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SJPYT-195: A Designed Nuclear Receptor Degrader That Functions as a Molecular Glue Degrader of GSPT1

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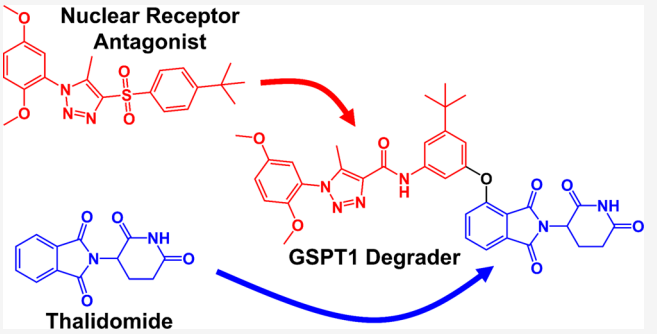
ABSTRACT: We previously reported a specific inverse agonist (SPA70) of the nuclear receptor pregnane X receptor (PXR). However, derivatization of SPA70 yielded only agonists and neutral antagonists, suggesting that inverse agonism of PXR is difficult to achieve. Therefore, we sought to design proteolysis targeting chimeras (PROTACs) aimed at inducing PXR degradation. Conjugation of a SPA70 derivative to ligands of the E3 substrate receptor cereblon (CRBN) resulted in one molecule, SJPYT-195, that reduced PXR protein level in an optimized degradation assay described here. Further analysis revealed that SJPYT-195 was a molecular glue degrader of the translation termination factor GSPT1 and that GSPT1 degradation resulted in subsequent reduction of PXR protein. GSPT1 has recently gained interest as an anticancer target, and our results give new insights into chemical determinants of drug-induced GSPT1 degradation. Additionally, we have developed assays and cell models for PXR degrader discovery that can be applied to additional protein targets.



KEYWORDS: *PROTAC, molecular glue, pregnane X receptor, GSPT1, acute myeloid leukemia*

Over the past decade, targeted protein degradation has emerged as a useful tool to expand the scope of druggable proteins encoded by the human genome. In 2010, the CUL4-RBX1-DDB1-CRBN ($CRL4^{CRBN}$) E3 ubiquitin ligase complex was identified as the molecular target of the immunomodulatory drug thalidomide,[1] and it was subsequently found that thalidomide and its derivatives act as "molecular glues" that bind to the E3 substrate receptor CRBN, thereby altering the substrate proteins recognized by $CRL4^{CRBN}$.[2,3] Shortly thereafter, linkage of thalidomide to various target protein ligands resulted in proteolysis targeting chimeras (PROTACs) that specifically induced $CRL4^{CRBN}$-mediated degradation of a range of proteins, such as the epigenetic readers BRD2, BRD3, and BRD4.[4] The demonstration that thalidomide-linked molecules could induce degradation of predetermined target proteins resulted in an explosion of the targeted protein degradation field. Importantly, this finding also led to small molecules that induce protein degradation by two distinct modes. PROTACs are bivalent molecules with an E3 ubiquitin ligase ligand linked to a ligand for the protein of interest; molecular glue-type degraders, on the other hand, are monovalent and facilitate specific protein−protein interactions.[5]

Thalidomide was widely used in the 1950s and 1960s as a sedative and morning sickness treatment; however, it was discontinued due to teratogenic effects. Because of extensive biological characterization, thalidomide and derivatives lenalidomide and pomalidomide have now been reclassified as immunomodulatory drugs and approved for use as multiple myeloma treatments.[6] Antimyeloma activity is attributed to $CRL4^{CRBN}$-mediated degradation of the transcription factors IKZF1 and IKZF3, which play critical roles in B cell development and are overexpressed in B cell malignancies.[2,3] Further derivatization of thalidomide resulted in CC-885 and CC-90009, potent cytotoxic agents that induce degradation of the translation termination factor G1 To S phase transition protein 1 homologue (GSPT1).[7,8] GSPT1 is upregulated in many cancers, particularly hematopoietic malignancies, and acute leukemia cells have been shown to be highly sensitive to GSPT1 degradation.[7−10] GSPT1 is therefore a potential drug target for future chemotherapies.

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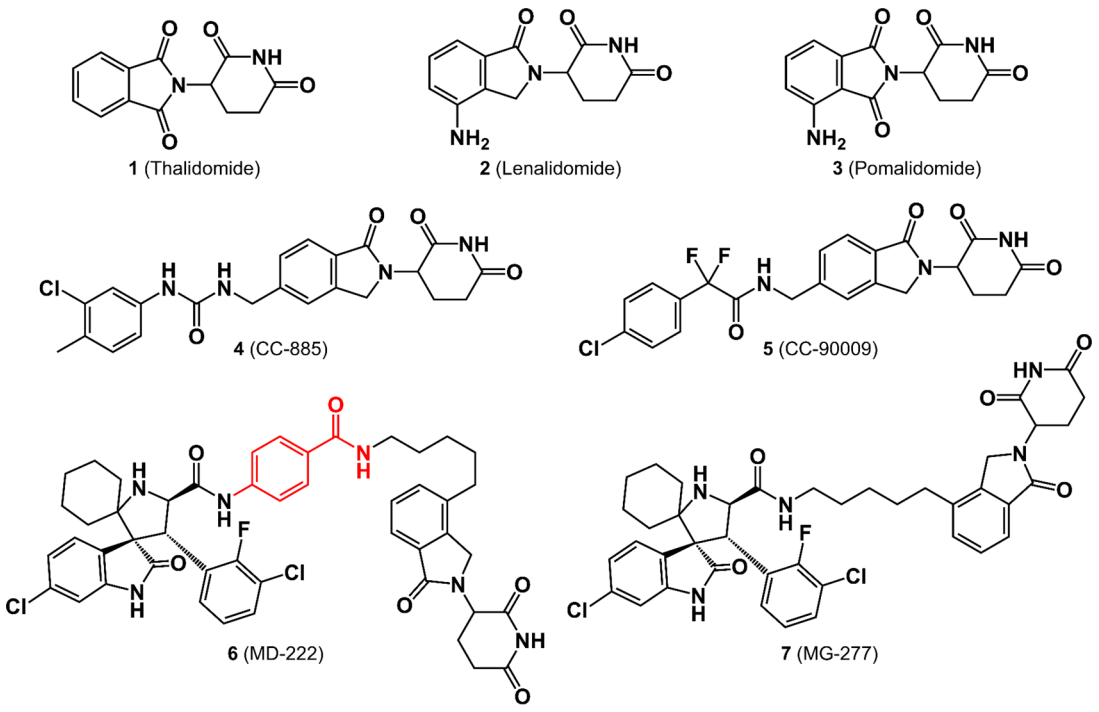

Figure 1. Thalidomide analogues and conjugates. In **6** (MD-222), the region removed to create **7** (MG-277) is colored in red.

