Journal of Medicinal Chemistry

Article

Cite This: J. Med. Chem. XXXX, XXX, XXX−XXX

pubs.acs.org/jmc

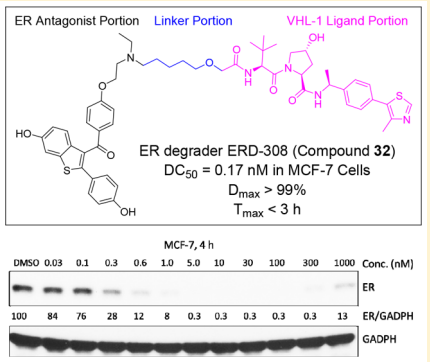
Discovery of ERD-308 as a Highly Potent Proteolysis Targeting Chimera (PROTAC) Degrader of Estrogen Receptor (ER)

Jiantao Hu,[†,‡,#] Biao Hu,[†,‡,#] Mingliang Wang,[†,‡,#] Fuming Xu,[†,‡,#] Bukeyan Miao,[†,‡,#] Chao-Yie Yang,[†,‡] Mi Wang,[†,‡] Zhaomin Liu,[†,‡] Daniel F. Hayes,[†,‡] Krishnapriya Chinnaswamy,[⊥] James Delproposto,[⊥] Jeanne Stuckey,[⊥] and Shaomeng Wang*[,†,‡,§,∥]

[†]The Rogel Cancer Center, [‡]Departments of Internal Medicine and [§]Pharmacology, University of Michigan Medical School, [∥]Department of Medicinal Chemistry, College of Pharmacy, [⊥]Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109, United States

Supporting Information

ABSTRACT: The estrogen receptor (ER) is a validated target for the treatment of estrogen receptor-positive (ER+) breast cancer. Here, we describe the design, synthesis, and extensive structure−activity relationship (SAR) studies of small-molecule ERα degraders based on the proteolysis targeting chimeras (PROTAC) concept. Our efforts have resulted in the discovery of highly potent and effective PROTAC ER degraders, as exemplified by ERD-308 (**32**). ERD-308 achieves DC_{50} (concentration causing 50% of protein degradation) values of 0.17 and 0.43 nM in MCF-7 and T47D ER+ breast cancer cell lines, respectively, and induces >95% of ER degradation at concentrations as low as 5 nM in both cell lines. Significantly, ERD-308 induces more complete ER degradation than fulvestrant, the only approved selective ER degrader (SERD), and is more effective in inhibition of cell proliferation than fulvestrant in MCF-7 cells. Further optimization of ERD-308 may lead to a new therapy for advanced ER+ breast cancer.



INTRODUCTION

Breast cancer (BC) is one of the most common malignancies in women worldwide. On the basis of the status of the tumor receptor, breast cancer can be further subdivided into estrogen receptor-positive (ER+), human epidermal growth factor receptor 2 (HER2)-positive (HER2+), and triple-negative subtypes.[1] ER+ breast cancer occurs in approximately 80% of newly diagnosed breast cancer cases.[2] As members of the nuclear receptor family, estrogen receptors ERα and ERβ are transcription factors regulating gene expression and mediating the biological effects of the estrogen. Both ERα and ERβ are widely expressed in different tissues, and ERα is considered to be the major mediator which transduces the estrogen signaling in the female reproductive tract and mammary glands.[3] ERα has therefore been pursued as a promising therapeutic target in multiple pathological settings, particularly in cancer and osteoporosis, and this is highlighted by the clinical success of tamoxifen for the treatment of ER+ BC and raloxifene for the prevention and treatment of osteoporosis in postmenopausal women.[4,5]

Although inhibition of estrogen synthesis by aromatase inhibitors and inhibition of ER pathway signaling by selective estrogen receptor modulators (SERM) (Figure 1) have demonstrated considerable clinical benefits in the treatment of ER+ BC, the development of intrinsic and acquired resistance to those drug classes presents an impediment for patients with advanced and metastatic breast cancer.[6,7] While there are clearly multiple resistance mechanisms to aromatase inhibitors and SERMs, recent studies have demonstrated that in most of the cases of drug-resistance, continued dependence on ERα signaling for tumor growth and disease progression is retained and the ER protein remains a principal driver in ER+ metastatic breast cancer.[8,9]

Selective estrogen receptor degraders (SERD) are small molecules that target ERα for proteasome-dependent degradation. Currently, fulvestrant (**5**, Figure 1) is the only SERD that has been approved for the treatment of postmenopausal women with advanced ER+ breast cancer with standard endocrine therapies.[10,11] The clinical success enjoyed by fulvestrant suggests that degradation of the ER protein is beneficial to patients with ER+ breast cancer, particularly those whose disease continues to progress after standard endocrine therapies. Because fulvestrant has poor solubility and is not orally bioavailable, it is administered clinically by a monthly intramuscular injection.[12,13] To address the shortcomings of fulvestrant, orally bioavailable SERD molecules have been developed and a number of them, shown in Figure 1, are currently being evaluated in clinical trials as new therapies for the treatment of ER+, metastatic BC.[14−19]

The proposed mechanism of action for traditional SERDs such as fulvestrant and those shown in Figure 1 is induction of misfolding of the ER protein, which ultimately leads to proteasome-dependent ERα protein degradation.[20] The SERD

Received: October 9, 2018

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

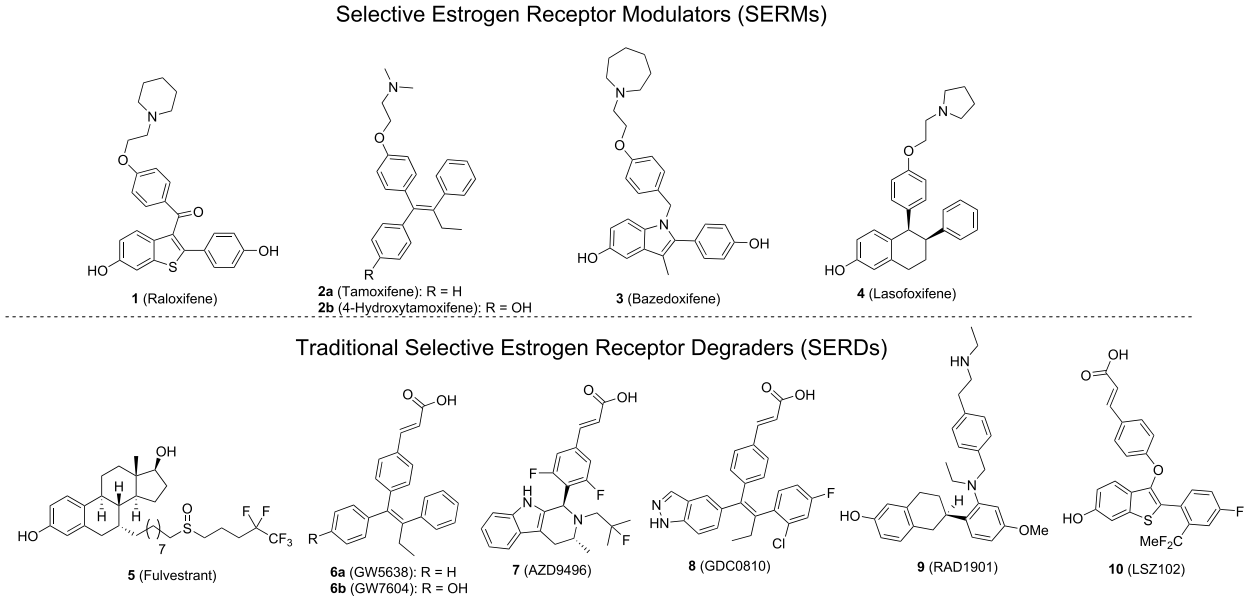


Figure 1. Representative SERMs and SERDs.

