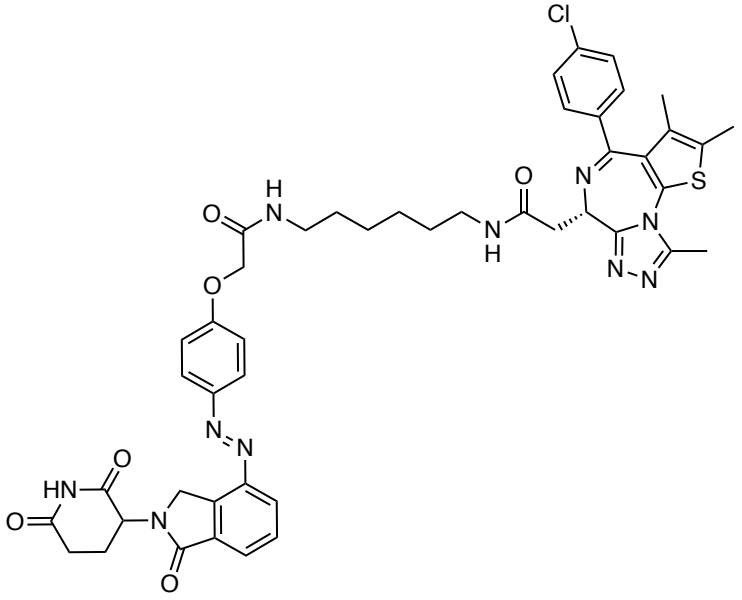
**HRMS** (ESI): calcd. for $C_{44}H_{43}ClKN_{10}O_6S^+$: 913.2408 m/z $[M+K]^+$

found: 913.2419 m/z $[M+K]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.48 min (*Z*). 875 m/z $[M+H]^+$.

$t_{ret}$ = 3.71 min (*E*). 875 m/z $[M+H]^+$.

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(6-(2-(4-((*E*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)hexyl)acetamide (PHOTAC-I-8)**



Into a round bottom flask with dry (+)-JQ1 free acid (6.0 mg, 0.015 mmol, 1 eq.) were added **S20** (19.0 mg, 0.030 mmol, 2 eq.) and HATU (8.7 mg, 0.027 mmol, 1.8 eq.) under nitrogen atmosphere. The solids were dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (13.9 mg, 0.108 mmol, 7.2 eq., 0.020 mL) the reaction was stirred for 18 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (30 mL), extracted with EtOAc (2x 20 mL), washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-I-8** (12.8 mg, 0.014 mmol, 95%) as a yellow solid.

$\mathbf{R}_f$ = 0.16 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.51 (d, *J* = 28.6 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 7.97 (d, *J* = 7.4 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.03 (d, *J* = 8.2 Hz, 2H), 6.76 – 6.66 (m, 1H), 6.61 (d, *J* = 4.7 Hz, 1H), 5.24 (d, *J* = 13.1 Hz, 1H), 4.84 (d, *J* = 18.0 Hz, 1H), 4.73 (d, *J* = 18.1 Hz, 1H), 4.59 (d, *J* = 11.4 Hz, 3H), 3.55 (dd, *J* = 14.0, 8.0 Hz, 1H), 3.29 (dd, *J* = 28.3, 5.6 Hz, 5H), 2.96 –

2.77 (m, 2H), 2.64 (s, 3H), 2.46 (dt, *J* = 13.0, 6.7 Hz, 1H), 2.38 (s, 3H), 2.30 – 2.18 (m, 1H), 1.65 (s, 3H), 1.51 (s, 4H), 1.31 (s, 4H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 171.27, 170.59, 169.77, 168.65, 167.62, 164.16, 160.14, 155.76, 150.07, 147.88, 147.09, 136.98, 136.69, 134.19, 133.41, 132.26, 131.05, 130.57, 129.97, 129.73, 129.67, 129.53, 128.86, 125.94, 125.11, 115.25, 67.69, 54.66, 52.07, 48.36, 39.58, 39.43, 38.92, 31.74, 29.47, 29.45, 26.34, 26.27, 23.58, 14.51, 13.23, 11.93 ppm.

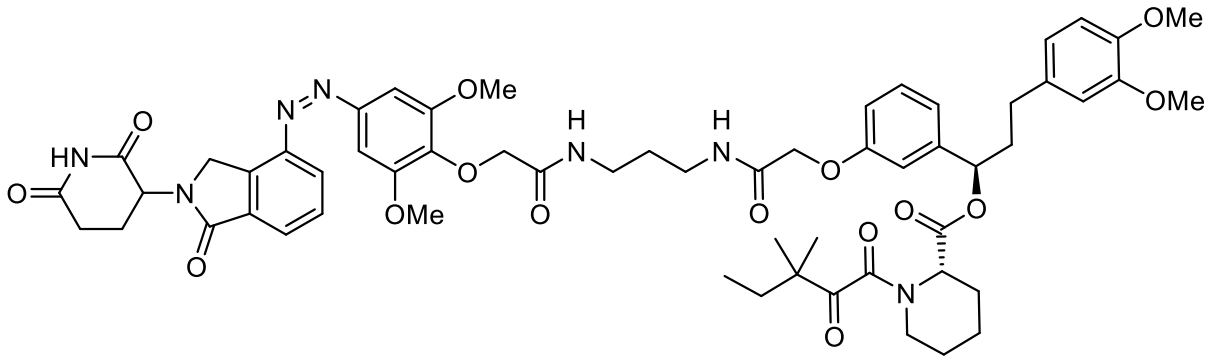
**HRMS** (ESI): calcd. for $C_{46}H_{47}ClN_{10}NaO_6S^+$: 925.2981 m/z $[M+Na]^+$

found: 925.2975 m/z $[M+Na]^+$.

**LCMS** (ESI): $t_{ret}$ = 3.57 min (*Z*) 903 m/z $[M+H]^+$.

$t_{ret}$ = 3.85 min (*E*) 903 m/z $[M+H]^+$.

**(1R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-((3-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)propyl)-amino)-2-oxoethoxy)phenyl)propyl-(2S)-1-(3,3-dimethyl-2-oxopentanoyl)-piperidine-2-carboxylate (PHOTAC-II-1)**



Into a round bottom flask with dry **2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid** (10.2 mg, 0.017 mmol, 1 eq.) were added **S9** (22.8 mg, 0.035 mmol, 2 eq.) and HATU (12 mg, 0.031 mmol, 1.8 eq.) under nitrogen. The reaction was dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (17 mg, 0.13 mmol, 7.5 eq., 0.023 mL) the reaction was stirred for 14 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-II-1** (17.8 mg, 0.016 mmol, 92%) as a yellow solid.

$\mathbf{R}_f$ = 0.59 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (600 MHz, Chloroform-*d*) δ = 8.33 (s, 1H), 8.20 (d, J = 7.7 Hz, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.84 (t, J = 6.3 Hz, 1H), 7.71 (t, J = 7.7 Hz, 1H), 7.37 (t, J = 6.3 Hz, 1H), 7.28 (t, J = 7.9 Hz, 1H), 7.21 (s, 2H), 7.00 – 6.92 (m, 2H), 6.87 (dd, J = 8.2, 2.8 Hz, 1H), 6.80 – 6.73 (m, 1H), 6.69 – 6.65 (m, 2H), 5.77 (dd, J = 8.0, 5.6 Hz, 1H), 5.30 (d, J = 4.9 Hz, 1H), 5.25 (dd, J = 13.4, 5.1 Hz, 1H), 4.85 (d, J = 17.8 Hz, 1H), 4.72 (d, J = 17.8 Hz, 1H), 4.60 (d, 2H), 4.46 (s, 2H), 3.96 (s, 6H), 3.86 – 3.81 (m, 6H), 3.47 – 3.33 (m, 5H), 3.16 (td, J = 13.2, 3.2 Hz, 1H), 2.95 – 2.81 (m, 2H), 2.64 – 2.42 (m, 3H), 2.36 (d, J = 13.9 Hz, 1H), 2.28 – 2.18 (m, 2H), 2.04 (dtd,

J = 11.6, 9.7, 4.8 Hz, 1H), 1.83 – 1.57 (m, 7H), 1.47 (qt, J = 13.0, 4.0 Hz, 1H), 1.35 (tt, J = 13.6, 3.6 Hz, 1H), 1.20 (d, J = 11.9 Hz, 6H), 0.87 (t, J = 7.4 Hz, 3H) ppm.

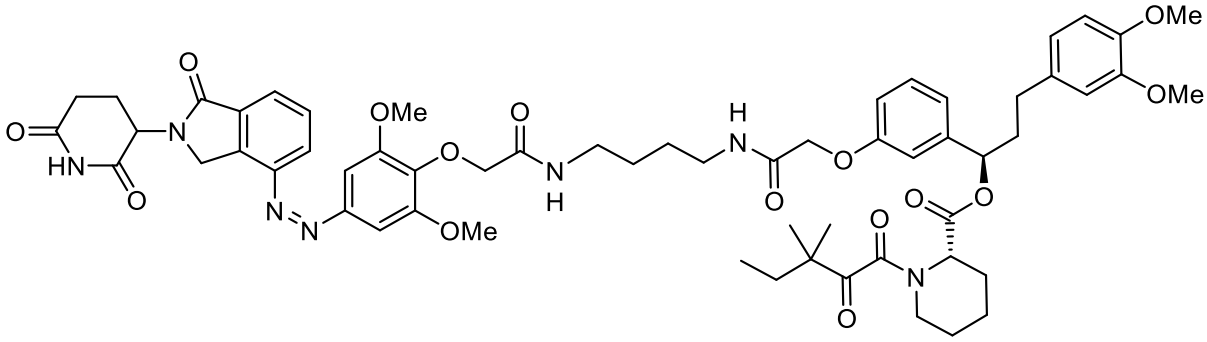
**$^{13}$C NMR** (150 MHz, $CDCl_3$) δ = 208.01, 171.22, 170.36, 169.77, 169.65, 168.55, 168.42, 167.38, 157.54, 152.75, 149.04, 148.99, 147.46, 146.88, 141.90, 139.76, 134.09, 133.49, 133.44, 130.08, 130.07, 129.61, 126.37, 120.25, 120.17, 114.23, 113.49, 111.81, 111.41, 100.56, 76.64, 72.73, 67.36, 56.50, 56.04, 55.96, 52.12, 51.39, 48.33, 46.82, 44.28, 38.28, 36.21, 36.08, 32.59, 31.73, 31.35, 29.84, 26.53, 25.06, 23.59, 23.57, 23.25, 21.32, 8.88 ppm.

**HRMS** (ESI): calcd. for $C_{58}H_{73}N_8O_{15}^+$: 1121.5189 m/z $[M+NH_4]^+$

found: 1121.5246 m/z $[M+NH_4]^+$.

**LCMS** (ESI): $t_{ret}$ = 4.56 min. 1104 m/z $[M+H]^+$.

**(1R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-((4-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)butyl)-amino)-2-oxoethoxy)phenyl)propyl(2S)-1-(3,3-dimethyl-2-oxopentanoyl)-piperidine-2-carboxylate (PHOTAC-II-2)**



Into a round bottom flask with dry **2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid** (10.2 mg, 0.018 mmol, 1 eq.) were added **3** (23.3 mg, 0.035 mmol, 2 eq.) and HATU (12 mg, 0.031 mmol, 1.8 eq.) under nitrogen. The reaction was dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (17 mg, 0.13 mmol, 7.5 eq., 0.023 mL) the reaction was stirred for 14 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-II-2** (17.7 mg, 0.016 mmol, 91%) as a yellow solid.