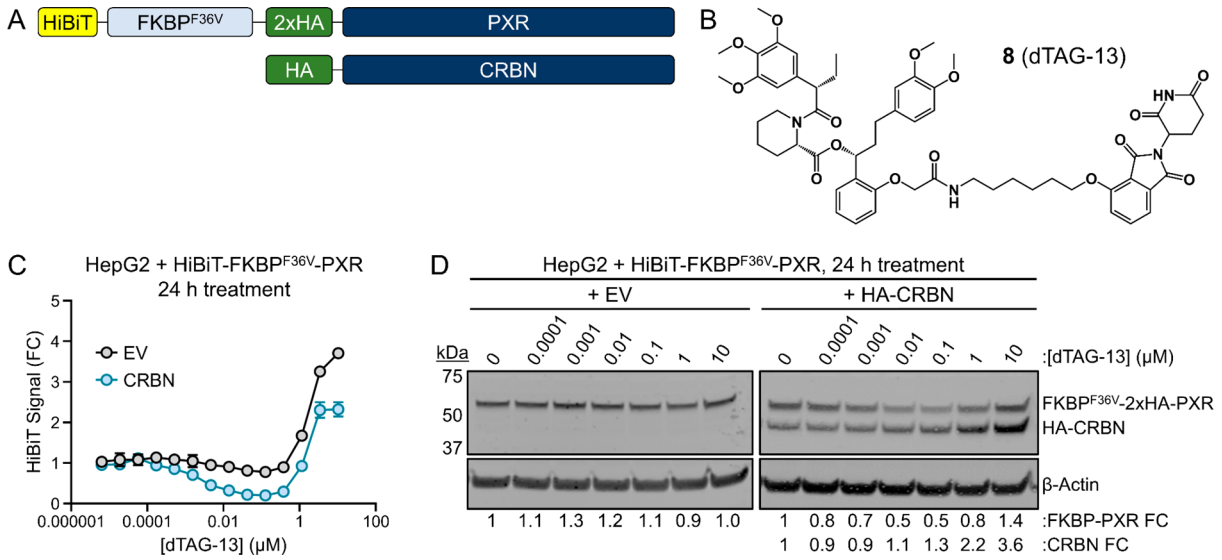

Figure 2. Assay development for PXR degrader screening. (A) Schematic of HiBiT-FKBP12^{F36V}-PXR and CRBN expression constructs. (B) Chemical structure of dTAG-13. (C) HepG2 cells were cotransfected with HiBiT-FKBP12^{F36V}-PXR and either an empty vector (EV) control or a CRBN-expressing vector. Cells were treated with dTAG-13 for 24 h and assessed for the HiBiT signal. FC, fold change relative to DMSO control. (D) HepG2 cells were cotransfected with HiBiT-FKBP12^{F36V}-PXR and either an empty vector (EV) control or a CRBN-expressing vector. Cells were treated with dTAG-13 for 24 h, and Western blot was performed with antibodies against HA or β-actin. Values represent the fold change compared to the DMSO-treated lane.

Because CRL4^{CRBN}-directed PROTACs contain a glutarimide moiety that binds CRBN,[11] these molecules have the potential to either (1) specifically induce degradation of the target protein of interest or (2) act as molecular glues that induce degradation of unintended targets. A class of phthalimide-conjugated molecules designed to degrade kinases was previously shown to degrade GSPT1 through a molecular glue mechanism rather than the intended kinases through the PROTAC mechanism.[12] Furthermore, a simple structural modification to the MDM2-degrading PROTAC MD-222 resulted in MG-277 with a loss of MDM2 degradation and a corresponding gain of GSPT1 degradation.[13] These findings indicate the importance of off-target evaluation in PROTAC development. However, conversion of PROTACs to molecular glues can also be a means of deriving new chemical matter that reprograms CRL4^{CRBN} substrate specificity. Representative phthalimide-based molecular glues and PROTACs are shown in Figure 1.

Pregnane X receptor (PXR) is a nuclear receptor that transcriptionally regulates genes encoding key drug-metaboliz-