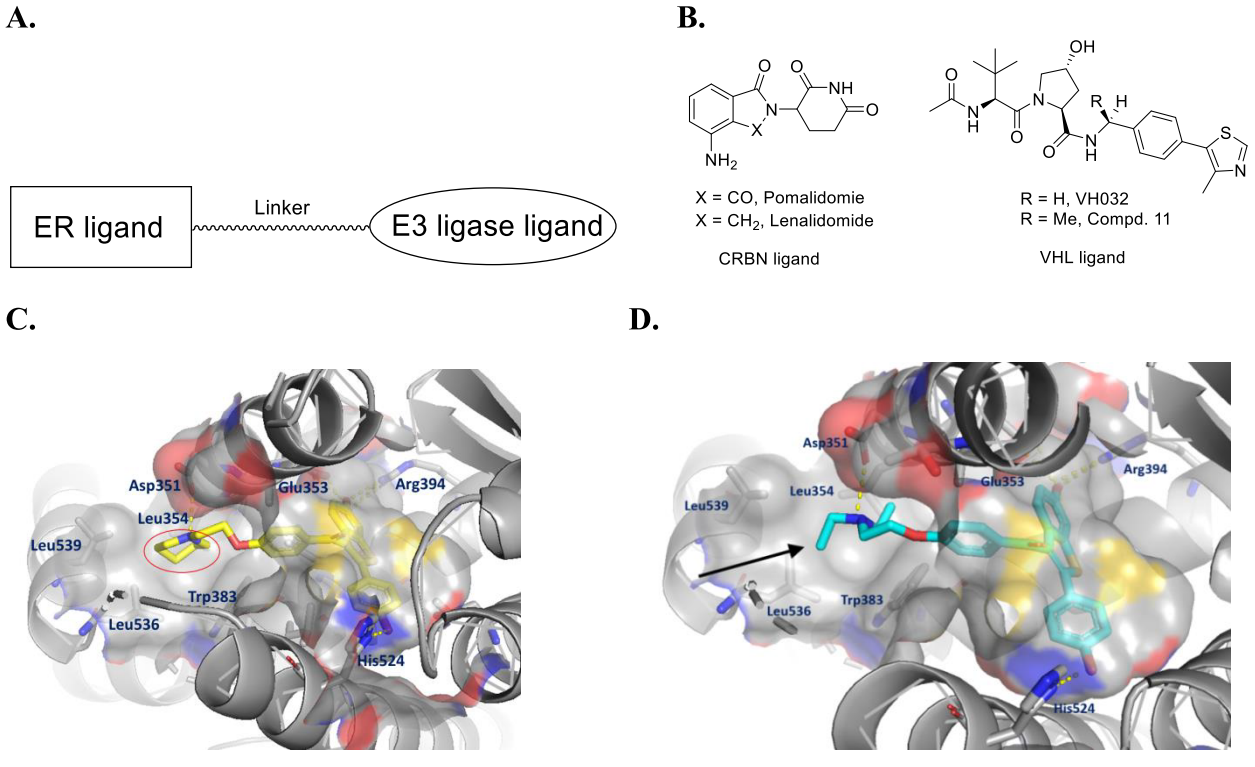


Figure 2. (A) A general scheme for the design of ER degraders based upon the PROTAC concept. (B) Structures of CRBN ligand and VHL ligand. (C) Crystal structure of ERα ligand-binding domain in a complex with raloxifene (**1**) (PDB 1ERR). (D) Predicted binding models of *N*,*N*-diethylamino analogue of raloxifene in complex with ERα LBD. Hydrogen bonds are depicted by yellow dashed lines.

molecules are typically potent and effective in inducing degradation of ER protein in ER+ breast cancer cells, but they are only able to achieve partial degradation of the ER protein.[21,22] Consequently, we propose that novel therapeutic agents, which can achieve more complete degradation of ER, could be more efficacious than the traditional SERD molecules for the treatment of ER+ metastatic breast cancer.

The proteolysis targeting chimera (PROTAC) concept was first introduced in 2001,[23] with the objective of induction of selective target protein degradation by hijacking the cellular E3 ubiquitination ligase systems.[24−28] PROTACs are heterobifunctional small molecules containing a ligand, which binds to the target protein of interest, and another ligand for an E3 ligase system. These two ligands are tethered together by a chemical linker (Figure 2A). The PROTAC strategy has recently gained momentum due in part to the availability of potent and druglike small-molecule ligands for a number of E3 ligase systems, and it has been employed for the design of small-molecule degraders for a number of proteins.[29−43] Recently, Naito et al. reported several PROTAC-like ERα

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

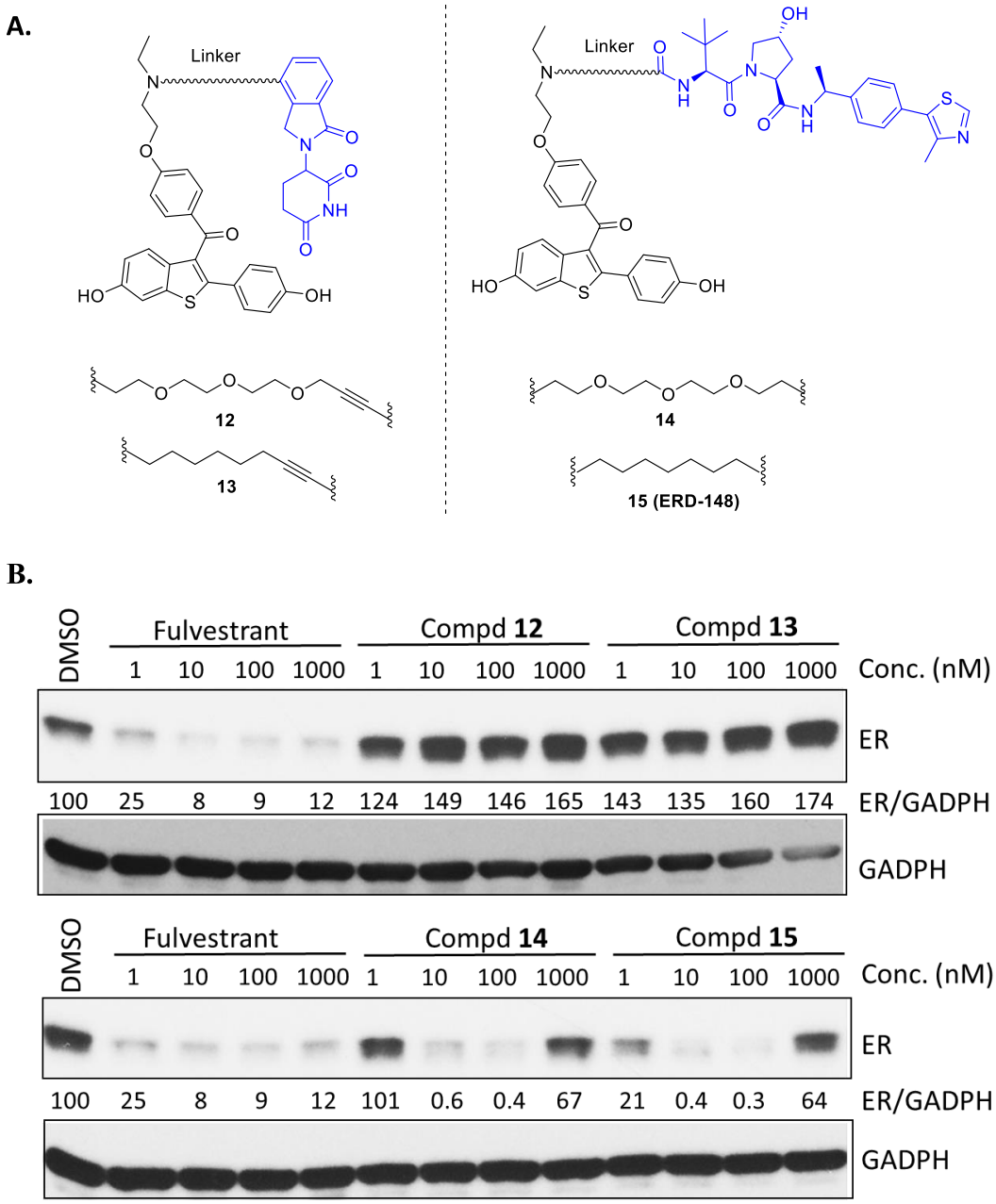


Figure 3. Degradation of ER protein by PROTACs. (A) Structures of synthetic putative ER degraders. (B) Western blotting analysis of ER protein in MCF-7 cells treated with putative PROTAC ER degraders and control compounds. Cells were treated with different compounds for 4 h, and whole cell lysates were then analyzed by Western blotting to examine the level of ER protein. The membranes were stripped and reblotted for GAPDH as the loading control. The numbers below the panel represent the ERα/GADPH ratio normalized with the DMSO control at 100.

degraders, which were named specific and nongenetic IAP-dependent protein erasers (SNIPERs).[44,45] They designed ERα SNIPER molecules using an ERα antagonist and a ligand for inhibitors of apoptosis protein (IAPs), which are E3 ligases. However, while SNIPER ER degraders effectively induce partial degradation of the ER protein, they also induce autoubiquitylation and proteasomal degradation of the E3 ligase, the cIAP1 protein, thus limiting their degradation and therapeutic efficacy.