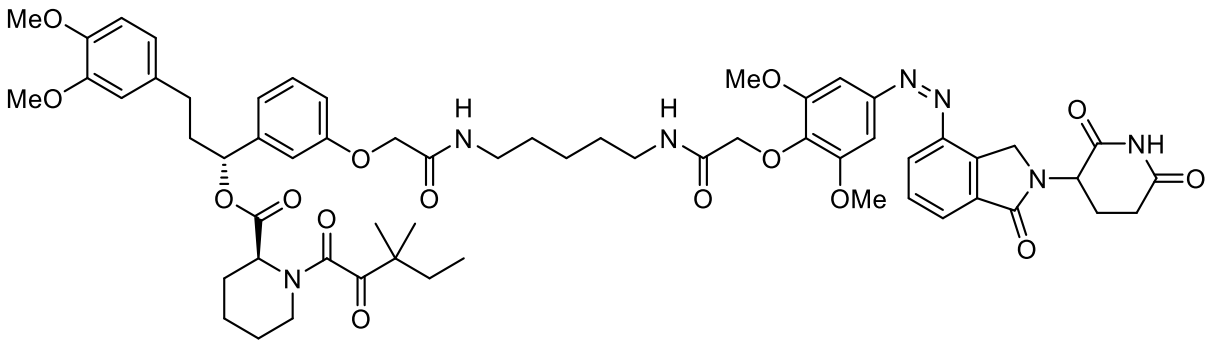
**$R_f$** = 0.59 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (600 MHz, Chloroform-*d*) δ = 8.35 (s, 1H), 8.19 (dd, J = 7.8, 1.0 Hz, 1H), 7.99 (dd, J = 7.5, 1.0 Hz, 1H), 7.74 – 7.67 (m, 2H), 7.28 (t, J = 7.9 Hz, 1H), 7.22 (s, 2H), 6.99 – 6.93 (m, 2H), 6.84 – 6.75 (m, 3H), 6.70 – 6.64 (m, 2H), 5.79 – 5.73 (m, 1H), 5.33 – 5.27 (m, 1H), 5.24 (dd, J = 13.4, 5.1 Hz, 1H), 4.85 (d, J = 17.9 Hz, 1H), 4.72 (d, J = 17.8 Hz, 1H), 4.60 (s, 2H), 4.48 (s, 2H), 3.98 (s, 6H), 3.86 – 3.82 (m, 6H), 3.44 – 3.32 (m, 5H), 3.16 (td, J = 13.2, 3.2 Hz, 1H), 2.92 – 2.80 (m, 2H), 2.65 – 2.43 (m, 3H), 2.36 (d, J = 14.0 Hz, 1H), 2.28 – 2.18 (m, 2H), 2.04 (ddt, J = 13.9, 10.1, 5.9 Hz, 1H), 1.78 – 1.59 (m, 9H), 1.48 (qt, J = 13.2, 4.4 Hz, 1H), 1.35 (tt, J = 13.3, 3.4 Hz, 1H), 1.20 (d, J = 8.2 Hz, 6H), 0.87 (t, J = 7.5 Hz, 3H) ppm.

**$^{13}$C NMR** (150 MHz, $CDCl_3$) δ = 208.03, 171.25, 169.78, 169.69, 169.68, 168.55, 168.20, 167.37, 157.43, 152.73, 149.00, 148.99, 147.47, 146.87, 142.04, 139.81, 134.13, 133.46, 133.44, 130.13, 130.01, 129.59, 126.33, 120.26, 120.24, 113.88, 113.63, 111.82, 111.41, 100.58, 76.54, 72.82, 67.37, 56.50, 56.04, 55.96, 52.15, 51.37, 48.38, 46.82, 44.27, 38.82, 38.69, 38.29, 32.59, 31.72, 31.34, 27.22, 27.20, 26.52, 25.06, 23.56, 23.53, 23.31, 21.29, 8.89 ppm.

| | |
|---|---|
| **HRMS** (ESI): calcd. for $C_{59}H_{72}N_7O_{15}^+$: | 1118.5081 m/z $[M+H]^+$ |
| found: | 1118.5081 m/z $[M+H]^+$. |
| **LCMS** (ESI): $t_{ret}$ = 4.56 min. | 1118 m/z $[M+H]^+$. |

**(1*R*)-3-(3,4-dimethoxyphenyl)-1-(3-(2-((5-(2-(4-((*Z*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)pentyl)amino)-2-oxoethoxy)phenyl)propyl (2*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (PHOTAC-II-3)**



Into a round bottom flask with dry **2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid** (10.0 mg, 0.018 mmol, 1 eq.) were added **S10** (25.1 mg, 0.034 mmol, 2 eq.) and HATU (11.7 mg, 0.031 mmol, 1.8 eq.) under nitrogen. The reaction was dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (15.5 mg, 0.12 mmol, 7 eq., 0.021 mL) the reaction was stirred for 14 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-II-3** (14.4 mg, 0.013 mmol, 74%) as a yellow solid.

**$R_f$** = 0.28 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (600 MHz, Chloroform-*d*) δ = 8.36 (d, *J* = 3.6 Hz, 1H), 8.20 (dd, *J* = 7.8, 1.0 Hz, 1H), 8.00 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.22 (s, 2H), 7.00 – 6.92 (m, 2H), 6.82 (dd, *J* = 8.2, 2.6 Hz, 1H), 6.80 – 6.72 (m, 2H), 6.71 – 6.63 (m, 2H), 5.77 (dd, *J* = 8.2, 5.5 Hz, 1H), 5.31 (d, *J* = 6.1 Hz, 1H), 5.25 (dd, *J* = 13.4, 5.1 Hz, 1H), 4.86 (d, *J* = 17.8 Hz, 1H), 4.72 (d, *J* = 17.8 Hz, 1H), 4.61 (s, 2H), 4.47 (s, 2H), 3.98 (s, 6H), 3.87 – 3.81 (m, 6H), 3.35 (q, *J* = 6.7 Hz, 5H), 3.21 – 3.12 (m, 1H), 3.04 – 2.90 (m, 1H), 2.85 (ddd, *J*

= 18.1, 13.3, 5.2 Hz, 1H), 2.59 – 2.41 (m, 3H), 2.36 (d, *J* = 14.0 Hz, 1H), 2.24 (m, 2H), 2.04 (m, 1H), 1.81 – 1.57 (m, 9H), 1.48 (m, 1H), 1.44 – 1.30 (m, 3H), 1.20 (d, *J* = 8.0 Hz, 6H), 0.87 (t, *J* = 7.4 Hz, 3H) ppm.

**$^{13}$C NMR** (150 MHz, $CDCl_3$) δ = 208.03, 171.19, 169.79, 169.68, 169.64, 168.55, 168.17, 167.38, 157.45, 152.72, 149.03, 149.00, 147.49, 146.89, 142.04, 139.85, 134.11, 133.45, 133.42, 130.14, 130.06, 129.60, 126.36, 120.27, 120.24, 113.85, 113.70, 111.83, 111.42, 100.59, 76.54, 72.81, 67.38, 56.51, 56.05, 55.97, 52.13, 51.37, 48.33, 46.83, 44.28, 39.04, 38.91, 38.30, 32.60, 31.75, 31.35, 29.44, 29.35, 26.53, 25.07, 24.24, 23.59, 23.57, 23.32, 21.30, 8.90 ppm.

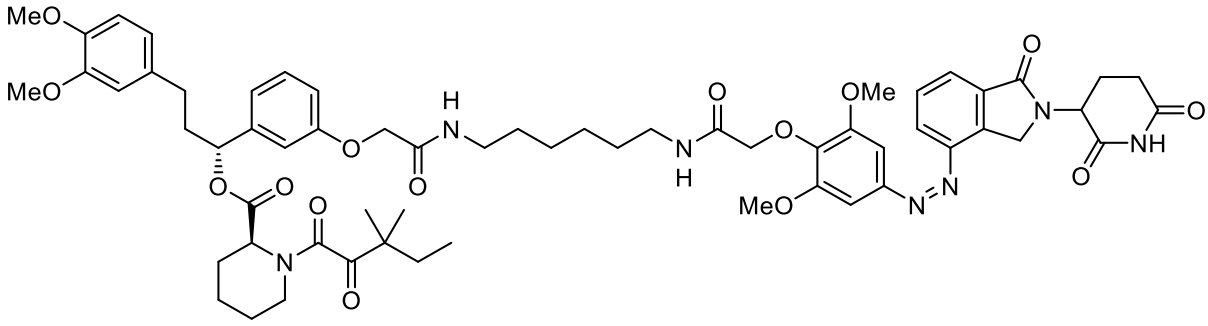
**HRMS** (APCI):calcd. for $C_{60}H_{74}N_7O_{15}^+$: 1132.5273 m/z $[M+H]^+$

found: 1132.5217 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 4.67 (*Z*) min. 566.7 m/z $[M+2H]^{2+}$.

$t_{ret}$ = 4.83 (E) min. 566.7 m/z $[M+2H]^{2+}$.

**(1*R*)-3-(3,4-dimethoxyphenyl)-1-(3-(2-((6-(2-(4-((*Z*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)hexyl)amino)-2-oxoethoxy)phenyl)propyl (2*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (PHOTAC-II-4)**



Into a round bottom flask with dry **2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid** (10.0 mg, 0.018 mmol, 1 eq.) were added **S11** (23.8 mg, 0.034 mmol, 2 eq.) and HATU (11.7 mg, 0.031 mmol, 1.8 eq.) under nitrogen. The reaction was dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (15.5 mg, 0.12 mmol, 7 eq., 0.021 mL) the reaction was stirred for 14 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-II-4** (16.9 mg, 0.015 mmol, 86%) as a yellow solid.

**R**$_f$ = 0.33 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (600 MHz, Chloroform-*d*) δ = 8.30 (s, 1H), 8.20 (dd, *J* = 7.9, 1.0 Hz, 1H), 8.00 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.71 (t, *J* = 7.7 Hz, 1H), 7.65 (t, *J* = 5.9 Hz, 1H), 7.29 (td, *J* = 7.9, 3.6 Hz, 1H), 7.22 (s, 2H), 7.00 – 6.88 (m, 2H), 6.85 – 6.75 (m, 2H), 6.72 (t, *J* = 6.0 Hz, 1H), 6.70 – 6.64 (m, 2H), 5.82 – 5.69 (m, 1H), 5.31 (d, *J* = 5.9 Hz, 1H), 5.26 (dd, *J* = 13.4, 5.1 Hz, 1H), 4.85 (d, *J* = 17.8 Hz, 1H), 4.72 (d, *J* = 17.8 Hz, 1H), 4.60 (s, 2H), 4.48 (d, *J* = 2.5 Hz, 2H), 3.98

(s, 6H), 3.85 (dd, *J* = 4.8, 2.5 Hz, 6H), 3.40 – 3.30 (m, 5H), 3.16 (td, *J* = 13.2, 3.1 Hz, 1H), 3.01 – 2.78 (m, 2H), 2.61 – 2.42 (m, 3H), 2.36 (d, *J* = 14.0 Hz, 1H), 2.24 (dddd, *J* = 17.8, 15.2, 7.8, 5.4 Hz, 2H), 2.10 – 1.99 (m, 1H), 1.80 – 1.52 (m, 9H), 1.48 (dt, *J* = 13.1, 4.0 Hz, 1H), 1.38 (tt, *J* = 10.4, 4.7 Hz, 5H), 1.21 (d, *J* = 8.3 Hz, 6H), 0.87 (t, *J* = 7.4 Hz, 3H) ppm.