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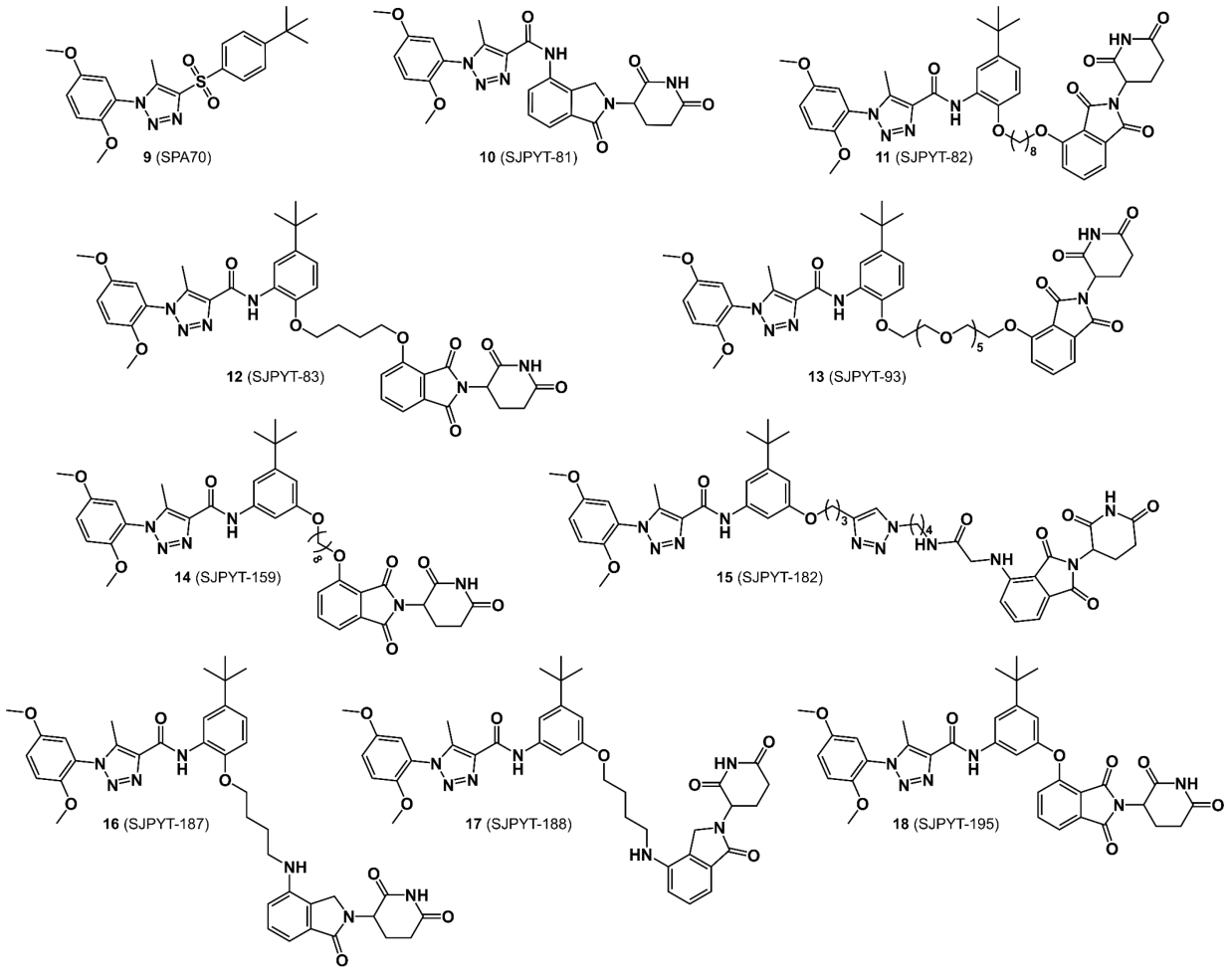

Figure 3. SPA70 and derived conjugates.

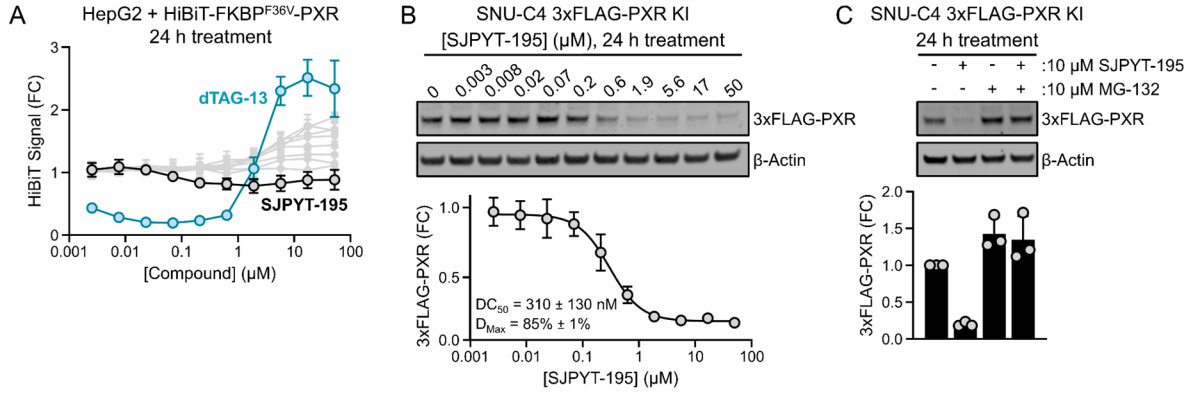

Figure 4. SJPYT-195 reduces PXR protein. (A) HepG2 cells were cotransfected with HiBiT-FKBP12^{F36V}-PXR and CRBN vectors. Cells were treated with compounds for 24 h and assessed for HiBiT signal. (B) SNU-C4 3xFLAG-PXR KI cells were treated with DMSO or SJPYT-195 for 24 h, and Western blot was performed with antibodies against FLAG or β-actin. (C) SNU-C4 3xFLAG-PXR KI cells were treated with the indicated compounds for 24 h, and Western blot was performed with antibodies against FLAG or β-actin.

ing enzymes and transporters.[14−23] We previously reported the discovery of a potent and selective inverse agonist (SPA70) of PXR.[24,25] Derivatization of SPA70 yielded only antagonists and agonists, suggesting that PXR inverse agonism is difficult to achieve.[26] Furthermore, a single PXR mutation (W299A) converts SPA70 from an inverse agonist to an agonist.[27] Therefore, as an alternative approach to developing compounds that inhibit transcriptional transactivation by PXR, we turned to the possibility of chemically induced protein degradation. No PXR degrader has been previously reported so to establish a method for discovery of PXR degraders in the absence of a known PXR-degrading control, we used the degradation tag (dTAG) system.[28] With this method, a protein of interest (PXR) is fused to a cytosolic prolyl isomerase, FKBP12, with Phe36 mutated to Val (FKBP12^{F36V}) (Figure 2A). A PROTAC molecule, dTAG-13 (Figure 2B), is then

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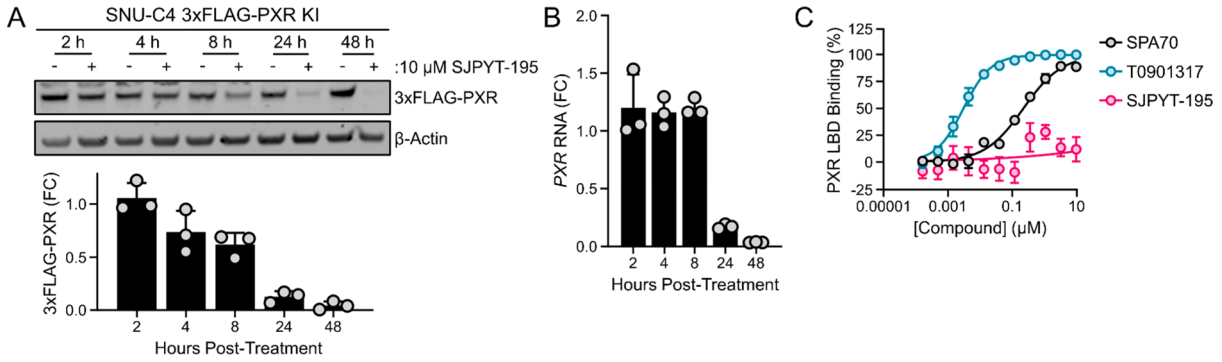