In the present study, we report our design, synthesis, and evaluation of PROTAC degraders of ERα protein by employing the cereblon/cullin 4A and VHL/cullin 2 neddylation degradation systems and several different classes of selective ER modulators/antagonists.[46] Our study has resulted in the discovery of highly potent PROTAC ERα degraders (hereafter called ER degraders) with DC_{50} < 1 nM and D_{max} > 95% in MCF-7 and T47D ER+ breast cancer cells. This study lays a foundation for the development of a completely new class of therapeutics for the treatment of ER+ metastatic breast cancer.

RESULTS AND DISCUSSION

Raloxifene (**1**, Figure 1), a highly potent SERM, has been approved for the treatment of osteoporosis and breast cancer in women and was employed in our design of PROTAC ER degraders. Both cereblon/cullin 4A and VHL/cullin 2 have been successfully used for the design of PROTAC degraders.[47,48] Therefore, we employed phthalimide analogues

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

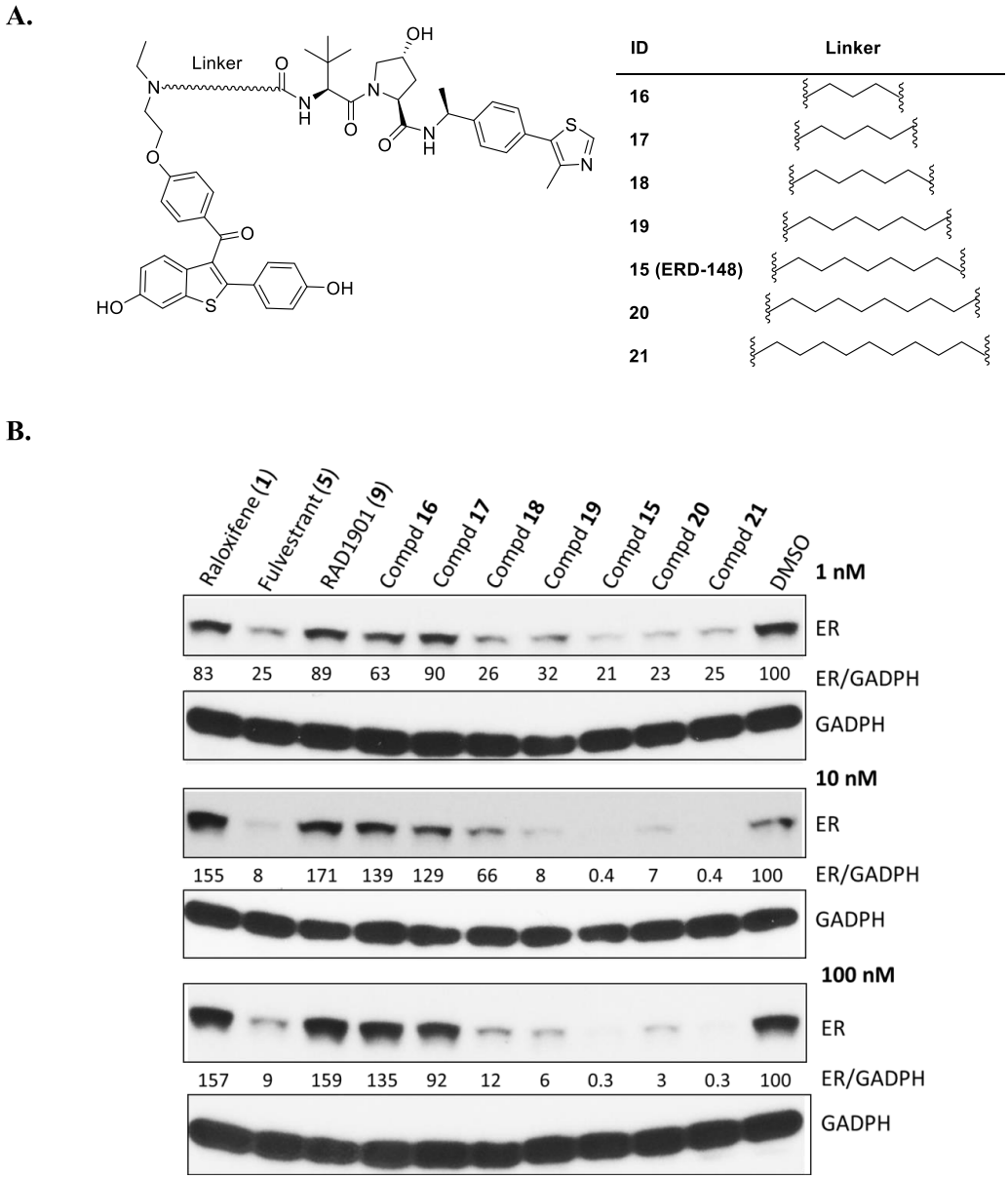


Figure 4. Optimization of linker length with linear aliphatic linkers. (A) Structures of ER degraders. (B) Western blotting analysis of ER protein in the MCF-7 cells treated with indicated compounds at 1, 10, and 100 nM. MCF-7 cells were treated with different compounds for 4 h and whole cell lysates were analyzed by Western blotting to examine the level of ER protein. The membranes were stripped and reblotted for GAPDH as the loading control. The numbers below the panel represent the ERα/GADPH ratio normalized with the DMSO control at 100.

to recruit cereblon and compound **11** to recruit VHL for the design of putative PROTAC ERα degraders (Figure 2B).

The cocrystal structure of human ER ligand-binding domain in a complex with raloxifene shows that the piperidine ring in raloxifene is buried in the hydrophobic pocket of ERα (Figure 2C).[49] For the synthesis of PROTAC ERα degraders, we replaced the piperidine group with an *N*,*N*-diethylamino group, similar to the *N*,*N*-dimethylamino group in tamoxifen (**2a**, Figure 1). Our modeling shows that one of the ethyl groups is exposed to solvent and is therefore suitable as the linking site in the design of PROTAC ER degraders (Figure 2D).

We first synthesized compounds **12** and **13** using a cereblon ligand and two different linkers and also prepared compounds **14** and **15** using the VHL ligand (**11**) and two different linkers as putative PROTAC ER degraders (Figure 3A). These four compounds (**12**−**15**) were evaluated for their ability to induce ER degradation in the MCF-7 ER+ breast cancer cell line, with fulvestrant used as the control.

Western blotting data (Figure 3B) showed that, consistent with its mode of action as a potent SERD molecule, fulvestrant very effectively induces ER protein degradation in MCF-7 cells. Interestingly, while compounds **14** and **15**, designed using a VHL ligand, are very effective in inducing ER degradation with 4 h treatment at concentrations as low as 1−10 nM in MCF-7 cells, compounds **12** and **13**, designed using a cereblon ligand, are completely ineffective in inducing ER degradation at concentrations of 1−1000 nM. Although further investigation is clearly needed before it can be concluded that the cereblon/cullin 4A system is less effective or ineffective for the design of ER degraders, our data for compounds **14** and **15** clearly demonstrate that the VHL/cullin 2 system can be successfully