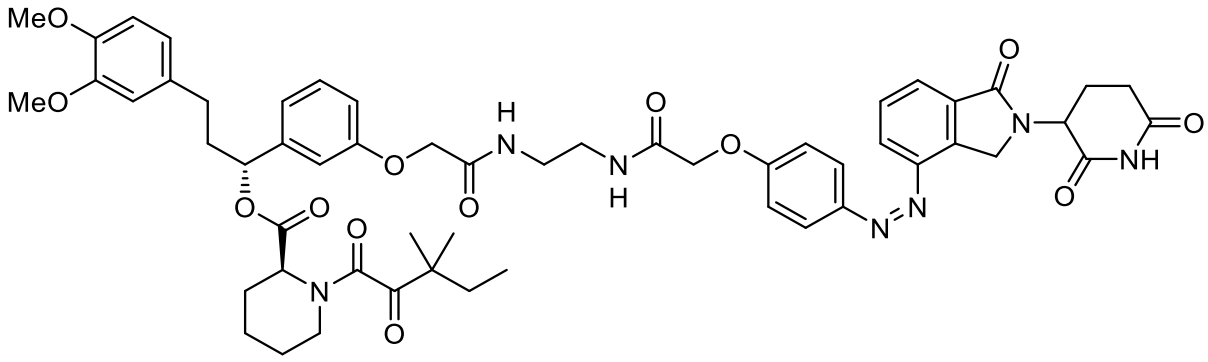
**$^{13}$C NMR** (150 MHz, $CDCl_3$) δ = 208.01, 171.17, 169.79, 169.63, 169.50, 168.54, 168.08, 167.37, 157.49, 152.74, 149.01, 148.99, 147.50, 146.91, 142.03, 139.91, 134.10, 133.45, 133.45, 130.14, 130.04, 129.61, 126.36, 120.27, 120.23, 113.89, 113.70, 111.84, 111.43, 100.59, 76.54, 72.88, 67.44, 56.50, 56.06, 55.97, 52.12, 51.37, 48.29, 46.83, 44.28, 39.12, 38.98, 38.30, 32.61, 31.74, 31.35, 29.74, 29.64, 26.69, 26.67, 26.52, 25.08, 23.61, 23.58, 23.32, 21.30, 8.90 ppm.

**HRMS** (APCI): calcd. for $C_{61}H_{76}N_7O_{15}^+$: 1146.5394 m/z $[M+H]^+$

found: 1146.5392 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 4.89 min. 573 m/z $[M+2H]^{2+}$.

**(1*R*)-3-(3,4-dimethoxyphenyl)-1-(3-(2-((2-(2-(4-((*Z*)-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)diazenyl)phenoxy)acetamido)ethyl)amino)-2-oxoethoxy)phenyl)propyl (2*S*)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (PHOTAC-II-5)**



Into a round bottom flask with dry **2-(3-((R)-3-(3,4-dimethoxyphenyl)-1-(((S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carbonyl)oxy)propyl)phenoxy)acetic acid** (8.0 mg, 0.014 mmol, 1 eq.) were added **S18** (15.9 mg, 0.027 mmol, 2 eq.) and HATU (9.4 mg, 0.025 mmol, 1.8 eq.) under nitrogen. The reaction was dissolved in dry DMF (1 mL). After addition of *i*-$Pr_2NEt$ (12.4 mg, 0.096 mmol, 7 eq., 0.02 mL) the reaction was stirred for 15 h at room temperature. The mixture was then diluted with EtOAc (20 mL), separated against 5% LiCl (20 mL), extracted with EtOAc (2x 20 mL) and washed twice with 10% LiCl (2x 20 mL) and brine (2x 20 mL). The combined organic phases were dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification of the resulting crude product by flash column chromatography ($CH_2Cl_2$/MeOH gradient, 0→20% MeOH) gave **PHOTAC-II-5** (9.1 mg, 0.009 mmol, 65%) as a yellow solid.

**$R_f$** = 0.17 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ = 11.02 (s, 1H), 8.30 (d, *J* = 5.0 Hz, 1H), 8.21 (d, *J* = 5.3 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.89 (d, *J* = 7.4 Hz, 1H), 7.78 (t, *J* = 7.6 Hz, 1H), 7.29 (q, *J* = 7.9 Hz, 1H), 7.19 – 7.15 (m, 2H), 6.96 (dd, *J* = 4.3, 2.2 Hz, 2H), 6.92 – 6.88 (m, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.76 – 6.73 (m, 1H), 6.65 (d, *J* = 8.2 Hz, 1H), 5.71 – 5.65 (m, 1H), 5.16 (m, 2H), 4.78 (d, *J* = 19.0 Hz, 1H), 4.67 (d, *J* = 18.9 Hz, 1H), 4.60 (s, 2H), 4.47 (s, 2H), 3.71 (s, 3H), 3.69 (s, 3H), 3.29 – 3.19 (m, 5H), 3.10 (t, *J* = 12.3 Hz, 1H), 2.94

(ddd, *J* = 17.9, 13.4, 5.4 Hz, 1H), 2.61 (d, *J* = 18.2 Hz, 1H), 2.57 – 2.45 (m, 4H), 2.23 (d, *J* = 13.3 Hz, 1H), 2.20 – 2.08 (m, 1H), 2.06 – 1.98 (m, 2H), 1.74 – 1.50 (m, 4H), 1.40 – 1.29 (m, 1H), 1.14 (s, 3H), 1.12 (s, 3H), 1.04 (d, *J* = 6.4 Hz, 1H), 0.78 (t, *J* = 7.4 Hz, 3H) ppm.

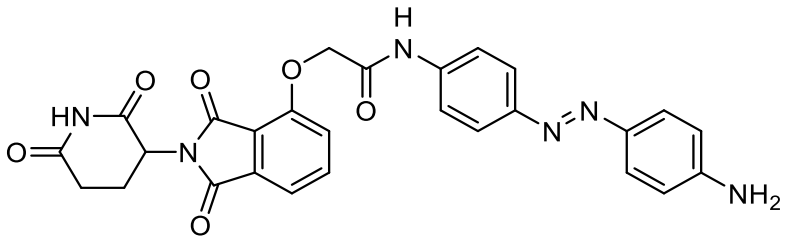
**$^{13}$C NMR** (150 MHz, DMSO) δ = 207.64, 172.91, 171.00, 169.32, 167.92, 167.46, 167.22, 166.82, 160.86, 157.72, 148.63, 147.06, 146.68, 146.58, 141.63, 134.28, 133.74, 133.13, 129.72, 129.57, 128.49, 124.99, 124.74, 119.91, 119.00, 115.41, 114.13, 112.98, 112.10, 111.87, 75.99, 67.10, 66.97, 55.47, 55.32, 51.66, 50.89, 48.24, 46.17, 43.84, 38.23, 38.16, 37.59, 31.93, 31.25, 30.62, 26.06, 24.36, 22.88, 22.60, 22.33, 20.77, 8.58 ppm.

**HRMS** (APCI): calcd. for $C_{55}H_{64}N_7O_{13}^+$: 1030.4557 m/z $[M+H]^+$

found: 1030.4565 m/z $[M+H]^+$.

**LCMS** (ESI): $t_{ret}$ = 4.70 min. 1030 m/z $[M+H]^+$.

## (*E*)-N-(4-((4-aminophenyl)diazenyl)phenyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (S21)



To a solution of thalidomide-4-hydroxyacetate[2] (16.7 mg, 50 µmol, 1.0 eq.) and 4,4'-diaminoazobenzene (32 mg, 150 µmol, 3.0 eq.) in THF (1.9 mL) was added HOBt (6.8 mg, 50 µmol, 1.0 eq.), PyBOP (52 mg, 100 µmol, 2.0 eq.) and triethylamine (35 µL, 26 mg, 250 µmol, 5.0 eq.) at room temperature. The reaction was stirred overnight, upon which the reaction solution was diluted with EtOAc, washed with water, sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate and concentrated in vacuo. The crude product was purified by column chromatography over $SiO_2$ using 0% → 10% MeOH in $CH_2Cl_2$ as the eluent to afford the desired product **S21** (21 mg, 40 µmol, 79%) as a highly insoluble brown solid.

**$R_f$** = 0.51 [$CH_2Cl_2$:MeOH, 19:1].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ = 11.13 (s, 1H), 10.35 (s, 1H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.76 (s, 4H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.51 (t, *J* = 7.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.05 (s, 2H), 5.15 (dd, *J* = 12.9, 5.4 Hz, 1H), 5.05 (s, 2H), 2.99 – 2.80 (m, 1H), 2.59 (t, 2H), 2.07 (d, *J* = 13.0 Hz, 2H) ppm.

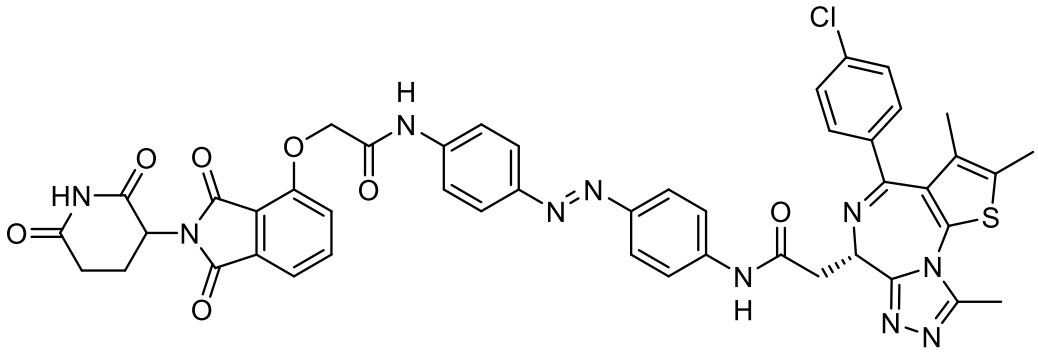
**$^{13}$C NMR** (100 MHz, DMSO) δ = 172.8, 169.9, 166.7, 165.9, 165.5, 155.2, 152.5, 148.5, 142.8, 139.4, 137.0, 133.1, 124.9, 122.6, 120.5, 119.6, 116.7, 116.1, 113.4, 67.6, 48.8, 31.0, 22.0 ppm.

**HRMS** (ESI): calcd. for $C_{27}H_{22}N_6NaO_6^+$: 549.1493 *m/z* $[M+Na]^+$

found: 549.1478 *m/z* $[M+Na]^+$.

**LCMS $T_R$** = 3.521 min

**2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-((E)-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)phenyl)diazenyl)phenyl)acetamide (PHOTAC-I-10)**




To a solution of **S37** (5.0 mg, 9.5 µmol, 1.0 eq.) and (+)-JQ1 free acid (4.2 mg, 10.4 µmol, 1.1 eq.) DCE (1.0 mL) was added TBTU (4.0 mg, 12.3 µmol, 1.3 eq.) and DIPEA (2 µL, 14.2 µmol, 1.5 eq.) at room temperature. The reaction was allowed to stir at room temperature overnight. Upon completion, the reaction was diluted with EtOAc and washed with water, NaHCO3 and brine. The organics were dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography over $SiO_2$ using 0% →10% MeOH in $CH_2Cl_2$ as the eluent to afford the desired product **PHOTAC-I-10** (3.0 mg, 3.3 µmol, 35%) as an orange amorphous solid.

**$R_F$** = 0.32 [$CH_2Cl_2$:MeOH, 95:5].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 9.46 (s, 1H), 9.36 (d, *J* = 6.0 Hz, 1H), 8.21 (d, *J* = 10.7 Hz, 1H), 7.82 (d, *J* = 1.9 Hz, 4H), 7.75 (dd, *J* = 8.9, 2.3 Hz, 2H), 7.69 (ddd, *J* = 8.5, 7.5, 2.0 Hz, 1H), 7.66 – 7.60 (m, 2H), 7.51 (dd, *J* = 7.4, 3.5 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.17 (d, *J* = 1.3 Hz, 1H), 4.97 (ddd, *J* = 12.3, 5.4, 2.0 Hz, 1H), 4.71 (s, 2H), 4.63 (dd, *J* = 8.7, 5.4 Hz, 1H), 3.87 – 3.76 (m, 1H), 3.49 (dd, *J* = 14.3, 5.3 Hz, 1H), 2.92 – 2.85 (m, 1H), 2.85 – 2.78 (m, 1H), 2.78 – 2.71 (m, 1H), 2.63 (s, 3H), 2.35 (s, 3H), 2.17 – 2.08 (m, 1H), 1.63 (s, 3H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 170.9, 170.9, 169.1, 168.1, 166.7, 166.5, 165.0, 164.4, 155.8, 154.4, 150.3, 149.6, 149.0, 140.9, 139.6, 137.4, 137.2, 136.5, 133.6, 132.2, 131.3,

131.1, 130.6, 130.1, 128.9, 124.0, 123.9, 120.1, 120.0, 119.9, 118.0, 68.5, 54.7, 49.6, 40.8, 31.6, 22.7, 14.6, 13.3, 12.0 ppm.