Figure 5. SJPYT-195 effects on PXR are inconsistent with direct PXR degradation. (A) SNU-C4 3xFLAG-PXR KI cells were treated with DMSO or 10 μM SJPYT-195 for the indicated times, and Western blot was performed with antibodies against FLAG or β-actin. (B) SNU-C4 3xFLAG-PXR KI cells were treated with DMSO or 10 μM SJPYT-195 for the indicated times, and RT-qPCR was used to assess the *PXR* RNA level. Results for (A) and (B) are shown as FC relative to the DMSO control at each time point. (C) TR-FRET assay was performed to assess binding of compounds to PXR LBD.

used to induce degradation of the fusion protein through recruitment of the $CRL4^{CRBN}$ E3 ubiquitin ligase to $FKBP12^{F36V}$ without affecting the endogenous wild-type FKBP12 protein. An HA tag was added for Western blot detection, and a HiBiT tag[29] was fused to the N-terminus to allow high-throughput plate-based quantification of HiBiT-$FKBP12^{F36V}$-PXR protein levels (Figure 2A). When overexpressed in HepG2 cells, only a slight reduction in HiBiT signal was observed with dTAG-13 (Figure 2C). However, when CRBN was co-overexpressed, dTAG-13 potently reduced HiBiT signal (Figure 2C). This was also observed when the HA tag was detected by Western blot (Figure 2D). The HiBiT detection was more sensitive to protein level changes than Western blot, and importantly, overexpression of CRBN was required to achieve effective dTAG-13-mediated degradation of the overexpressed HiBiT-$FKBP12^{F36V}$-PXR protein. A hook effect was observed as expected, and the potency of dTAG-13 in this assay was comparable to previously reported results.[28]

After successful validation of our HiBiT-based high-throughput assay, we next performed a focused screen for compounds that reduce PXR protein level. We synthesized nine compounds that linked PXR ligands to CRBN ligands (Figure 3) and tested them against HiBiT-$FKBP12^{F36V}$-PXR, using dTAG-13 as an assay control. Most compounds either had no effect or increased HiBiT signal, but one compound, SJPYT-195, dose-dependently reduced HiBiT signal (Figure 4A). To assess SJPYT-195 activity against endogenous PXR, we used CRISPR/Cas9 to knock in a 3xFLAG tag to the PXR N-terminus in the colorectal SNU-C4 cell line (henceforth referred to as SNU-C4 3xFLAG-PXR KI cells). SJPYT-195 potently and efficaciously reduced endogenous PXR protein in this system, with a half maximal degradation concentration (DC_{50}) of 310 ± 130 nM and maximum degradation efficacy (D_{Max}) of 85 ± 1% (Figure 4B). The loss of PXR protein was dependent on the proteasome, evidenced by a rescue of PXR levels by the proteasome inhibitor MG-132 (Figure 4C).

We next performed time-course analyses of PXR protein and mRNA levels to further study the mechanism of PXR protein loss. The kinetics of PXR protein decrease in response to SJPYT-195 was quite slow (Figure 5A), contrary to the expectation that a direct protein degrader generally results in rapid protein loss. Furthermore, SJPYT-195 drastically reduced the *PXR* RNA level after 24 h of treatment (Figure 5B). Lastly, we found that unlike SPA70 and the potent PXR ligand T0901317, SJPYT-195 only weakly bound the PXR ligand binding domain (LBD) (Figure 5C). These results suggest that SJPYT-195 may indirectly reduce PXR protein through, for example, a transcriptional or translational mechanism. However, the observation that PXR protein decrease occurs at earlier time points than RNA decrease indicates that the effect is at least partially independent of transcription defects.

To determine additional potential cellular SJPYT-195 targets, we used tandem mass tag mass spectrometry (TMT-MS) to quantify protein changes in SNU-C4 3xFLAG-PXR KI cells at the whole-proteome scale. First, we identified a treatment time point that markedly decreased PXR protein without affecting RNA (Figure 6A). At 12 h, 5 μM SJPYT-195 reduced PXR protein ~50% with negligible reduction of PXR RNA. Next, we performed TMT-MS analysis for cells treated for 12 h with either 5 μM SJPYT-195 or DMSO control (Figure 6B and Table S1). The analysis identified five downregulated proteins (GSPT1, GSPT2, ZFP91, CYP1A1, and BRIP1) and one upregulated protein (FOS). SJPYT-195 was remarkably selective, only downregulating five proteins at the relatively high treatment concentration (5 μM) and long duration (12 h). However, we cannot rule out that additional targets exist that were simply not detected by this approach. Nevertheless, our data set had high coverage of 9692 unique proteins, representing the majority of expressed human proteome in a specific cell type. Proteins at extremely low abundance, such as PXR, were not observed in the data.

GSPT1 is a translation termination factor that participates in nascent protein release from ribosomes.[30−32] Loss of GSPT1 results in translation defects that could lead to the decrease of unstable or lowly abundant proteins, such as PXR. Using Western blot, we validated that SJPYT-195 does indeed deplete GSPT1 at concentrations much lower than those required to reduce PXR (Figure 6C). The activity of SJPYT-195 on GSPT1 was ~100-fold lower than that of the previously reported GSPT1 degrader CC-885, which is the most potent GSPT1 degrader known to date.[7] MG-132 rescued GSPT1 in the presence of SJPYT-195, indicating that GSPT1 loss was proteasome-dependent (Figure 6D). Furthermore, siRNA-mediated knockdown of CRBN protected GSPT1 from degradation, showing that the protein loss was dependent on CRBN (Figure 6E). In addition to degrading GSPT1, CC-885 also reduced PXR protein, suggesting that PXR reduction is a secondary effect of GSPT1 degradation