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

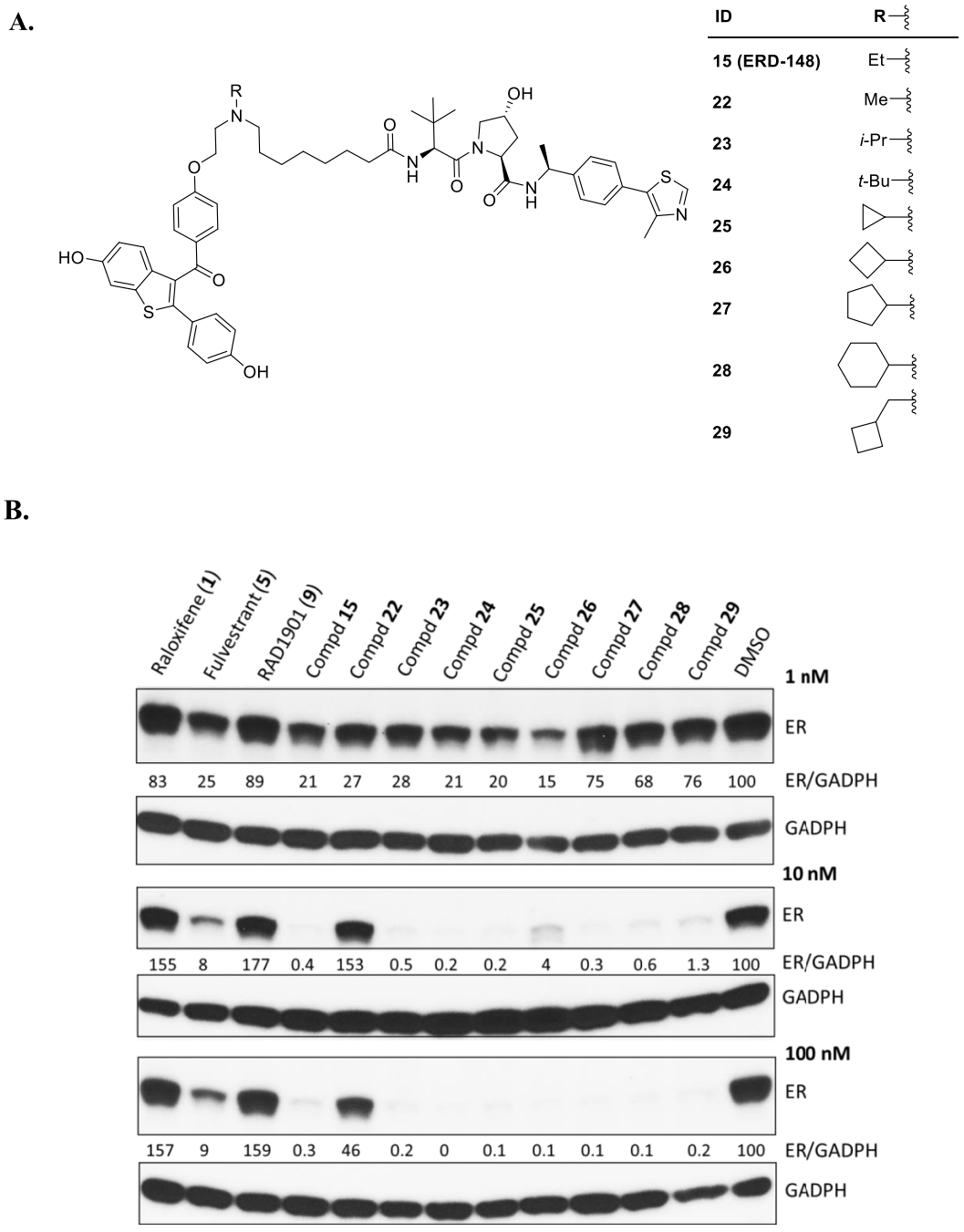


Figure 5. Optimization of N-substituent groups (A) Structures of ER degraders. (B) Western blotting analysis of ER protein in the MCF-7 cells treated with indicated compounds at 1, 10, and 100 nM. MCF-7 cells were treated with different compounds for 4 h, and whole cell lysates were analyzed by Western blotting to examine the level of ER protein. The membranes were stripped and reblotted for GAPDH as the loading control. The numbers below the panel represent the ERα/GADPH ratio normalized with the DMSO control at 100.

employed for the design of potent and highly effective PROTAC ER degraders. We have therefore focused on our further design and optimization of PROTAC ER degraders using VHL ligands.

Our previous studies on bromodomain and extra-terminal (BET) degraders demonstrated that the length and chemical composition of the linker in a PROTAC degrader molecule were critical in achieving effective protein degradation.[48,50,51] We therefore synthesized a series of analogues of compound **15** with the linker length varying from 3 to 9 atoms (Figure 4A). These compounds were evaluated for their ability to induce ER degradation in MCF-7 cells at concentrations of 1, 10, and 100 nM, with compound **15**, fulvestrant (**5**), another SERD molecule RAD1901 (**9**), and raloxifene (**1**) included as controls. Our Western blotting data (Figure 4B) showed that compounds **16** and **17** with a $(CH_2)_3$ or $(CH_2)_4$ linker are ineffective in inducing ER degradation at 1−100 nM concentrations. However, compounds **15**, **18**, **19**, **20**, and **21**, with linkers containing 6−9 carbon atoms, are all highly effective in inducing ER degradation at concentrations as low as 1 nM. These compounds display similar potencies, with **15** being slightly more potent than the other compounds. Fulvestrant effectively induces ER degradation at concentrations of 1, 10, and 100 nM, but RAD1901 fails to induce ER