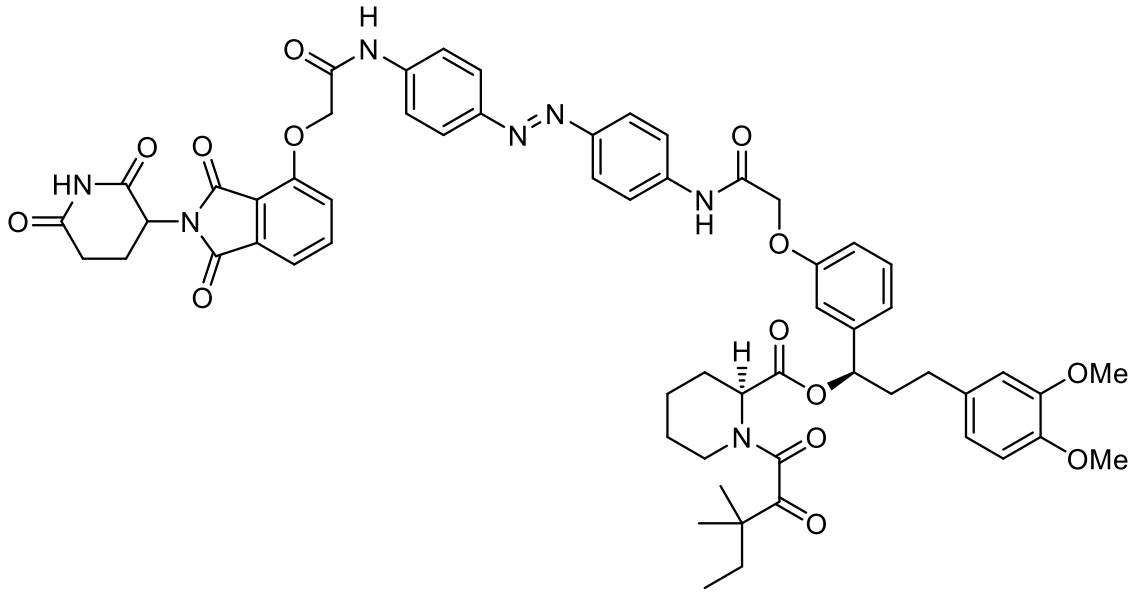
**HRMS** (ESI): calcd. for $C_{46}H_{37}ClN_{10}O_7SNa^+$: 931.2148 *m/z* $[M+Na]^+$

found: 931.2167 *m/z* $[M+Na]^+$.

**LCMS** $T_R$ = 4.642 min

Method: Azo 5% to 100%B over 5 min 1250 max mass

**(1R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-((4-((E)-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)phenyl)diazenyl)phenyl)amino)-2-oxoethoxy)phenyl)propyl (2S)-1-(3,3-dimethyl-2-oxopentanoyl)piperidine-2-carboxylate (PHOTAC-II-6)**



To a solution of **S37** (6.0 mg, 11.4 μmol, 1.0 eq.) and SLF free acid (7.3 mg, 12.5 μmol, 1.1 eq.) in DMF (1.2 mL) was added HATU (5.6 mg, 14.8 μmol, 1.3 eq.) and DIPEA (3 μL, 17.1 μmol, 1.5 eq.) at room temperature. The reaction was allowed to stir at room temperature overnight. Upon completion, the reaction was diluted with EtOAc and washed with water, $NaHCO_3$ and brine. The organics were dried over sodium sulfate and concentrated to afford an orange amorphous solid. The residue was purified by column chromatography using 0% →50% acetone in $CH_2Cl_2$ to afford the desired product **PHOTAC-II-6** (8.1 mg, 7.4 μmol, 65%) as an orange amorphous solid.

**$R_f$** = 0.15 [$CH_2Cl_2$:Acetone, 9:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 9.55 (s, 1H), 8.55 (d, *J* = 22.1 Hz, 1H), 8.24 (d, *J* = 3.7 Hz, 1H), 7.98 – 7.90 (m, 6H), 7.82 – 7.72 (m, 3H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.34 (t, *J* = 7.9 Hz,

1H), 7.24 (s, 1H), 7.11 – 7.03 (m, 1H), 7.03 – 6.99 (m, 1H), 6.93 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.82 – 6.73 (m, 1H), 6.67 (d, *J* = 6.2 Hz, 2H), 5.81 (dd, *J* = 8.0, 5.3 Hz, 1H), 5.34 (d, *J* = 5.6 Hz, 1H), 5.04 (dd, *J* = 12.2, 5.3 Hz, 1H), 4.79 (s, 2H), 4.65 (s, 2H), 3.85 (d, *J* = 5.9 Hz, 6H), 3.35 (s, 2H), 3.22 – 3.11 (m, 1H), 3.01 – 2.74 (m, 4H), 2.67 – 2.51 (m, 2H), 2.39 – 2.20 (m, 2H), 2.17 (d, *J* = 2.1 Hz, 4H), 2.07 (td, *J* = 9.0, 7.2, 3.9 Hz, 1H), 1.84 – 1.56 (m, 5H), 1.52 – 1.28 (m, 0H), 1.25 (s, 7H), 1.21 (d, *J* = 1.8 Hz, 5H), 0.87 (t, *J* = 7.4 Hz, 4H) ppm.

**$^{13}$C NMR** (100 MHz, $CDCl_3$) δ = 208.1, 170.9, 169.9, 168.0, 167.4, 166.6, 166.5, 166.3, 165.0, 157.3, 154.4, 149.5, 149.5, 149.0, 147.5, 142.2, 139.9, 139.3, 137.4, 133.6, 133.4, 130.3, 124.1, 124.0, 120.7, 120.3, 120.3, 120.1, 120.1, 118.6, 118.0, 114.1, 113.8, 111.9, 111.9, 111.4, 76.5, 68.5, 67.7, 56.1, 56.0, 53.9, 53.6, 51.4, 49.6, 46.9, 44.3, 38.3, 32.6, 31.6, 31.3, 31.1, 29.4, 26.5, 25.1, 23.5, 23.4, 22.7, 21.2, 14.3, 8.9 ppm.

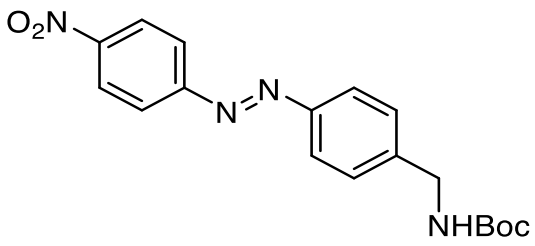
**HRMS** (ESI): calcd. for $C_{59}H_{62}N_7O_{14}^+$: 1092.4355 *m/z* $[M+H]^+$

found: 1092.4334 *m/z* $[M+H]^+$.

**LCMS** $T_R$ = 5.265 min

Method: Azo 5% to 100%B over 5 min 1250 max mass

## *tert*-butyl (*E*)-(4-((4-nitrophenyl)diazenyl)benzyl)carbamate (S22)




The following procedure was carried out in two steps:

**Oxone Oxidation**:

To a solution of 4-nitroaniline (566 mg, 4.1 mmol, 1.0 eq) in $CH_2Cl_2$ (14.6 mL) was added a solution of oxone (2.5 g, 4.1 mmol, 1.0 eq) in water (14.6 mL). The biphasic mixture was stirred vigorously under $N_2$ atmosphere. After 3 hours, phases were separated and the aqueous phase was extracted with $CH_2Cl_2$. The organic phases were combined and then washed with 1 M HCl, sat. NaCl, dried over $Na_2SO_4$ and concentrated under reduced pressure to approximately 5 mL. The resulting yellow-black solution of nitrosobenzene in $CH_2Cl_2$ was carried on to the next step immediately.

**Mills Reaction**:

To the nitrosobenzene solution in $CH_2Cl_2$, prepared as described above, was added sequentially tert-butyl (4-aminobenzyl)carbamate[3] (910 mg, 4.1 mmol, 1.0 eq) and glacial AcOH (1.2 mL, 20 mmol, 5.000 eq). The reaction mixture was allowed to stir for 15 hours under N2 atmosphere, after which time the reaction mixture was found to be an orange-black suspension. EtOAc was added and the organic phase was washed with 1 M NaOH, sat. $NaHCO_3$, sat. NaCl. The organic phase was then dried over $Na_2SO_4$ and concentrated under reduced pressure. Crude material was purified by flash column chromatography over $SiO_2$ using a gradient from 1% → 5% → 10% EtOAc in Hexanes as the eluent, affording **S22** (900 mg, 2.5 mmol, 62%) as a crystalline red solid.

$\mathbf{R}_f$ = 0.24 [Hexanes:EtOAc, 85:15].

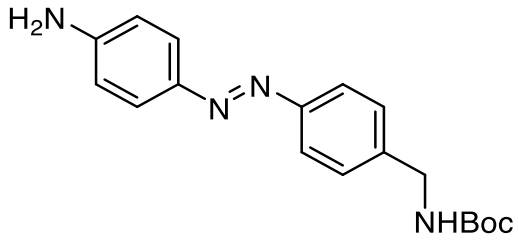
**$^{1}$H NMR** (400 MHz, Chloroform-*d*) δ = 8.37 (d, *J* = 8.8 Hz, 2H), 8.02 (d, *J* = 8.9 Hz, 2H), 7.94 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 4.98 (s, 1H), 4.42 (d, *J* = 5.3 Hz, 2H), 1.48 (s, 2H) ppm.

**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 156.0, 155.8, 151.8, 148.8, 144.0, 128.2, 124.9, 123.9, 123.6, 80.0, 44.5, 28.5 ppm.

**HRMS** (APCI): calcd. for $C_{13}H_{13}N_4O_2^+$: 257.1033 *m/z* $[M\text{-Boc}+H]^+$
found: 257.1041 *m/z* $[M\text{-Boc}+H]^+$.

**LCMS** $T_R$ = 4.736 min

## *tert*-butyl (*E*)-(4-((4-aminophenyl)diazenyl)benzyl)carbamate (S23)




To a solution of **S22** (100.0 mg, 270 µmol, 1.0 eq.) in dioxane (4.0 mL) and water (0.4 mL) in a pressure tube was added $Na_2S·9H_2O$ (202 mg, 842 µmol, 3.0 eq.). The reaction was sealed and heated to 85 °C. After 1 hour, the reaction was diluted with water and the aqueous layer was extracted 3 times with EtOAc. The organics were combined, washed with brine, dried over sodium sulfate and concentrated in vacuo to afford an orange-red solid. The reaction was loaded onto isolute and purified by column chromatography over $SiO_2$ using a stepped gradient from 9:1 →1:1 Hexanes/EtOAc as the eluent to afford **S23** (70.0 mg, 215 µmol, 76%) as a pale orange solid.

**R**$_f$ = 0.14 [Hexanes:EtOAc, 8:2].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 7.80 (dd, *J* = 8.6, 3.0 Hz, 4H), 7.37 (d, *J* = 8.1 Hz, 2H), 6.72 (d, *J* = 8.7 Hz, 2H), 4.96 (s, 1H), 4.36 (d, *J* = 6.0 Hz, 2H), 4.21 – 3.92 (m, 2H), 1.47 (s, 10H).