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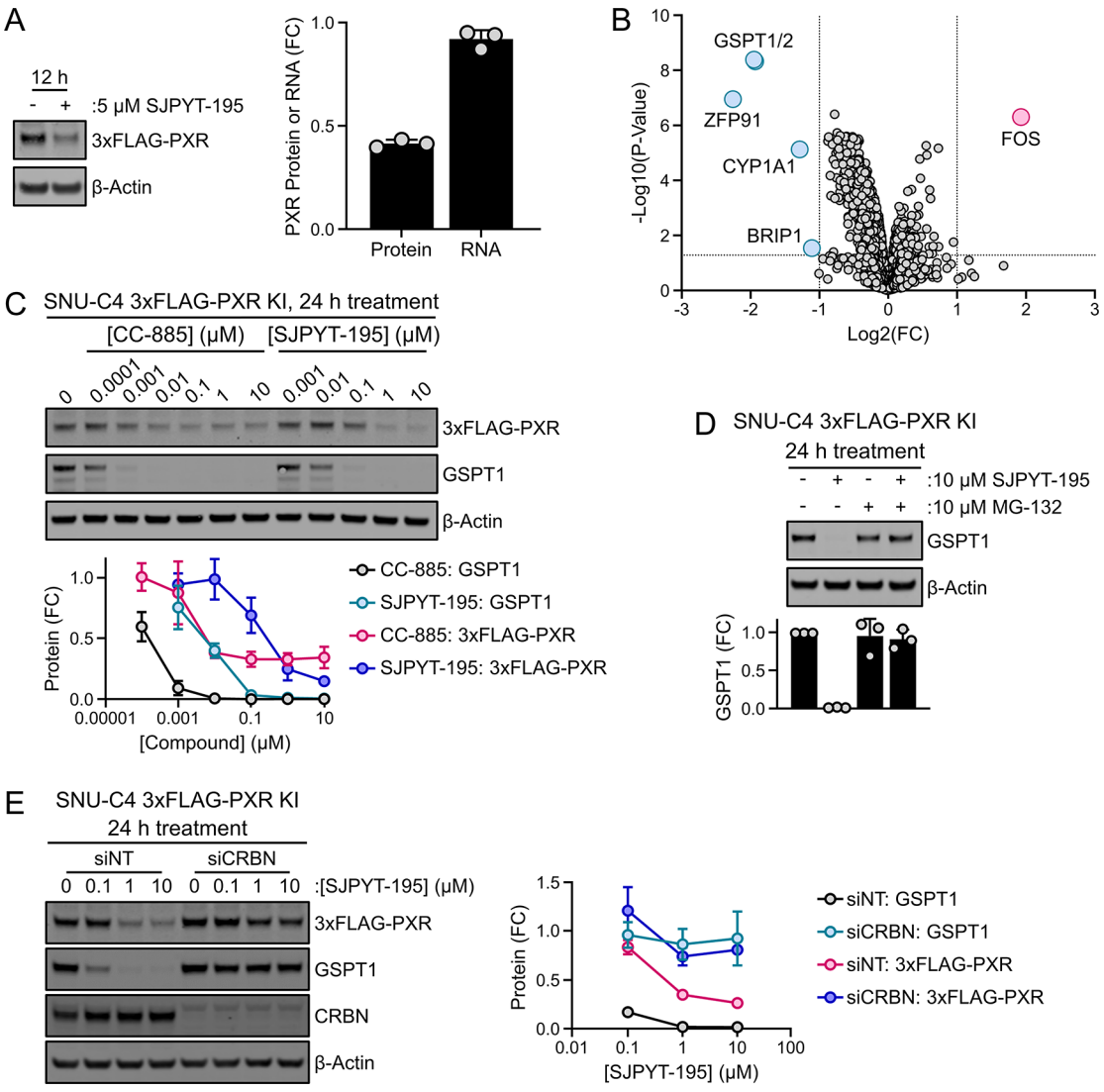

Figure 6. SJPYT-195 significantly reduces GSPT1 protein level. (A) SNU-C4 3xFLAG-PXR KI cells were treated with DMSO or 5 μM SJPYT-195 for 12 h, Western blot was performed with antibodies against FLAG or β-actin, and RT-qPCR was used to assess the *PXR* RNA level. Results in the bar graph are fold changes relative to the respective DMSO control samples. (B) SNU-C4 3xFLAG-PXR KI cells were treated with DMSO or 5 μM SJPYT-195 for 12 h, and TMT-MS was performed. The volcano plot compares SJPYT-195 vs DMSO. (C) SNU-C4 3xFLAG-PXR KI cells were treated with CC-885 or SJPYT-195 for 24 h, and Western blot was performed with antibodies against FLAG, GSPT1, or β-actin. (D) SNU-C4 3xFLAG-PXR KI cells were treated with the indicated compounds for 24 h, and Western blot was performed with antibodies against GSPT1 or β-actin. (E) SNU-C4 3xFLAG-PXR KI cells were transfected with either nontargeting (NT) control siRNA or siRNA targeting CRBN. After 72 h, cells were treated with the indicated concentrations of SJPYT-195 for 24 h, and Western blot was performed with antibodies against FLAG, GSPT1, CRBN, or β-actin.

(Figure 6C). Interestingly, though, SJPYT-195 reduced PXR to a greater extent than CC-885. Therefore, SJPYT-195 may impact PXR protein level by more than one mechanism. This conclusion is supported by the observation that CRBN knockdown completely rescued GSPT1 from SJPYT-195-mediated degradation but only partially rescued PXR (Figure 6E).

GSPT1 degraders are actively studied as potential anticancer agents, and CC-885 is a potent killer of various cell models.[7] We tested the cytotoxicity of CC-885 and SJPYT-195 in the SNU-C4 3xFLAG-PXR KI cells and found half maximal cytotoxic concentration (CC_{50}) values of 3.3 ± 0.3 and 440 ± 80 nM, respectively (Figure 7A). The 133-fold difference in CC_{50} values correlated well with the observed differences in potencies for GSPT1 degradation in Figure 6C. To assess the role of GSPT1 degradation in mediating the cytotoxicity of SJPYT-195, we generated SNU-C4 3xFLAG-PXR KI cell lines stably expressing either empty vector (EV), HA-tagged wild-type GSPT1, or HA-tagged $GSPT1^{G575N}$, a mutant that was previously shown to be resistant to molecular glue-mediated degradation.[7] Overexpression of $GSPT1^{G575N}$ rendered the cells partially resistant to both CC-885 and SJPYT-195, suggesting that compound cytotoxicity is mediated through GSPT1 degradation (Figure 7B,C). Full rescue could not be achieved because the plasmid-derived GSPT1 was expressed at a significantly lower level than the endogenous GSPT1 (Figure 7D). By Western blot, the overexpressed GSPT1 was well-separated from endogenous GSPT1 because we utilized the short isoform that lacks residues 1−138 of the long isoform. In SNU-C4 cells, the long isoform appears to be the dominantly expressed protein and therefore migrates more slowly in the gel than the overexpressed short isoform.