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

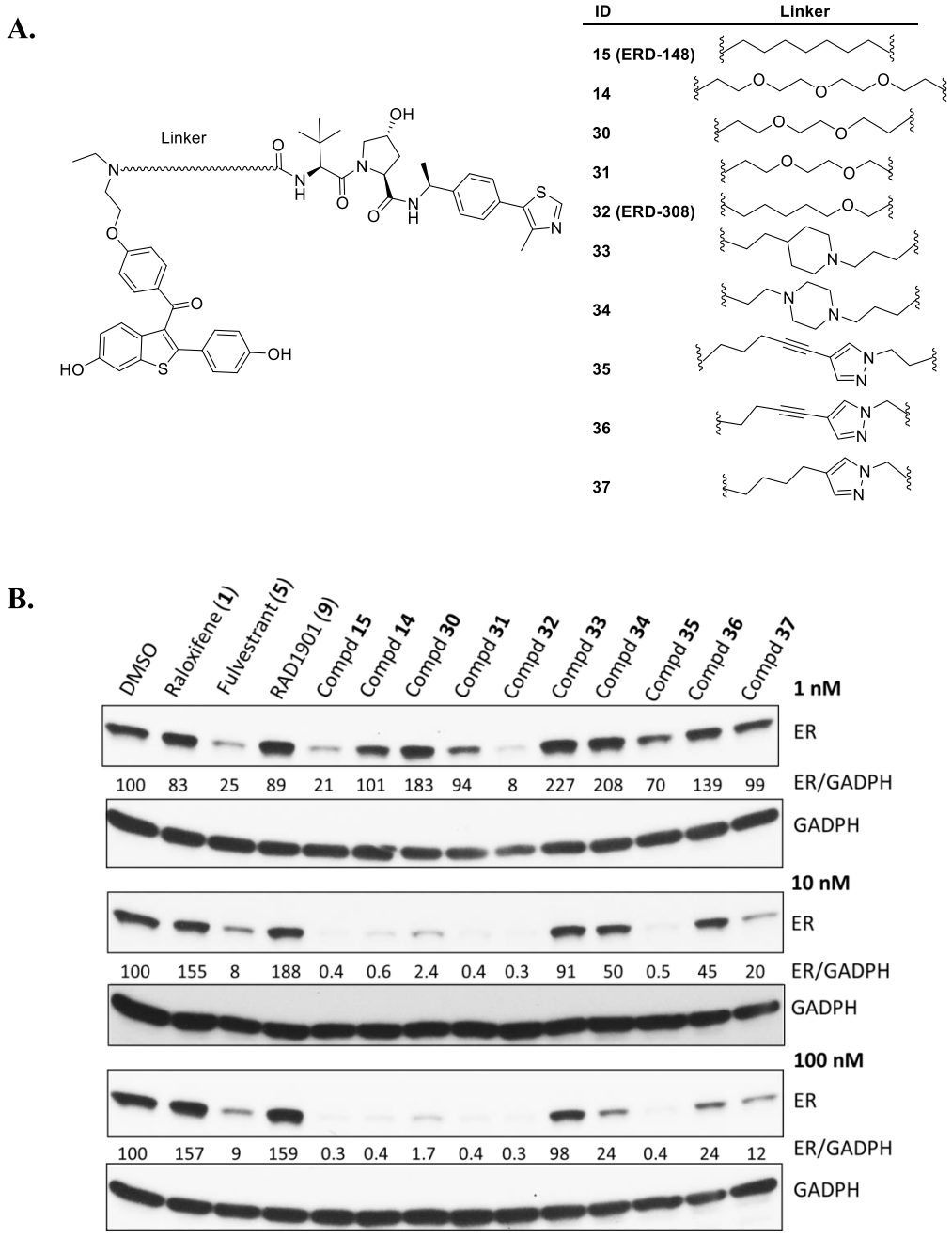


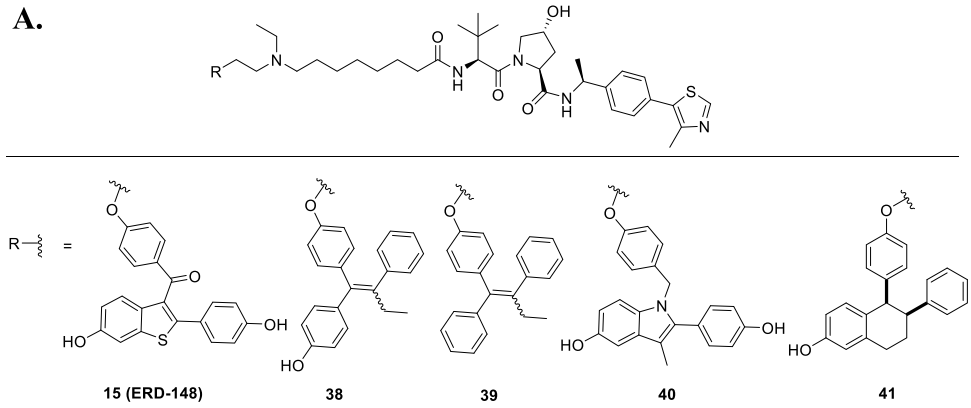
Figure 6. Optimization of the linker composition. (A) Structures of the ER degraders. (B) Western blotting analysis of ER protein in the MCF-7 cells treated with indicated compounds at 1, 10, and 100 nM. MCF-7 cells were treated with different compounds for 4 h, and whole cell lysates were analyzed by Western blotting to examine the level of ER protein.The membranes were stripped and reblotted for GAPDH as the loading control. The numbers below the panel represent the ERα/GADPH ratio normalized with the DMSO control at 100.

degradation at 1−100 nM. Consistent with its mode of action as a SERM, raloxifene fails to induce any ER degradation.

Upon the basis of our modeling (Figure 2D), an *N*-ethyl group in the ER antagonist portion of compound **15** binds to a hydrophobic pocket in the ER protein and presumably contributes to its binding to ER. We therefore synthesized and tested a series of analogues of **15** with different *N*-substituent alkyl groups (Figure 5A). As shown in Figure 5B, compound **22** with a *N*-methyl group is ineffective in inducing ER degradation at 1 and 10 nM and induces only moderate ER degradation at 100 nM. In contrast, compounds **23**−**29**, with an isopropyl, *tert*-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cyclobutylmethyl group, respectively, all induce essentially complete ER degradation in MCF-7 cells at concentrations of 10 and 100 nM. Upon the basis of the Western blotting data from these compounds at a 10 nM concentration, compound **26** with a cyclobutyl group is the most potent ER degrader among this series of compounds, followed by compound **25** with a cyclopropyl group, and compounds **15** and **24** with an ethyl or a *tert*-butyl group. However, in view of the fact that compound **15** possesses the lowest molecular weight (MW: 1002.3) and achieves an excellent degradation potency, we employed it as the template molecule for further optimization.

Our previous studies showed that in addition to the linker length, the linker composition plays a key role for protein

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX



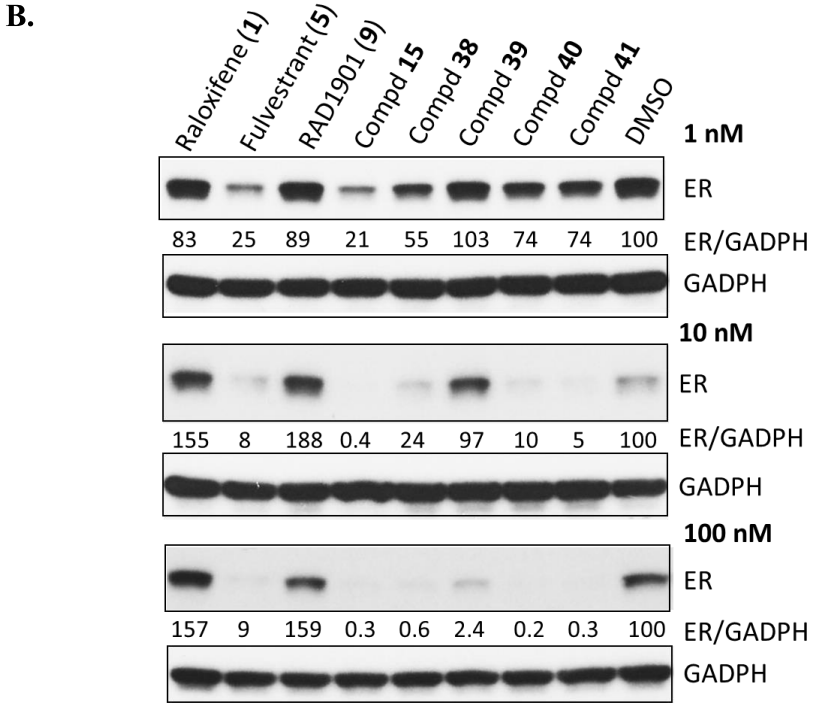


Figure 7. SAR study for ER ligands. (A) Structures of ER degraders. (B) Western blotting analysis of ER protein in the MCF-7 cells treated with indicated compounds at 1, 10, and 100 nM. MCF-7 cells were treated with different compounds for 4 h, and whole cell lysates were analyzed by Western blotting to examine the level of ER protein. The membranes were stripped and reblotted for GAPDH as the loading control. The numbers below the panel represent the ERα/GADPH ratio normalized with the DMSO control at 100.

degradation potencies in our BET PROTAC molecules.[48] In view of the fact that the linker in compound **15** is quite hydrophobic and that this compound displays poor aqueous solubility, we synthesized a series of new analogues with linkers to improve solubility of the resulting compounds (Figure 6A). These new analogues were evaluated together with **14** and **15** for their ability to induce ER degradation in MCF-7 cells (Figure 6B).

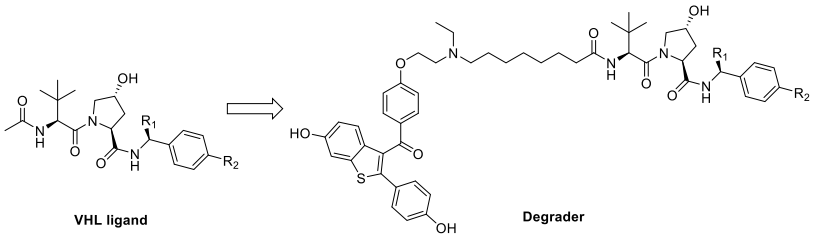
Compounds **14** and **30**−**32** with a polyethylene glycol unit (PEG) embedded in the linker are all effective in inducing ER degradation at 10 and 100 nM in MCF-7 cells. Upon the basis of the Western blotting data from these compounds tested at 1 nM, compound **32** is the most potent compound, followed by compound **31**.

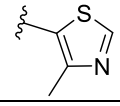
Compounds **33** and **34** containing a positively charged piperidinyl or piperazinyl group are much less potent than compound **15** and fail in fact to induce any ER degradation at 1 and 10 nM in MCF-7 cells. While **33** is still ineffective in inducing ER degradation at 100 nM, compound **34** at 100 nM clearly induces ER degradation.

Compound **15** has a very flexible linker. We sought to determine if introduction of a conformationally rigid linker using a charge-neutral, aromatic ring can result in potent PROTAC ER degraders. This led to the design and synthesis of compounds **35**−**37**. Western blotting analysis showed that while compounds **35**−**37** effectively induce ER degradation, these three compounds are much less potent than compounds **15** and **32**.

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

A.



ID (VHL ligand)	Binding affinity IC_{50} (nM)	ID (degrader)	R_1	R_2
11	196±6	**15 (ERD-148)**	Me	
VH032	454±24	**42**	H	
43a	7867±642	**43**	Me	Cl
44a	4827±418	**44**	Me	CN
45a	879±97	**45**	Me	ethynyl
46a	6112±820	**46**	Me	*c*-Pr
47a	41001±6665	**47**	Me	*i*-pr
48a	58897±5264	**48**	Me	*t*-Bu

B.