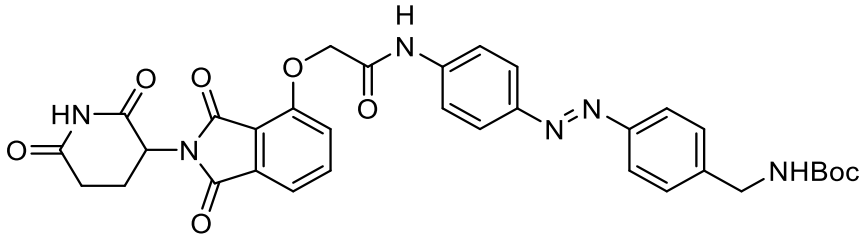
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 156.0, 152.3, 149.8, 145.6, 140.8, 128.1, 125.2, 122.7, 114.7, 79.7, 44.5, 28.5.

**HRMS** (APCI): calcd. for $C_{18}H_{23}N_4O_4^+$: 327.1816 *m/z* $[M+H]^+$

found: 327.1804 *m/z* $[M+H]^+$.

**LCMS** $T_R$ = 4.032 min

## *tert*-butyl (*E*)-(4-((4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)phenyl)diazenyl)benzyl)carbamate (S24)




Thalidomide-4-hydroxyacetate [2] (20.0 mg, 60.2 µmol, 1.0 eq) and **S23** (29.5 mg, 90.3 µmol, 1.5 eq) were dissolved in DMF (600 µL) followed by the addition of HATU (25.2 mg, 66.2 µmol, 1.1 eq) and DIPEA (21 µL, 120 µmol, 2.0 eq) at rt. The reaction was allowed to stir at rt overnight. The reaction was diluted with water, extracted 3 times with EtOAc, organics were combined, washed with bicarb and brine and dried over sodium sulfate. Concentration of organics and purification by column chromatography over $SiO_2$ using 8:1.5:0.5 DCM/EtOAc/MeOH as the mobile phase afforded **S24** (37 mg, 57.8 µmol, 96%) as an orange amorphous solid.

**R**$_f$ = 0.06 [Hexanes:EtOAc, 1:1].

**$^1$H NMR** (400 MHz, Acetone-$d_6$) δ = 10.03 (s, 1H), 9.84 (s, 1H), 8.05 – 7.93 (m, 4H), 7.93 – 7.82 (m, 3H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.51 (d, *J* = 8.1 Hz, 2H), 5.25 (dd, *J* = 12.5, 5.4 Hz, 1H), 5.00 (s, 2H), 4.39 (d, *J* = 6.3 Hz, 2H), 3.09 – 2.91 (m, 1H), 2.92 – 2.78 (m, 3H), 2.39 – 2.20 (m, 1H), 1.46 (s, 9H) ppm.

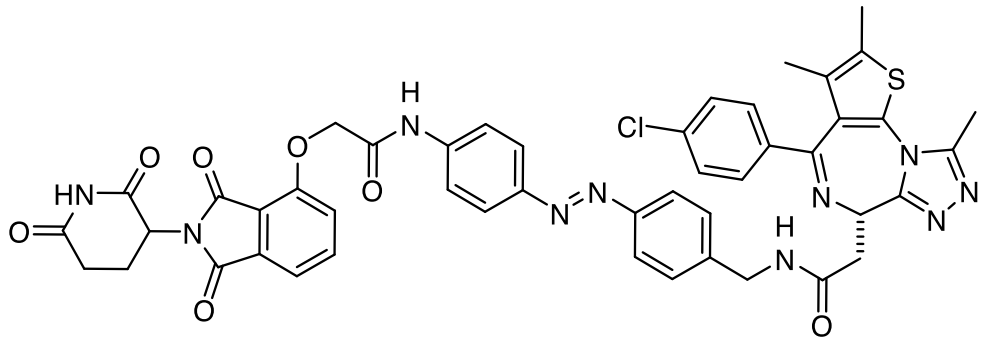
**$^{13}$C NMR** (101 MHz, Acetone) δ = 172.7, 170.1, 167.7, 167.6, 166.8, 157.0, 155.8, 152.6, 149.8, 144.7, 142.0, 138.1, 134.4, 128.9, 124.7, 123.6, 122.1, 120.5, 119.3, 117.9, 79.1, 69.7, 50.5, 44.6, 32.1, 28.7, 23.4 ppm ppm.

**HRMS** (ESI): calcd. for $C_{33}H_{32}N_6NaO_8^+$: 663.2174 *m/z* $[M+Na]^+$

found: 663.2178 *m/z* $[M+Na]^+$.

**LCMS** $T_R$ = 4.505 min

**2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-((*E*)-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)phenyl)diazenyl)benzyl)acetamide (PHOTAC-I-11)**



**S24** (10.0 mg, 15.6 µmol, 1.0 eq) was dissolved in formic acid (1.6 mL), immediately turning the solution to a deep red color, and allowed to stir overnight at rt. After this period, the solvent was evaporated in vacuo to afford an orange amorphous solid. To this was added (+)-JQ1 free acid (6.6 mg, 16.4 µmol, 1.05 eq) in DMF (0.66 mL), HATU (8.9 mg, 23.4 µmol, 1.5 eq), followed by DIPEA (5 µL, 31.2 µmol, 2.0 eq) and the reaction was allowed to stir overnight at room temperature. The reaction was diluted with water, extracted 3 times with EtOAc, organics were combined, washed with bicarb and brine and dried over sodium sulfate. The organic layer was concentrated in vacuo and the residue was purified by semi-preparative reverse phase HPLC (50% → 70% MeCN gradient + 0.01% formic acid) affording **PHOTAC-I-11** (6.0 mg, 6.5 µmol, 42%) as a yellow orange amorphous solid.

$\mathbf{R}_f$ = 0.29 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 9.58 (d, *J* = 8.8 Hz, 1H), 8.29 (d, *J* = 18.0 Hz, 1H), 7.96 (qd, *J* = 9.0, 2.8 Hz, 4H), 7.89 – 7.76 (m, 3H), 7.61 (dd, *J* = 7.3, 1.4 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.36 – 7.29 (m, 3H), 7.26 (d, *J* = 8.8 Hz, 1H), 5.06 (dd, *J* = 12.2, 5.2 Hz, 1H), 4.82 (d, *J* = 4.0 Hz, 2H), 4.79 – 4.65 (m, 2H), 4.46 (dd, *J* = 15.3, 5.3 Hz, 1H), 3.67 – 3.49 (m, 2H), 3.03 – 2.76 (m, 3H), 2.70 (s, 3H), 2.42 (s, 3H), 2.30 – 2.16 (m, 1H), 1.68 (s, 3H) ppm.

**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 170.9, 170.9, 170.7, 168.0, 166.6, 166.5, 165.1, 164.3, 155.7, 154.4, 152.1, 150.1, 149.5, 141.5, 140.0, 137.4, 137.1, 136.5, 133.6, 132.3, 131.1,

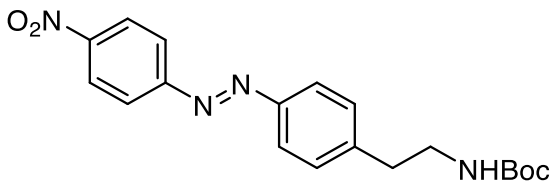
130.6, 130.0, 128.9, 128.5, 124.2, 123.2, 120.0, 120.0, 118.0, 68.5, 54.6, 49.6, 43.4, 41.2, 39.4, 31.6, 22.8, 14.6, 13.3, 12.0 ppm.

**HRMS** (APCI) calcd. for $C_{47}H_{43}ClN_{11}O_7S^+$: 940.2751 *m/z* $[M+NH_4]^+$

found: 940.2736 *m/z* $[M+NH_4]^+$.

**LCMS** $T_R$ = 4.422 min

## *tert*-butyl (*E*)-(4-((4-nitrophenyl)diazenyl)phenethyl)carbamate (S25)




The following procedure was carried out in two steps:

**Oxone Oxidation**:

To a solution of 4-nitroaniline (462 mg, 3.3 mmol, 1.0 eq.) in $CH_2Cl_2$ (12.0 mL) was added a solution of oxone (2.1 g, 3.3 mmol, 1.0 eq.) in water (12.0 mL). The biphasic mixture was stirred vigorously under $N_2$ atmosphere. After 3 hours, phases were separated, and the aqueous phase was extracted with $CH_2Cl_2$. The organic phases were combined and then washed with 1 M HCl, sat. NaCl, dried over $Na_2SO_4$ and concentrated under reduced pressure to approximately 5 mL. The resulting yellow-black solution of nitrosobenzene in $CH_2Cl_2$ was carried on to the next step immediately.

**Mills Reaction**:

To the nitrosobenzene solution in DCM, prepared as described above, was added sequentially *tert*-butyl (4-aminophenethyl)carbamate[4] (791.0 mg, 3.3 mmol, 1.0 eq.) and glacial AcOH (0.96 mL, 17 mmol, 5.0 eq.). The reaction mixture was allowed to stir for 15 hours under N2 atmosphere, after which time the reaction mixture was found to be an orange-black suspension. EtOAc was added and the organic phase was washed with 3x 1-M-NaOH, 2x sat. $NaHCO_3$, 2x sat. NaCl. The organic phase was then dried over $Na_2SO_4$ and concentrated under reduced pressure. Crude material was purified by flash column chromatography over $SiO_2$ using a gradient from 1% → 5% → 10% EtOAc in Hexanes as the eluent, affording affording **S25** (724.0 mg, 1.955 mmol, 58%) as a crystalline red solid.

$\mathbf{R}_f$ = 0.23 [Hexanes:EtOAc, 9:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.36 (dd, *J* = 8.8, 1.6 Hz, 2H), 8.00 (dd, *J* = 8.7, 1.4 Hz, 2H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.2 Hz, 2H), 4.62 (s, 1H), 3.43 (d, *J* = 5.1 Hz, 2H), 2.90 (t, *J* = 7.0 Hz, 2H), 1.44 (s, 9H) ppm.

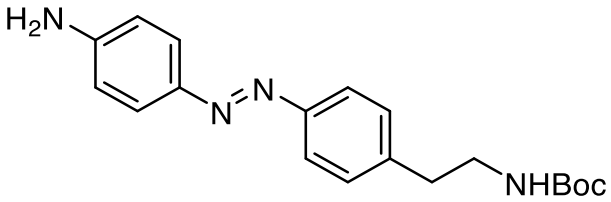
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 155.9, 155.9, 151.3, 148.7, 144.3, 129.9, 124.8, 123.8, 123.5, 79.6, 41.7, 36.4, 28.5 ppm.

**HRMS** (APCI): calcd. for $C_{14}H_{15}N_4O_2^+$: 271.1195 *m/z* $[M\text{-Boc}+H]^+$

found: 271.1190 *m/z* $[M\text{-Boc}+H]^+$.

**LCMS** $T_R$ = 4.858 min

## *tert*-butyl (*E*)-(4-((4-aminophenyl)diazenyl)phenethyl)carbamate (S26)




To a solution of **S25** (100.0 mg, 270 µmol, 1.0 eq.) in dioxane (4.0 mL) and water (0.4 mL) in a pressure tube was added $Na_2S{\cdot}9H_2O$ (195 mg, 810 µmol, 3.0 eq.). The reaction was sealed and heated to 85 °C. After 1 hour, the reaction was diluted with water and the aqueous layer was extracted 3 times with EtOAc. The organics were combined, washed with brine, dried over sodium sulfate and concentrated in vacuo to afford an orange-red solid. The reaction was loaded onto isolute and purified by column chromatography over $SiO_2$ using a stepped gradient from 9:1 →1:1 Hexanes/EtOAc as the eluent to afford **S26** (77.0 mg, 226 µmol, 84%) as a pale orange solid.

**$R_f$** = 0.64 [Hexanes:EtOAc, 6:4].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 7.80 (d, *J* = 4.5 Hz, 2H), 7.78 (d, *J* = 3 .9 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 6.73 (d, *J* = 8.8 Hz, 2H), 4.58 (s, 1H), 4.06 (s, 2H), 3.41 (d, *J* = 6.7 Hz, 1H), 2.86 (t, *J* = 7.0 Hz, 2H), 1.44 (s, 9H) ppm.