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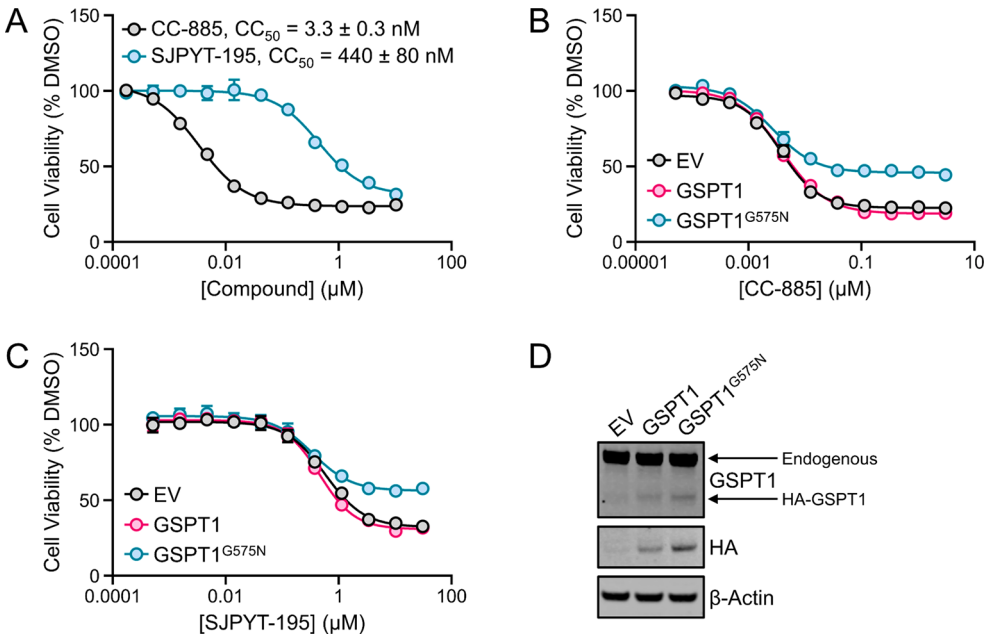

Figure 7. SJPYT-195 is a cytotoxic GSPT1 degrader. (A) SNU-C4 3xFLAG-PXR KI cells were treated with CC-885 or SJPYT-195 for 72 h, and cell viability was assessed by CellTiter-Glo. (B,C) SNU-C4 3xFLAG-PXR KI cells stably overexpressing empty vector (EV), HA-GSPT1, or HA-GSPT1^{G575N} were treated with CC-885 or SJPYT-195 for 72 h, and cell viability was assessed by CellTiter-Glo. (D) SNU-C4 3xFLAG-PXR KI cells stably overexpressing empty vector (EV), HA-GSPT1, or HA-GSPT1^{G575N} were subjected to Western blot with antibodies against GSPT1, HA, or β-actin.

To evaluate the relationship between SJPYT-195 structure and GSPT1 degradation, we synthesized nine analogues with varying linker lengths and compositions and truncations of the PXR ligand-derived component (Figure 8). Western blot analysis showed that all analogues had substantially reduced activity for both PXR and GSPT1 (Figure 9A,B and Table 1). However, most compounds showed similar binding affinity to CRBN itself (Figure 9C and Table 1), suggesting that either recruitment or ubiquitination of GSPT1 is impaired in the inactive analogues. In fact, SJPYT-231 had the strongest CRBN binding of all tested compounds but did not affect either PXR or GSPT1 levels.

The results seem to suggest that short linkers and large substituents are favored for GSPT1 degradation by this particular class of chemicals. Importantly, CRBN binding did not correlate with GSPT1 degradation. For example, by truncating the dimethoxyphenyl triazole part of the PXR ligand, SJPYT-216, SJPYT-217, and SJPYT-231 still maintained strong CRBN binding but lost the degradation activity for GSPT1. This indicates that the integrity of the PXR ligand is crucial to the degradation of GSPT1, possibly by influencing the molecular glue-induced CRBN-GSPT1 interface. On the other hand, although SJPYT-223 had greatly reduced CRBN binding, it still exhibited strong GSPT1 degradation activity, indicating that the short three-atom linker was beneficial to GSPT1 degradation activity (compared to the longer linkers of SJPYT-220 and SJPYT-226). For SJPYT-226 (four-atom linker), amino acetylation on the CRBN ligand also greatly reduced CRBN binding. The other four-atom linker compounds (SJPYT-219, SJPYT-220, SJPYT-228, and SJPYT-229) all retained strong binding of CRBN, with SJPYT-219, SJPYT-228, and SJPYT-229 showing strong GSPT1 degradation. SJPYT-220 only weakly degraded GSPT1, possibly due to an unstable ester bond of the phenolic hydroxyl group. Placement of hydrogen bond donors and acceptors in the linker had a profound impact on CRBN binding and GSPT1 degradation (SJPYT-219 versus SJPYT-220; SJPYT-217 versus SJPYT-231). In SJPYT-220, a linker hydrogen bond donor NH (SJPYT-219) was replaced with the hydrogen bond acceptor O. This substitution did not alter CRBN binding affinity but substantially reduced the GSPT1 degrading activity. Interestingly, substitution at this same position of molecules with single atom linkers and truncated PXR ligands had different effects. SJPYT-231 (NH) had 3-fold better CRBN binding but 2-fold worse GSPT1 degradation than SJPYT-217 (O). These observations indicate that the combined compositions of linkers and the linked substituents impact biological outcomes in context-specific manners related to the total R group. With the full PXR ligand and only a one-atom linker, SJPYT-195 was the most potent inducer of GSPT1 degradation in the group of chemicals.