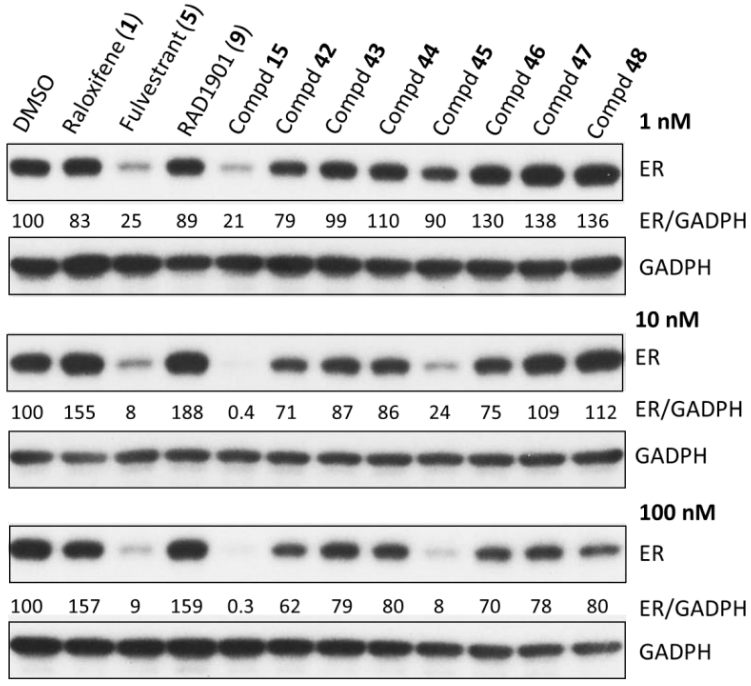


Figure 8. SAR study for VHL ligands. (A) Structures of ER degraders and binding affinities of relative VHL ligands against VHL protein by fluorescence polarization (FP) assay. (B) Western blotting analysis of ER protein in the MCF-7 cells treated with indicated compounds at 1, 10, and 100 nM. MCF-7 cells were treated with different compounds for 4 h, and whole cell lysates were analyzed by Western blotting to examine the level of ER protein. The membranes were stripped and reblotted for GAPDH as the loading control. The numbers below the panel represent the ERα/GADPH ratio normalized with the DMSO control at 100.

Compound **15** contains the core structure of the ER antagonist raloxifene (**1**), and we next designed and synthesized a series of ER degraders using the core structures of several different classes of selective ER modulators, including tamoxifen (**2a**), 4-hydroxytamoxifen (**2b**), bazedoxifene (**3**), and lasofoxifene (**4**). These efforts resulted in compounds **38**–**41** (Figure 7A), which are all very effective in inducing ER degradation with compound **39**, with the tamoxifen core structure being the least potent. Our data showed that compound **15** is still more potent than compounds **38**–**41** in induction of ER degradation (Figure 7B).

We next investigated the effects of the VHL ligand portion in our PROTAC ER degraders on ER degradation, focusing particularly on the benzylamine moiety because this portion is known to be critical in achieving high binding affinities to VHL (Figure 8A).[52−55] First, we synthesized a series of VHL ligands. We established a fluorescence polarization (FP) assay for VHL (Figure S1 in Supporting Information) and tested the binding affinities of VHL ligands **11** and **43a**–**48a**, with a previously

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

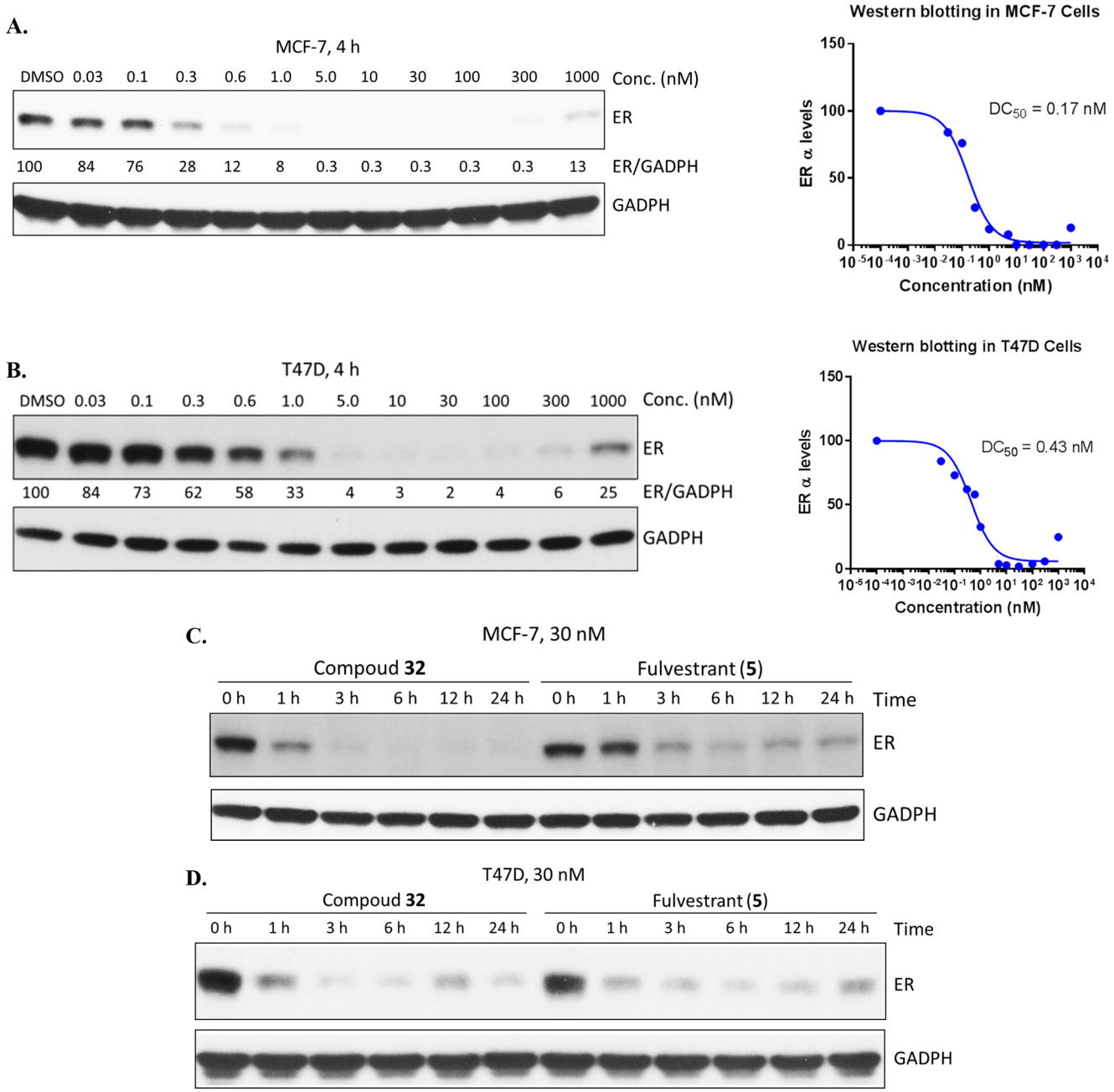


Figure 9. Further characterization of compound **32** (ERD-308). (A) ERα degradation dose-response for compound **32** with a treatment time of 4 h in MCF-7 cells. ER protein was examined by Western blotting and ER protein level was quantified by densitometry and normalized to the corresponding density of GAPDH protein. (B) ERα degradation dose-response for compound **32** with a treatment time of 4 h in T47D cells. ER protein was examined by Western blotting and ER protein level was quantified by densitometry and normalized to the corresponding density of GAPDH protein. (C) Time course of ERα degradation by compound **32** (30 nM) and fulvestrant (30 nM) in the MCF-7 cells. (D) Time course of ERα degradation by compound **32** (30 nM) and fulvestrant (30 nM) in the T47D cells.

reported VHL ligand (VH032)[54] included as a control (Figure 8A, Figure S2 in Supporting Information). Among these VHL ligands, compound **11** is twice as potent as VH032 with an IC_{50} value of 196 nM in our FP assay. However, substitution of the 4-methylthiazole group with other smaller aliphatic groups decreases the binding affinity significantly. Among them, an ethynyl group substituent displayed acceptable binding affinity with an IC_{50} value of 879 nM.

Next, we employed those VHL ligands in the design of ER degraders to investigate the influence of VHL binding affinity on ER degradation. As shown in Figure 8B, compound **42**, which lacks the chiral methyl group in **15**, is much less potent than **15** in inducing ER degradation. Replacement of the methylthiazole group in **15** with other hydrophobic groups, such as chloro (**43**), cyano (**44**), cyclopropyl (**46**), isopropyl (**47**), and *tert*-butyl (**48**), led to significantly reduced ER degradation. An exception is compound **45** with an ethynyl group, which displays good or moderate degradation activity at 10 and 100 nM. Collectively, our data show that, consistent with the mechanism of action of PROTAC molecules, the binding affinity of degraders to VHL protein plays an important role in induction of ER degradation.

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

Through extensive optimization of the three structural moieties in our PROTAC ER degraders, we have obtained a number of highly potent ER PROTAC molecules in MCF-7 cells, as exemplified by **32** (ERD-308). We next performed further investigations to characterize the action of compound **32**.