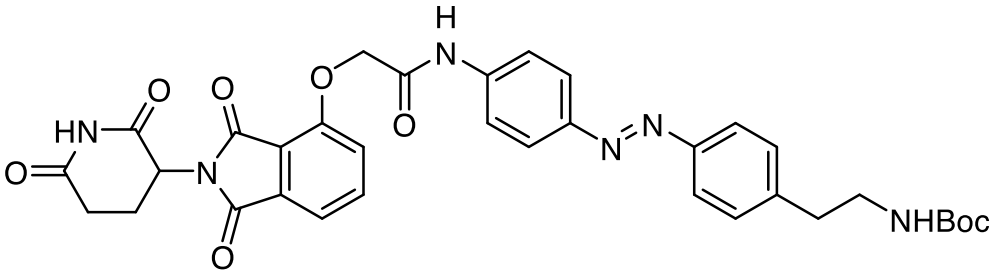
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 156.0, 151.8, 149.7, 145.7, 141.1, 129.5, 125.2, 122.7, 114.7, 79.4, 41.8, 36.1, 28.5 ppm.

**HRMS** (APCI): calcd. for $C_{19}H_{25}N_4O_2^+$: 341.1972 *m/z* $[M+H]^+$

found: 341.1973 *m/z* $[M+H]^+$.

**LCMS** $T_R$ = 4.141 min

## *tert*-butyl (*E*)-(4-((4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)phenyl)diazenyl)phenethyl)carbamate (S27)




Thalidomide-4-hydroxyacetate[2] (20.0 mg, 60.2 µmol, 1.0 eq.) and **S26** (20.0 mg, 60.2 µmol, 1.0 eq.) were dissolved in DMF (600 µL) followed by the addition of TBTU (25 mg, 66.2 µmol, 1.10 eq.) and DIPEA (21 µL, 120.4 µmol, 2.0 eq.) at rt. The reaction was allowed to stir at room temperature overnight upon which the reaction was diluted with EtOAc, the organics were washed three times with equal portions of water, saturated sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate and concentrated. The crude product was purified by column chromatography over $SiO_2$ using a gradient of 9:1 hexanes/EtOAc to 100% EtOAc as the eluent to afford product **S27** (38.0 mg, 58.0 µmol, 96%) as an orange amorphous solid.

**$R_f$** = 0.29 [Hexanes:EtOAc, 6:4].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 9.54 (s, 1H), 8.41 (d, *J* = 13.7 Hz, 1H), 7.92 (t, *J* = 2.8 Hz, 4H), 7.83 (dd, *J* = 8.3, 1.7 Hz, 2H), 7.80 – 7.70 (m, 1H), 7.63 – 7.51 (m, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 1H), 5.04 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.78 (d, *J* = 1.9 Hz, 2H), 4.60 (s, 1H), 3.41 (t, *J* = 6.7 Hz, 2H), 2.97 – 2.78 (m, 6H), 2.25 – 2.15 (m, 1H), 1.44 (s, 9H) ppm.

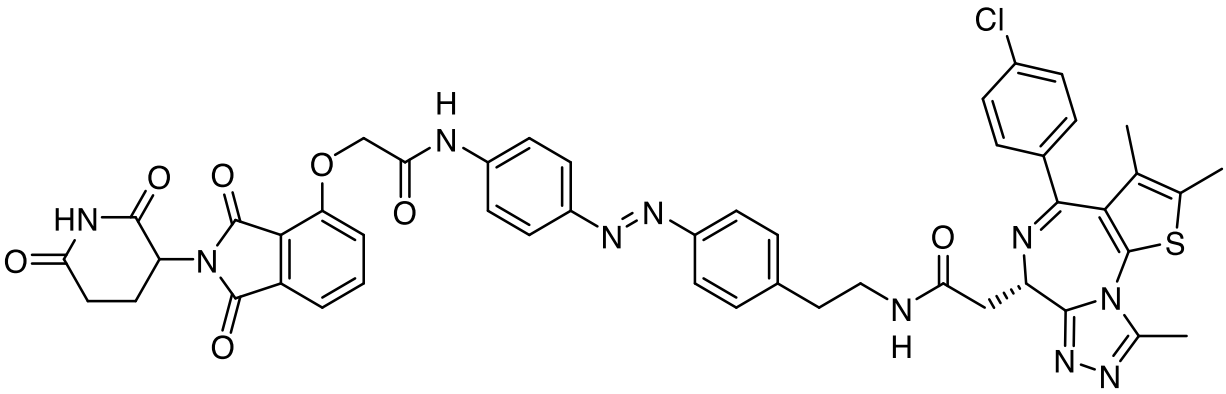
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 171.0, 168.1, 166.6, 166.5, 165.1, 156.0, 154.4, 151.5, 149.5, 142.4, 139.9, 137.4, 133.6, 129.7, 124.1, 123.1, 120.0 (overlap of two signals), 118.6, 118.0, 79.5, 68.5, 49.6, 41.8, 36.2, 31.6, 28.5, 22.7 ppm.

**HRMS** (ESI): calcd. for $C_{34}H_{35}N_6O_8$: 655.2511 *m/z* $[M+H]^+$

found: 655.2530 *m/z* $[M+H]^+$.

**LCMS** $T_R$ = 4.599 min

**2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-((E)-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)phenyl)diazenyl)phenethyl)acetamide (PHOTAC-I-12)**



**S27** (5.0 mg, 9.0 µmol, 1.0 eq) was dissolved in formic acid (1.5 mL0 and allowed to stir overnight. After this period the reaction was concentrated and azeotroped to afford an orange residue. This crude material and (+)-JQ1-free acid (4.0 mg, 9.9 µmol, 1.1 eq) were dissolved in DMF (400 µL) followed by the addition of HATU (4.5 mg, 11.7 µmol, 1.3 eq) and DIPEA (2.0 µL, 13.5 µmol, 1.5 eq) at rt. The reaction was allowed to stir at rt overnight. The reaction was diluted with water and extracted 3 times with EtOAc. The organic layers were combined, washed with saturated sodium bicarbonate and brine and dried over sodium sulfate. Concentration of organics and purification by column chromatography over $SiO_2$ using 8:1.5:0.5 DCM/EtOAc/MeOH as the mobile phase afforded **PHOTAC-I-12** (7.9 mg, 8.4 µmol, 94%) as an orange amorphous solid.

**$R_f$** = 0.17 [$CH_2Cl_2$:EtOAc:MeOH, 8:1.5:0.5].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 9.57 (d, *J* = 2.4 Hz, 1H), 8.13 (d, *J* = 2.8 Hz, 1H), 8.03 – 7.89 (m, 4H), 7.78 (dd, *J* = 8.0, 4.0 Hz, 3H), 7.61 (d, *J* = 2.0 Hz, 1H), 7.40 – 7.26 (m, 7H), 6.77 (s, 1H), 5.05 (dd, *J* = 11.9, 5.0 Hz, 1H), 4.81 (s, 2H), 4.56 (t, *J* = 6.9 Hz, 1H), 3.76 – 3.45 (m, 3H), 3.31 (dd, *J* = 14.2, 5.8 Hz, 1H), 3.02 – 2.76 (m, 5H), 2.66 (s, 3H), 2.37 (s, 3H), 2.27 – 2.18 (m, 1H), 1.66 (s, 3H) ppm.

**$^{13}$C NMR** (101 MHz, Chloroform-*d*) δ = 170.8, 170.5, 167.9, 166.6, 166.5, 165.1, 164.2, 155.6, 154.4, 151.4, 150.0, 149.5, 142.3, 139.9, 137.4, 137.1, 136.4, 133.6, 132.1, 131.2, 131.1, 130.6,

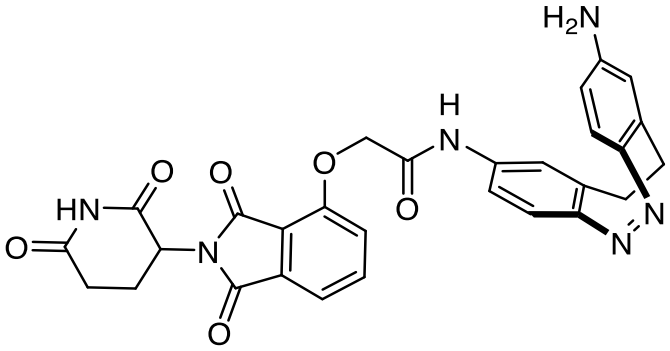
130.0, 129.6, 128.9, 124.1, 123.0, 120.1 (overlap 2 peaks), 118.7, 118.1, 68.5, 54.6, 49.6, 40.5, 39.6, 35.6, 31.6, 22.8, 14.5, 13.2, 12.0 ppm.

**HRMS** (ESI): calcd. $C_{48}H_{42}ClN_{10}O_7S$: 937.2642 *m/z* $[M+H]^+$

found: 937.2675 *m/z* $[M+H]^+$.

**LCMS** $T_R$ = 4.599 min

## (Z)-N-(9-amino-11,12-dihydrodibenzo[c,g][1,2]diazocin-2-yl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamide (S28)



Thalidomide-4-hydroxyacetate (20.0 mg, 60 µmol, 1.0 eq.) and (Z)-11,12-dihydrodibenzo[c,g][1,2]diazocine-2,9-diamine[5] (21.5 mg, 90 µmol, 1.5 eq.) were dissolved in DMF (0.6 mL) followed by the addition of TBTU (21.3 mg, 66 µmol, 1.1 eq.) and DIPEA (21 µL, 120 µmol, 2.0 eq.) at rt. The reaction was allowed to stir at rt overnight. The reaction was diluted with $CH_2Cl_2$ and successively washed with water, saturated sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate and concentrated. The residue was purified by column chromatography over silica using a 1% to 3% MeOH in $CH_2Cl_2$ as the eluent to afford **S28** (17.0 mg, 31 µmol, 51%) as an amorphous yellow solid. This product was contaminated with an unknown impurity and used in the next step.

**$R_f$** = 0.08 [$CH_2Cl_2$:MeOH, 95:5].

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.12 (s, 1H), 10.10 (s, 1H), 7.80 (ddd, *J* = 8.5, 7.3, 4.2 Hz, 1H), 7.50 (d, *J* = 7.2 Hz, 1H), 7.47 – 7.38 (m, 2H), 7.38 – 7.30 (m, 1H), 6.81 (dd, *J* = 30.2, 8.4 Hz, 1H), 6.63 – 6.51 (m, 1H), 6.36 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.20 (d, *J* = 2.3 Hz, 1H), 5.13 (q, *J* = 6.5, 5.5 Hz, 2H), 4.96 (d, *J* = 3.2 Hz, 2H), 2.90 (s, 2H), 2.76 – 2.55 (m, 6H), 2.08 – 1.92 (m, 1H) ppm.

**$^{13}$C NMR** (101 MHz, DMSO) δ = 172.8, 169.9, 166.7, 165.6, 165.5, 162.3, 155.2, 151.4, 147.9, 145.4, 136.9, 136.7, 133.0, 129.5, 128.5, 121.0, 120.5, 119.5, 117.4, 116.7, 116.0, 113.6, 111.9, 67.4, 48.8, 31.6, 31.2, 30.9, 22.0 ppm.