In summary, through efforts to obtain chemicals that induce degradation of the nuclear receptor PXR, we instead identified SJPYT-195 as a new subclass of GSPT1-degrading small molecules. The small molecule CC-885 was previously identified as a GSPT1 degrader and potent cytotoxic agent in acute myeloid leukemia (AML) cell lines,[7] suggesting that GSPT1 is a potential anticancer target. RNA sequencing analysis in pediatric tumor samples has also shown that GSPT1 is overexpressed in hematopoietic malignancies like AML.[10] These observations have led to interest in developing GSPT1-degrading small molecules. SJPYT-195 shows promise as a lead compound for further modification and study, and we have already shown that a chemical truncation of this molecule produces a higher affinity CRBN binder than thalidomide or pomalidomide (SJPYT-231, Figure 9C and Table 1). Thus, our study has identified chemical matter for future use as (1) GSPT1 degraders or (2) high-potency CRBN ligands in PROTAC synthesis.

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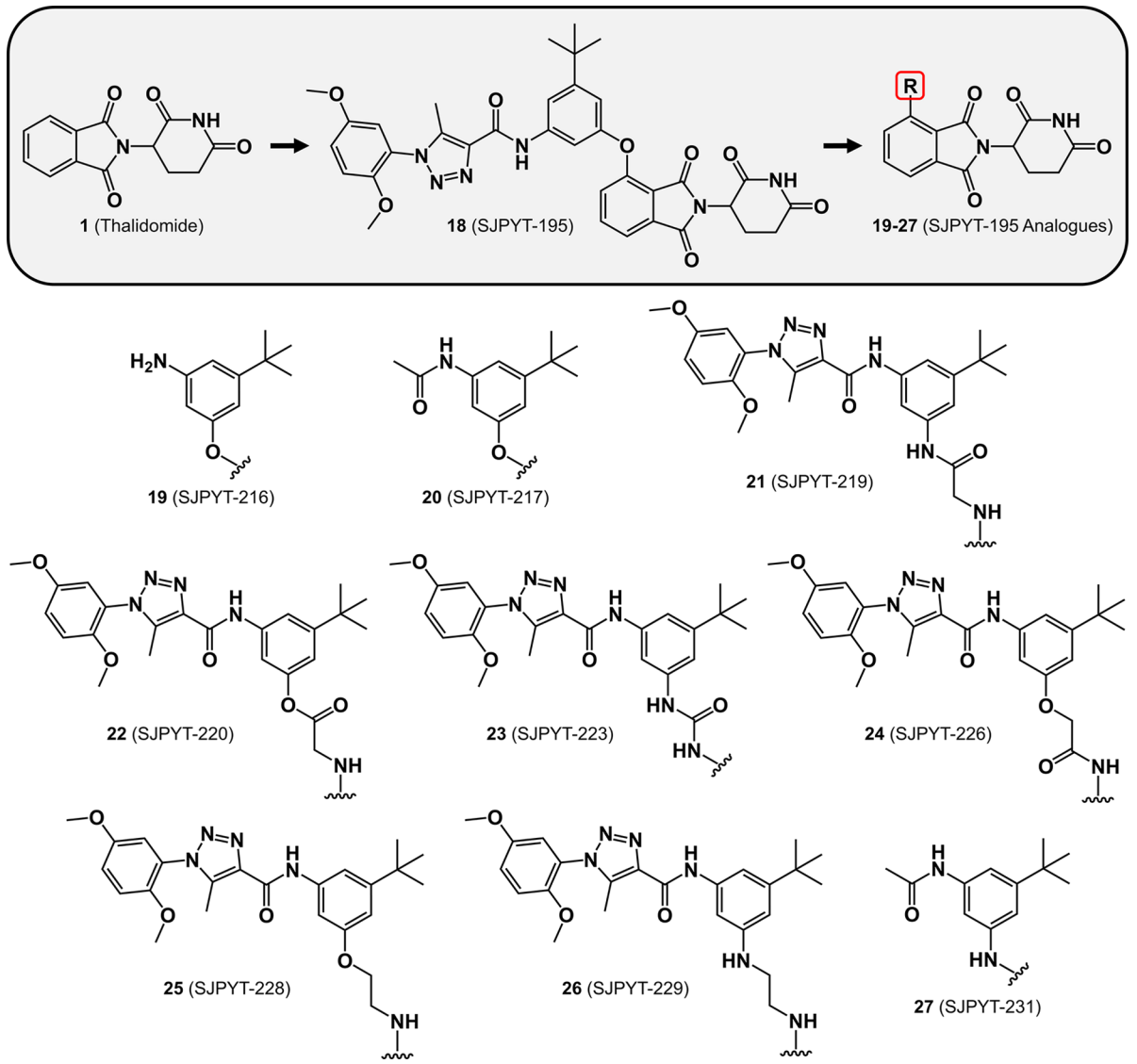

Figure 8. Chemical structures of SJPYT-195 analogues.