We first examined the ER degradation by compound **32** in a wide range of concentrations to determine its DC_{50} (concentration to achieve 50% of protein degradation) in MCF-7 cells (Figure 9A). Quantification of our Western blotting data showed that compound **32** achieves a DC_{50} value of 0.17 nM in the MCF-7 cells with a 4 h treatment. Compound **32** achieves a maximum ER degradation of >95% based upon our quantification at concentrations as low as 5 nM. It is noteworthy that compound **32** induces less ER degradation at 1 μM than at lower concentrations, a known "hook" effect that has been observed previously in PROTAC degraders.[56,57]

We also evaluated compound **32** for its ability to induce ER degradation in the T47D ER+ breast cancer cell line. As shown in Figure 9B, compound **32** achieves a DC_{50} value of 0.43 nM and a maximum degradation of >95% at 5 nM. Compound **32** at 1 μM also demonstrates a hook effect in the T47D cells.

Next, we examined the kinetics of ER degradation induced by compound **32** in MCF-7 cells. As shown in Figure 9C, at a concentration of 30 nM, compound **32** reduces >80% of the ER protein level with a 1 h treatment and achieves essentially complete ER degradation at the 3 h time-point, indicating fast kinetics. In comparison, fulvestrant, a conventional SERD, has only a modest effect on reduction of the level of ER at 1 h and achieves a maximum of approximately 90% of ER degradation after a 24 h treatment. The kinetic data obtained for **32** and fulvestrant in the T47D cells were similar to those observed in MCF-7 cells (Figure 9D). Taken together, our data demonstrate that compound **32** displays faster degradation kinetics and more complete degradation than fulvestrant.

Upon the basis of the PROTAC design, degradation of ER protein induced by the PROTAC degrader **32** should depend upon its binding to both the ER protein and the VHL protein. Furthermore, the induced ER degradation by a PROTAC ER degrader such as **32** should be proteasome-dependent. We therefore investigated the mechanism of action of ER degradation induced by **32** (Figure 10). Consistent with the PROTAC design, ER degradation induced by compound **32** at a 30 nM concentration is significantly reduced by addition of 1 μM of raloxifene or 1 μM of the proteasome inhibitor carfilzomib, but raloxifene or carfilzomib alone have no effect on the ER protein levels. Interestingly, 1 μM of the VHL ligand (**11**) blocks the degradation by 30 nM of compound **32** only slightly (Figure 10A). To further confirm that the degradation is VHL-dependent, we performed a dose−response experiment with VHL ligand **11**. As shown in Figure 10B, the degradation by compound **32** was completely blocked with 5 or 10 μM of **11**. Taken together, these mechanistic data provide clear evidence that compound **32** acts as a bona fide PROTAC ER degrader.

Because compound **32** is highly potent and effective in induction of ER degradation, we used the WST-8 cell proliferation assay to evaluate its ability to inhibit cell proliferation in MCF-7 cells, with raloxifene and fulvestrant included as controls (Figure 11A). Our data showed that compound **32** is highly potent and effective in inhibition of cell proliferation and achieves an IC_{50} value of 0.77 nM and a

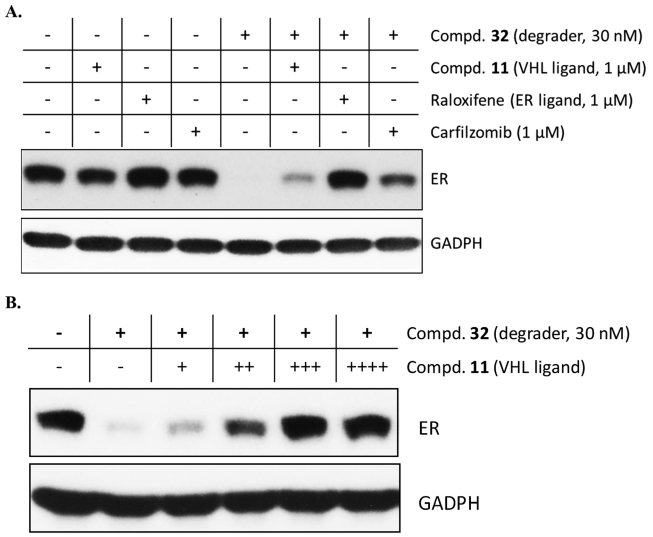


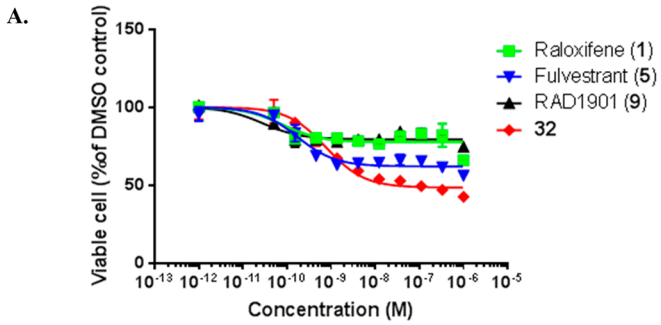
Figure 10. Degradation is dependent on VHL, ER, and proteasome. (A) MCF-7 cells were pretreated with VHL ligand **11** (1 μM), or ER ligand raloxifene (**1**) (1 μm), or the proteasome inhibitor carfilzomib (1 μM) for 2 h, followed by treatment with DMSO or compound **32** (30 nM) for 4 h. Then whole-cell lysates were analyzed by Western blotting. (B) MCF-7 cells were pretreated with VHL ligand **11** (+, 0.5 μM; ++, 1 μM; +++, 5 μM; ++++, 10 μM) for 2 h, followed by treatment with DMSO or compound **32** (30 nM) for 4 h. Then whole-cell lysates were analyzed by Western blotting.

maximum inhibition (I_{max}) of 57.5% in MCF-7 cells. Fulvestrant is also very effective and potent in inhibition of cell proliferation, but it achieves an I_{max} value of 43.8%. Raloxifene, on the other hand, achieves an I_{max} value of only 34.0%. Interestingly, RAD1901, a previously reported SERD molecule,[18] achieves an I_{max} value of only 25.7%. Hence the more complete ER degradation achieved by compound **32** translates into a greater maximum cell growth inhibition than that of fulvestrant or RAD1901, two conventional SERD molecules. Furthermore, compound **32** achieves a much greater maximum cell growth inhibition than raloxifene, a SERM molecule. As expected, compound **32** does not exhibit the cell proliferation inhibition effects in triple-negative breast cancer cell MDA-MB-231 (Figure S3 in Supporting Information) and primary human mammary epithelial cells (Figure S4 in Supporting Information).

To visually evaluate the cellular effect, we conducted a crystal violet staining experiment for compound **32** at 10, 100, and 300 nM with raloxifene and fulvestrant as controls (Figure 11B). Consistent with the WST-8 cell proliferation assay, treatment of MCF-7 cells with compound **32** reduced cell proliferation more significantly than raloxifene or fulvestrant at all three of the concentrations tested (Figure 11B).

We next performed quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis to evaluate the ability of compound **32** to suppress the mRNA levels of pGR and GREB1, two ER-regulated genes in MCF-7 cells. As shown in Figures 11C,D, the expression of both genes is strongly suppressed by compound **32**. Compound **32** is only slightly more effective than fulvestrant in suppressing the expression of pGR and GREB1 at both 10 and 100 nM, but it is significantly more effective than raloxifene.