**HRMS** (APCI): calcd. $C_{29}H_{25}N_6O_6$: 553.1836 *m/z* $[M+H]^+$

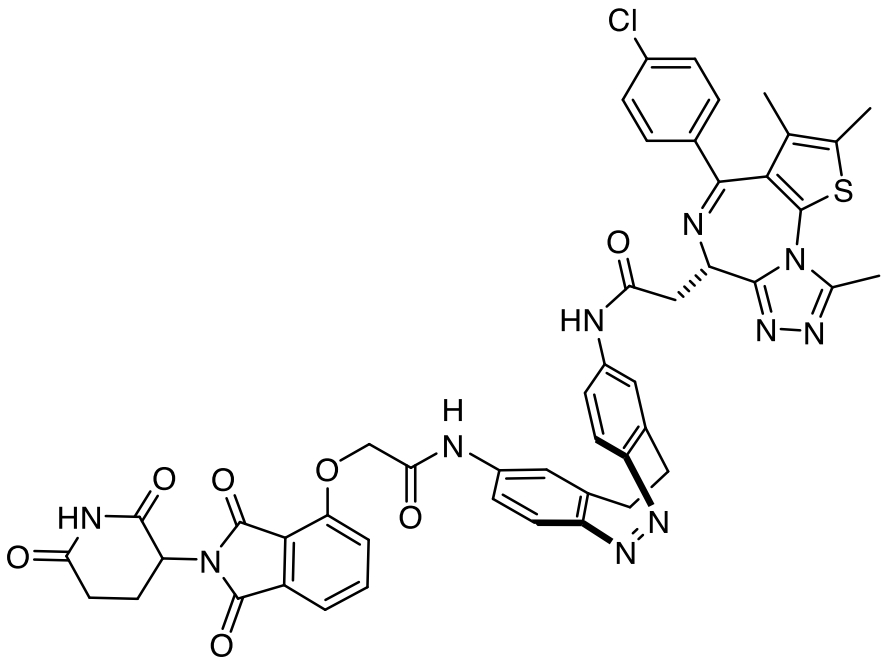
found: 553.1828 *m/z* $[M+H]^+$.

Chemical Formula: C29H25N6O6

Exact Mass: 553.1836

**LCMS** $T_R$ = 2.931 min

**2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-((Z)-9-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)-11,12-dihydrodibenzo[c,g][1,2]diazocin-2-yl)acetamide (PHOTAC-I-13)**




To a solution of **S28** (5.0 mg, 9.0 µmol, 1.0 eq.) and (+)-JQ1 free acid (4.0 mg, 10 µmol, 1.1 eq.) in DCE (1.8 mL) was added TBTU (3.8 mg, 11.8 µmol, 1.3 eq.) followed by DIPEA (1.8 mg, 2.0 µL, 13.6 µmol, 1.5 eq.) at room temperature. The reaction was allowed to stir overnight upon which the reaction was diluted with EtOAc, washed with equal portions of water, saturated sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography over $SiO_2$ using a gradient of 0% → 10% MeOH in $CH_2Cl_2$ as the eluent to afford **PHOTAC-I-13** (5.9 mg, 5.9 µmol, 65%) as a amorphous yellow solid.

**$R_f$** = 0.39 [$CH_2Cl_2$: MeOH, 95:5].

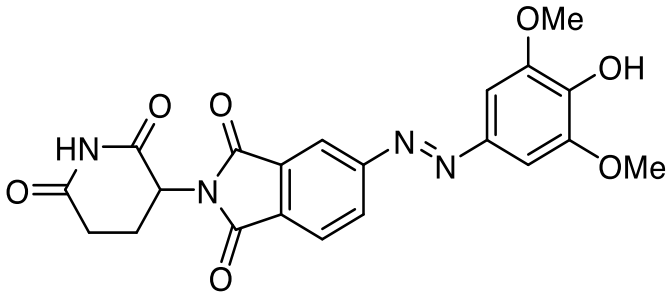
**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 9.23 (d, *J* = 122.5 Hz, 2H), 7.84 – 7.74 (m, 1H), 7.75 – 7.63 (m, 1H), 7.60 (dd, *J* = 7.4, 3.4 Hz, 1H), 7.42 (dd, *J* = 8.6, 2.6 Hz, 2H), 7.38 – 7.32 (m, 2H), 7.25 (dd, *J* = 8.5, 6.2 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 5.12 – 4.93 (m, 1H), 4.73 (d, *J* = 8.6 Hz, 2H), 4.61 (dd, *J* = 9.2, 4.7 Hz, 1H), 3.79 (ddd, *J* = 22.4, 14.1,

9.1 Hz, 1H), 3.44 (ddd, *J* = 19.4, 14.0, 4.8 Hz, 1H), 3.04 – 2.75 (m, 7H), 2.68 (d, *J* = 4.3 Hz, 3H), 2.64 (d, *J* = 0.8 Hz, 5H), 2.42 (d, *J* = 2.4 Hz, 3H), 2.31 – 2.15 (m, 1H), 1.72 – 1.60 (m, 3H) ppm.

**$^{13}$C NMR** (101 MHz, Chloroform-*d*) δ = 168.5, 168.3, 166.5, 164.8, 164.7, 155.4, 154.4, 152.1, 150.1, 137.2, 136.2, 133.5, 131.3, 131.0, 130.5, 129.9, 129.9, 129.2, 128.8, 120.5, 120.2, 118.7, 118.0, 117.8, 68.8, 68.5, 54.6, 54.4, 49.4, 41.0, 31.5, 31.5, 22.6, 14.4, 14.4, 13.1, 11.7 ppm.

| | | |
|---|---|---|
| **HRMS** (APCI): | calcd. $C_{48}H_{40}ClN_{10}O_7S$: | 935.2491 *m/z* $[M+H]^+$ |
| | found: | 935.2478 *m/z* $[M+H]^+$. |

## (E)-2-(2,6-dioxopiperidin-3-yl)-5-((4-hydroxy-3,5-dimethoxyphenyl)diazenyl)isoindoline-1,3-dione (S29)




To a solution of 5-aminothalidomide (200 mg, 0.73 μmol, 1.0 eq) and $NaNO_2$ (424 μl, 848 μmol, 1.16 equiv) in acetone/water (4:1, 8 mL) was added 4 equiv. of HCl (4.0 M in 1,4-dioxane, 732 μL, 2.9 mmol, 4.0 equiv) at 0 °C. After stirring for 1 h, the solution was added in a dropwise fashion to a mixture of 2,6-dimethoxyphenol, (135 mg, 0.88 mmol, 1.2 equiv), $NaHCO_3$ (1.5 g, 18.1 mmol, 24.7 equiv), $Na_2CO_3$ (3.7 g, 34.5 mmol, 47.2 equiv) in water/MeOH (5:2, 28 mL) at 0 °C and allowed to stir for an additional hour. After this time period, the reaction was quenched with sat. $NH_4Cl$ and extracted with EtOAc. The organic layers were combined and washed with brine and concentrated under reduced pressure. The residue was purfied by column chromatography over $SiO_2$ using 4:6 Hexanes/EtOAc as the eluent to afford **S29** (49.0 mg, 112 μmol, 15%) as a red solid.

**$R_f$** = 0.23 [Hexanes:EtOAc, 4:6].

**$^1$H NMR** (400 MHz, Acetone-$d_6$) δ = 9.82 (s, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.08 (d, *J* = 2.1 Hz, 1H), 6.98 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.97 (s, 2H), 5.04 (dd, *J* = 12.6, 5.4 Hz, 1H), 3.02 – 2.88 (m, 1H), 2.81 (s, 6H), 2.79 – 2.76 (m, 3H), 2.75 – 2.68 (m, 1H), 2.26 – 2.12 (m, 1H) ppm.

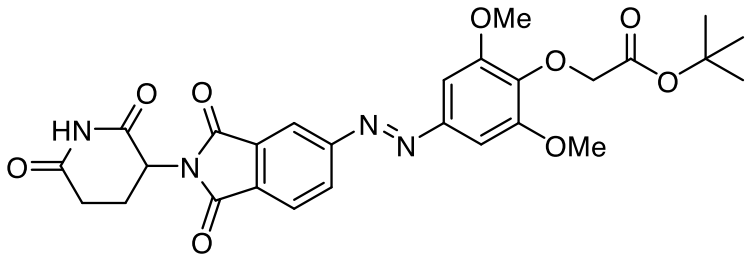
**$^{13}$C NMR** (101 MHz, Acetone) δ = 171.7, 169.0, 166.6, 166.6, 156.7, 148.2, 144.9, 141.1, 133.4, 132.1, 129.7, 124.6, 115.1, 101.7, 55.8, 49.7, 31.1, 22.4 ppm.

**HRMS** (APCI): calcd. for $C_{21}H_{19}N_4O_7^+$: 439.1248 *m/z* $[M+H]^+$

found: 439.1251 *m/z* $[M+H]^+$.

**LCMS $T_R$** = 3.180 min

## tert-butyl (E)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)diazenyl)-2,6-dimethoxyphenoxy)acetate (S30)




**S29** (35.0 mg, 78.8 µmol, 1.0 eq) was dissolved in DMF (0.8 mL) at room temperature. To this was added $K_2CO_3$ (16.6 mg, 0.12 mmol, 1.5 equiv), immediately turning the solution a blue-black color, followed by the addition of *tert*-butyl bromoacetate (16.4 mg, 83.8 µmol, 1.05 eq). The reaction was allowed to stir at room temperature for 2 hours upon which the reaction was quenched with saturated aqueous $NH_4Cl$. The aqueous layer was extracted three times with EtOAc, the organics were combined, washed with brine, dried over sodium sulfate and concentrated. The residue was purified by column chromatography over $SiO_2$ using a gradient of 0 → 30% EtOAc in DCM to afford **S30** (17.0 mg, 30.8 µmol, 39%) as an orange film.

**$R_f$** = 0.47 [$CH_2Cl_2$:EtOAc, 7:3].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.37 (d, *J* = 1.6 Hz, 1H), 8.29 (dd, *J* = 7.9, 1.7 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 8.00 (s, 1H), 7.34 (s, 2H), 5.05 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.74 (s, 2H), 3.99 (s, 6H), 3.02 – 2.70 (m, 4H), 2.31 – 2.14 (m, 1H), 1.51 (s, 9H) ppm.

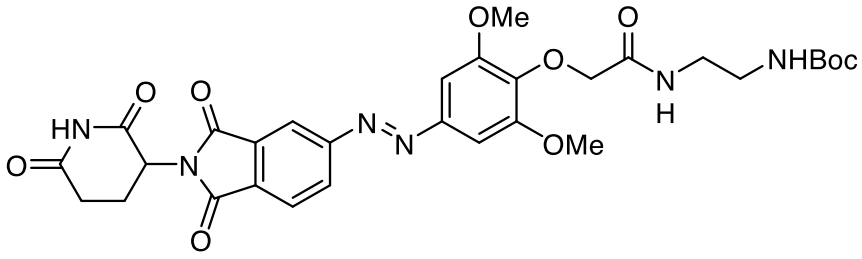
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 170.7, 168.3, 167.8, 166.8, 166.7, 156.9, 152.8, 147.9, 140.6, 133.2, 132.3, 130.1, 129.8, 125.2, 125.0, 119.4, 117.0, 101.5, 81.9, 70.0, 56.5, 49.7, 31.6, 29.9, 28.3, 22.8 ppm.

**HRMS** (APCI): calcd. for $C_{23}H_{21}N_4O_9^+$: 497.1303 *m/z* $[M\text{-}t\text{Bu}+H]^+$

found: 497.1292 *m/z* $[M\text{-}t\text{Bu}+H]^+$.

**LCMS $T_R$** = 3.383 min

## ***tert*-butyl (*E*)-(2-(2-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)ethyl)carbamate (S31)**




**S30** (8.0 mg, 14.5 µmol, 1.0 eq.) was dissolved in formic acid (1.4 mL), immediately turning the solution to a deep red color, and allowed to stir overnight at rt. After this period, the solvent was evaporated in vacuo to afford an orange-red amorphous solid. To this was added *N*-Boc-ethylene diamine (2.8 mg, 17.4 µmol, 1.2 eq.) in DMF (1.4 mL), HATU (8.3 mg, 21.7 µmol, 1.5 eq.), followed by DIPEA (5 µL, 3.7 mg, 28.7 µmol, 2.0 eq.) and the reaction was allowed to stir overnight at room temperature. The reaction was diluted with water, extracted 3 times with EtOAc, organics were combined, washed with bicarb and brine and dried over sodium sulfate. The organic layer was concentrated in vacuo and the residue was purified column chromatography over $SiO_2$ using 0% → 3% MeOH in $CH_2Cl_2$ as the eluent to affod **S31** (5.7 mg, 8.9 µmol, 62%) as an orange film.