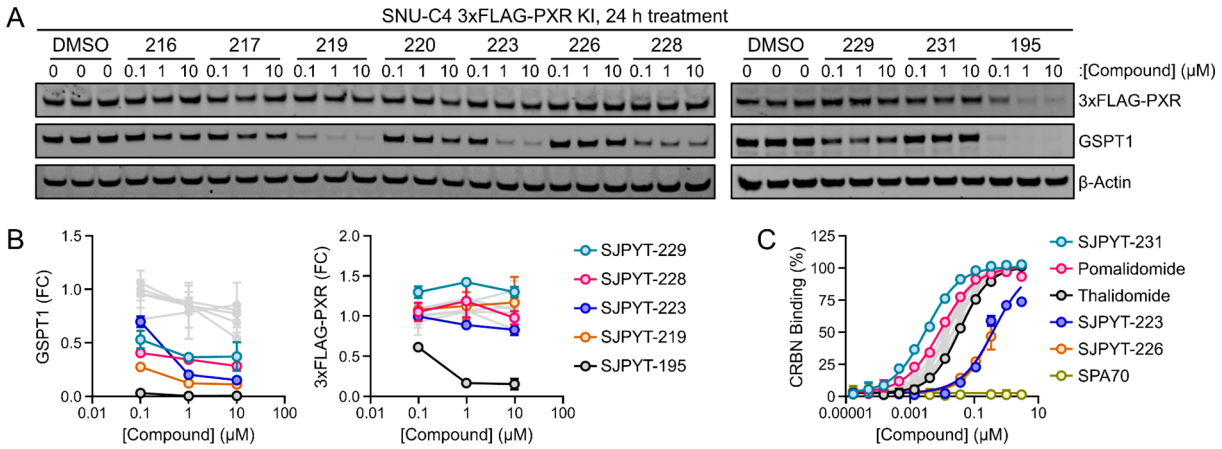

Figure 9. SJPYT-195 structure−activity relationship. (A) SNU-C4 3xFLAG-PXR KI cells were treated with DMSO or compounds for 24 h, and Western blot was performed with antibodies against FLAG, GSPT1, or β-actin. (B) GSPT1 or 3xFLAG-PXR bands from (A) were quantified as fold change relative to DMSO controls. Compounds that decreased GSPT1 protein by >50% are indicated by the legend, and other compounds are displayed in gray. (C) TR-FRET assay was performed to assess binding of compounds to CRBN. Compounds that showed no substantial change from thalidomide are displayed in gray. Controls and compounds that differ from thalidomide are indicated by the legend.

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Table 1. CRBN Binding and GSPT1 Degrading Activities of Compounds

Compound	CRBN binding EC_{50} ± SD (nM)[a]	GSPT1 loss at 10 μM (% ± SD)[c]
SJPYT-195	31 ± 1.7	100 ± 0.1
SJPYT-216	14 ± 1.0	23 ± 2.8
SJPYT-217	11 ± 0.6	31 ± 9.8
SJPYT-219	15 ± 1.1	89 ± 3.1
SJPYT-220	17 ± 1.7	46 ± 1.5
SJPYT-223	410 ± 37	85 ± 2.2
SJPYT-226	>333[b]	20 ± 4.8
SJPYT-228	23 ± 1.4	72 ± 2.3
SJPYT-229	24 ± 1.9	63 ± 13
SJPYT-231	3.2 ± 0.2	15 ± 21
SPA70	>3000[b]	NT[d]
thalidomide	36 ± 1.2	NT
pomalidomide	9.0 ± 0.6	NT

[a]CRBN binding half maximal effective concentrations (EC_{50}) are derived from Figure 9C. [b]The EC_{50} for SJPYT-226 and SPA70 could not be derived because the fitted curves did not reach 50%. For SJPYT-226, assay interference was observed at concentrations greater than 333 nM, and these points were excluded from the analysis. SPA70 was inactive. [c]The percentage of GSPT1 protein reduction at 10 μM compound was calculated from the Western blots in Figure 9A. [d]NT: not tested.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00223.

Synthetic schemes; general information; experimental procedures; ^{1}H NMR, ^{13}C NMR, HRMS, and HPLC of **SJPYT-81, 82, 83, 93, 159, 182, 187, 188, 195, 216, 217, 219, 220, 223, 226, 228, 229, 231** (PDF)

Supplemental Tables: (1) Differential expression analysis of TMT-MS. (2) SMILES strings for **SJPYT-81, 82, 83, 93, 159, 182, 187, 188, 195, 216, 217, 219, 220, 223, 226, 228, 229, 231**. (3) Sequences of plasmids used in this study. (4) Editing construct sequences and relevant primers for generation of SNU-C4 3xFLAG-PXR KI cells by CRISPR/Cas9 (XLSX)

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Notes

The authors declare the following competing financial interest(s): The authors have the following patents related to this manuscript: (1) Chen T, Lin W, Wang Y. 1,4,5-Substituted 1,2,3-Triazole Analogues as Antagonists of the Pregnane X Receptor. International Patent Application published as WO/2017/165139, 2017; US Patent Application published as US 2019/0077770 A1, 2019. US patent No. 10,550,091 B2 issued, 2020. (2) Chen T, Huber A, Li Y, Lin W. Proteolysis Targeting Chimeras for Human Pregnane X Receptor and for Degradation of GSPT1. US Provisional Application No. 63/333,925. Filing date: April 22, 2022.

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■ ABBREVIATIONS

ACN, acetonitrile; AML, acute myeloid leukemia; BRD2, bromodomain-containing protein 2; BRD3, bromodomain-

containing protein 3; BRD4, bromodomain-containing protein 4; BRIP1, BRCA1 interacting protein 1; CC_{50}, half maximal cytotoxic concentration; CRBN, cereblon; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9; $CRL4^{CRBN}$, CUL4-RBX1-DDB1-CRBN complex; CUL4, cullin 4; CV, column volume; CYP1A1, cytochrome P450 family 1 subfamily A member 1; DC_{50}, half maximal degradation concentration; DDB1, DNA damage-binding protein 1; D_{Max}, maximum degradation efficacy; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate-buffered saline; dTAG, degradation tag; DTT, dithiothreitol; EC_{50}, half maximal effective concentration; ELSD, evaporative light scattering detector; EMEM, Eagle's minimum essential medium; ESI, electrospray ionization; EV, empty vector; FA, formic acid; FC, fold change; FDR, false discovery rate; FKBP12, FK506-binding protein 12; FOS, Fos proto-oncogene; GSPT1, G1 To S phase transition protein 1 homologue; GSPT2, G1 To S phase transition protein 2; HA, hemagglutinin; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; IKZF1, IKAROS family zinc finger 1; IKZF3, IKAROS family zinc finger 3; KI, knock-in; LBD, ligand binding domain; MDM2, mouse double minute 2 homologue; MS, mass spectrometry; NMR, nuclear magnetic resonance; PCA, principal component analysis; PDA, photodiode-array detection; PPM, parts per million; PROTAC, proteolysis targeting chimera; PSM, peptide-spectrum match; PXR, pregnane X receptor; RBX1, RING-box protein 1; RNA, ribonucleic acid; RPMI, Roswell Park Memorial Institute; RT, room temperature; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SAR, structure−activity relationship; TBST, tris-buffered saline + Tween 20; TCEP, tris(2-carboxyethyl)phosphine; TMT-MS, tandem mass tag mass spectrometry; TR-FRET, time-resolved fluorescence resonance energy transfer; UHPLC, ultrahigh-performance liquid chromatography; UPLC, ultraperformance liquid chromatography; ZFP91, zinc finger protein 91 homologue

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