**$R_f$** = 0.09 [$CH_2Cl_2$:MeOH, 97:3].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.35 (d, *J* = 1.5 Hz, 1H), 8.28 (dd, *J* = 7.9, 1.7 Hz, 1H), 8.17 (s, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.91 – 7.83 (m, 1H), 7.33 f(s, 2H), 5.03 (dd, *J* = 12.2, 5.5 Hz, 1H), 4.90 (s, 1H), 4.63 (s, 2H), 4.01 (s, 6H), 3.49 (d, *J* = 6.0 Hz, 2H), 3.38 – 3.24 (m, 2H), 3.02 – 2.63 (m, 3H), 2.28 – 2.09 (m, 1H), 1.43 (s, 9H) ppm.

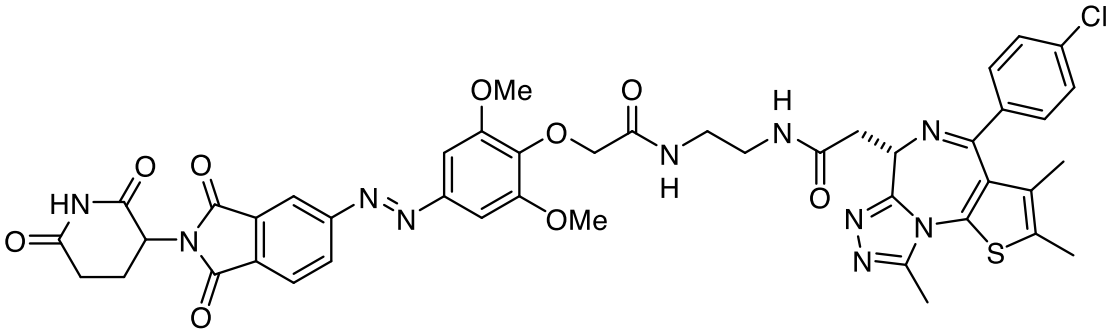
**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 170.8, 170.3, 167.9, 166.7, 166.7, 156.7, 156.2, 152.8, 148.6, 140.4, 133.2, 132.6, 130.2, 125.0, 116.9, 101.3, 79.7, 72.8, 56.5, 49.7, 40.9, 39.2, 31.6, 28.5, 22.8 ppm.

**HRMS** (APCI): calcd. for $C_{25}H_{27}N_6O_8^+$: 539.1885 *m/z* $[M\text{-}Boc+H]^+$

found: 539.1873 *m/z* $[M\text{-}Boc+H]^+$.

**LCMS** $T_R$ = 3.953 min

## 2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(2-(2-(4-((E)-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)diazenyl)-2,6-dimethoxyphenoxy)acetamido)ethyl)acetamide (PHOTAC-I-9)



**S31** (5.7 mg, 8.9 µmol, 1.0 equiv) was dissolved in formic acid and allowed to stir at room temperature for 30 min. After this period, the reaction was concentrated *in vacuo* and azeotroped. The crude residue was used immediately without further purification. This crude material and (+)-JQ1-free acid (3.9 mg, 9.8 µmol, 1.1 eq) were dissolved in DMF (400 µL) followed by the addition HATU (3.7 mg, 9.8 µmol, 1.1 eq) and DIPEA (2.3 µL, 17.9 µmol, 2.0 eq) at rt. The reaction was allowed to stir at rt overnight. The reaction was diluted with water and extracted 3 times with EtOAc. The organic layers were combined, washed with saturated sodium bicarbonate and brine and dried over sodium sulfate. Concentration of organics and purification by column chromatography over $SiO_2$ using 0% → 10% MeOH in DCM as the mobile phase afforded **PHOTAC-I-9** (4.1 mg, 4.4 µmol, 50%) as an orange amorphous solid.

**$R_f$** = 0.23 [$CH_2Cl_2$:MeOH, 9:1].

**$^1$H NMR** (400 MHz, Chloroform-*d*) δ = 8.32 (d, *J* = 1.5 Hz, 1H), 8.26 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.16 (s, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 6.4 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 3H), 7.31 (s, 2H), 5.03 (dd, *J* = 12.3, 5.4 Hz, 1H), 4.69 (t, *J* = 6.8 Hz, 1H), 4.62 (s, 2H), 3.99 (s, 6H), 3.65 – 3.35 (m, 6H), 3.02 – 2.71 (m, 3H), 2.69 (s, 3H), 2.40 (s, 3H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.67 (s, 3H), 1.67 – 1.55 (m, 1H) ppm.

**$^{13}$C NMR** (101 MHz, $CDCl_3$) δ = 176.9, 171.0, 170.8, 170.5, 167.9, 166.8, 166.7, 164.7, 156.7, 155.4, 152.8, 150.3, 148.6, 140.5, 137.5, 136.0, 135.9, 133.1, 132.5, 131.3, 130.2, 130.2, 129.0, 129.0, 125.0, 117.0, 101.4, 72.8, 56.6, 53.6, 49.7, 40.0, 38.8, 33.7, 32.1, 31.6, 24.9, 22.8, 22.8, 14.6, 13.3, 11.9 pmm.

**HRMS** (APCI): calcd. for $C_{44}H_{42}ClN_{10}O_9S^+$: 921.2545 *m/z* $[M+H]^+$

found: 921.2519 *m/z* $[M+H]^+$.

**LCMS $T_R$** = 4.089 min

Method: Azo 5% to 100%B over 5 min 1250 max mass

## Cell culture procedures

The human acute lymphoblastic leukemia RS4;11 (ATCC® CRL1873™) cell line was purchased from the American Type Culture Collection and cultured in RPMI1640 medium (Gibco) with 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin (PS) in a humidified incubator at 37 °C with 5% $CO_2$ in air. For the experiments compounds were serially diluted in RPMI1640 without the dye phenol red (Gibco) to reduce the influence of the dye by absorption. Azobenzene stocks and dilutions were strictly kept in the dark and prepared under red light conditions.

For immunoblotting analysis, cells ($2x10^6$ for RS4:11) were incubated for the indicated times with PHOTACs, placed in a light-proof box and preirradiated for 1 min at 390 nm followed by 100 ms pulses every 10 s or were kept in the dark for 4 h. After incubation, cells were collected in the dark by centrifugation (150 g, 5 min) at 4 °C and the pellets were washed twice with ice cold PBS (1 mL).

## Colorimetric MTT Assays

The activity of dehydrogenase enzymes in metabolically active cells, as a quantitative measurement for cytotoxicity and proliferation, was determined by colorimetric measurement of the reduction of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to the formazan derivative. The absorbance of formazan at 490 nm was measured on a FLUOstar Omega microplate reader (BMG Labtech). Cells were treated with different concentrations, ranging of our compounds (from 10 $\mu$M to 1 nM/1 pM) in triplicates or sextuplicates, using 1% DMSO as cosolvent, and incubated on a 96-well plate for 72 h. They were placed in light-proof boxes and exposed to the lighting conditions specified in the experiment for 72 h. Next, 10 $\mu$L of Promega CellTiter 96® $AQ_{ueous}$ One Solution Reagent was added to each well and incubated for further 4-7 hours at 37 °C. The absorbance at 490 nm was then recorded with a 96-well plate reader.

Data was analyzed using GraphPad Prism Version 5.01 (GraphPad Software Inc) and fitted using the Sigmoidal dose-response (variable slope) fit. Results represent the mean viability ± SEM relative to the 1% DMSO treated control.

## Immunoblotting Analysis

Cells were lysed in RIPA buffer containing protease and phosphatase inhibitor and protein concentration was determined using BCA (Thermo Fisher). Immunoblotting was performed as previously described (Marzio et al., 2019).[6] Briefly, samples were resolved under denaturing and reducing conditions using 4%–12% Bis-Tris gels (NuPAGE) and transferred to a PDVF membrane (Immobilon-P, Millipore). Membranes were blocked with 5% nonfat dried milk, incubated with primary antibodies overnight at 4°C. After washing the membranes, secondary antibodies coupled with horseradish peroxidase were applied (Amersham-GE). Immunoreactive bands were visualized by enhanced chemiluminescence reagent (Thermo Fisher Scientific) and signal was acquired using ImageQuant LAS 400 (GE).

| Antibodies | Source | Identifier |
|---|---|---|
| BRD2 | Bethyl | A302-583A |
| BRD3 | Bethyl | A302-368A-1 |
| BRD4 | Cell Signaling Technology | #13440 |
| c-MYC | Cell Signaling Technology | #5605 |
| p21 | Cell Signaling Technology | #2947 |
| p27 | BD Biosciences | cat. No. 610241 |
| PARP1 | Cell Signaling Technology | cat. No. 9542S |
| PCNA | dako | cat. No. M0879 |
| αTUBULIN | Sigma Aldrich | T6557 |
| γTUBULIN | Sigma Aldrich | T6074 |
| FKBP12 | Santa Cruz Biotechnology | cat. No. sc-133067 |
| CUL4A | Bethyl | A300-739A |
| MCM2 | Santa Cruz Biotechnology | cat. No. sc-9839 |
| Skp1 | Michele Pagano's Lab | N/A |
| anti-Rabbit IgG, peroxidase-linked antibody | Thermo Fisher | NA934 |
| anti-Mouse IgG, peroxidase-linked antibody | Thermo Fisher | NA931 |
| anti-goat IgG-HRP | Santa Cruz Biotechnology | cat. No. sc-2354 |

## LED illumination

For illumination of the cells we used the cell disco system as previously described in the literature.[7] 5 mm LEDs 370 nm (XSL-370-5E), 390 nm (VL390-5-15), 410 nm (VL410-5-15), 430 nm (VL430-5-15), 450 nm (ELD-450-525), 465 nm (RLS-B465), 477 nm (RLS-5B475-5), 490 nm (LED490-03), 505 nm (B5-433-B505), 525 nm (B5-433-B525), 545 nm (LED545-04), 572 nm (B5-433-20) and 590 nm (CY5111A-WY) were purchased from Roithner Lasertechnik. For experiments using 390 nm, cells were preirradiated for 1 min at

390 nm to quickly switch the photoswitches in the active state. Pulsed irradiation was performed using 100 ms pulses every 10 s in 96- or 6-well plates, controlled by an Arduino system.

## Determination of Photophysical properties

For UV-VIS studies on the Varian Cary 60 UV-Visible Spectrophotometer, samples were stored and prepared under red light to avoid formation of the (*Z*)-isomers. 10 mM stock solutions were prepared in the dark and diluted to a final concentration of 25 µM for measurement. UV-VIS spectra of **PHOTACs** following irradiation with different wavelengths for 5 min using a monochromator. Measurement was started from the dark adapted state followed by 370 nm and further increasing the wavelength. By increasing the wavelength from low to high we can observe how much we can switch from (*Z*)- to (*E*)-isomers, whereas going from high to low wavelength, the PSS might not be reached due to low absorptivity above 500 nm. **PHOTAC-I-1–8** were measured in PBS with 10% DMSO, whereas **PHOTAC-II-1–6** were measured in DMSO.

Thermal relaxation was measured by preirradiating **PHOTACs** with 390 nm irradiation and observing the absorption at 370 nm over 12 h at 37 °C in DMSO in tightly sealed cuvettes.

Reversible switching and photochemical stability of **PHOTAC-I-3** was demonstrated in DMSO, cycling the irradiation of the monochromator between 390 nm (3 min) and 500 nm (7 min).

Separated spectra of the (*Z*)- and (*E*)-isomers could be obtained from the internal UV-VIS detector of the LCMS, by irradiating the sample before injection, and were normalized at the isosbestic point. In the case of non-solvochromic photoswitches, photostationary states were calculated in the region of largest absorption difference between 330 and 390 nm, from the separated spectra obtained by LCMS and the spectra obtained following irradiation with different wavelengths for 5 min, all normalized to the isosbestic point.

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