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX



ID	GI_{50} (nM)	Imax (%)
Raloxifene (**1**)	0.079	34.0
Fulvestrant (**5**)	0.17	43.8
RAD1901 (**9**)	0.028	25.7
ERD-308 (**32**)	0.77	57.5

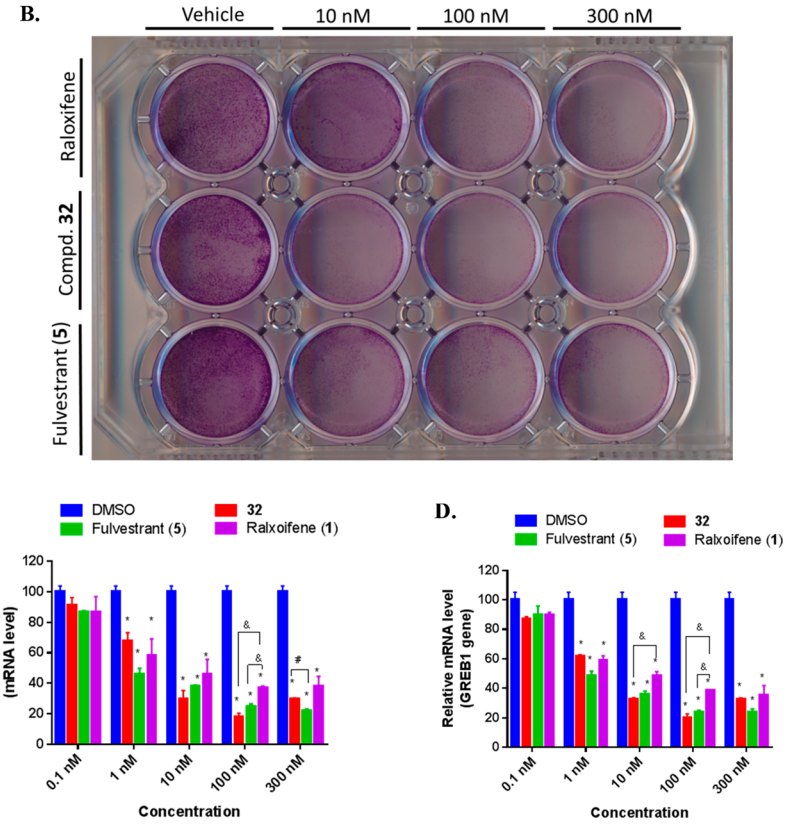


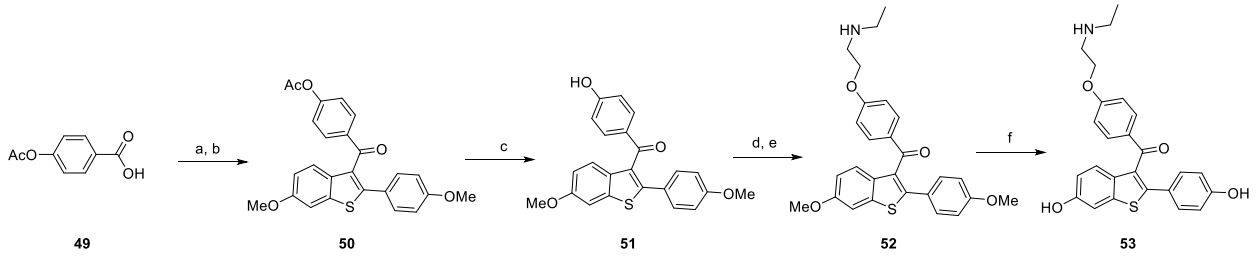
Figure 11. Cellular effects of compound **32** (ERD-308) in MCF-7 cells. (A) Cell viability assay. Cells were treated with indicated doses for 4 days. Cell viability was determined by colorimetric WST-8 assay. GI_{50} values were calculated using the GraphPad Prism 7 software. (B) Crystal violet staining. Cells were treated with indicated doses for 5 days, and then fixed and stained with crystal violet. (C,D) qRT-PCR analysis for pGR gene and GREB1 mRNA expression in MCF-7 cells. Cells were treated with DMSO, raloxifene (**1**), fulvestrant (**5**), or compound **32** at indicated doses for 8 h. Multiple student's *t* tests were conducted using the Holm−Sidak method. * Significant difference between the compound treated group and the vehicle treated control group (DMSO). & Significant difference between the compound treated group and the ER inhibitor (raloxifene) treated group. # Significant difference between fulvestrant and PROTAC degrader treated groups.

CHEMISTRY

The syntheses of the presented final compounds were outlined in Schemes 3−5. First, key common intermediates **53** and **58** were synthesized as shown in Schemes 1 and 2, respectively. The commercial 4-acetoxybenzoic acid (**49**) was converted to the acyl chloride, which, after Friedel−Crafts acylation of commercial 6-methoxy-2-(4-methoxyphenyl)benzo-[*b*]-thiophene, furnished compound **50**. After deacetylation of compound **50** under basic and aqueous conditions, compound **51** was converted to the alkyl bromide, which was substituted with excess ethylamine to afford the secondary amine (**52**). Cleavage of both aryl methoxy ethers in **52** with boron tribromide furnished the dihydroxy intermediate **53**. Following the literature reported procedure,[47] the synthesis of compound **58** commenced with the *tert*-butyloxycarbonyl protection of commercial (*S*)-1-(4-bromophenyl)ethan-1-amine (**54**). Subsequent Suzuki coupling with 4-methylthiazole afforded compound **55**, and this was followed by deprotection under acidic conditions and amide coupling with commercial (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine-2-car-

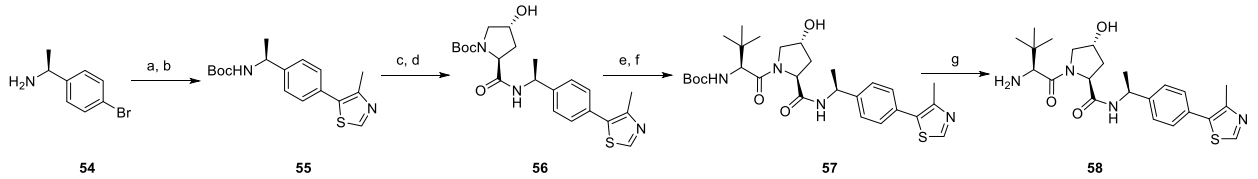
DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of the Key Intermediate 53[a]



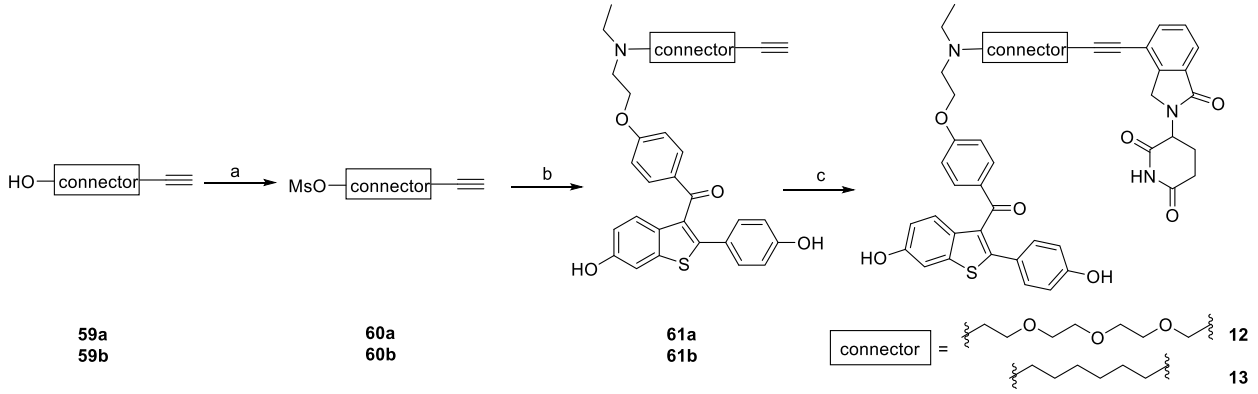
[a]Reagents and conditions: (a) oxalyl chloride, DMF, DCM, 0 °C to RT, 1 h; (b) 6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thiophene, $AlCl_3$, DCM, 0 °C to RT, 1 h; (c) NaOAc, EtOH/H_2O, 80 °C, 12 h; (d) 1,2-dibromoethane, Cs_2CO_3, MeCN, reflux, 12 h; (e) $EtNH_2$, DIPEA, DMF, 80 °C, 12 h; (f) BBr_3, DCM, 0 °C to RT, 1 h.

Scheme 2. Synthesis of the Key Intermediate 58[a]



[a]Reagents and conditions: (a) Boc_2O, $NaHCO_3$, EtOAc/H_2O, 2 h; (b) 4-methylthiazole, $Pd(OAc)_2$, KOAc, DMA, 90 °C, 12 h; (c) 4 N HCl in dioxane/MeOH, RT, 12 h; (d) (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid, HATU, DIPEA, DMF, 0 °C to RT, 12 h; (e) 4 N HCl in dioxane/MeOH, RT, 12 h; (f) (*S*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid, HATU, DIPEA, DMF, 0 °C to RT, 12 h; (g) 4 N HCl in dioxane/MeOH, RT, 12 h.

Scheme 3. Synthesis of Compounds 12 and 13[a]



[a]Reagents and conditions: (a) MsCl, TEA, DCM, 0 °C to RT, 1 h; (b) **53**, DIPEA, DMF, 80 °C, 12 h; (c) 3-(4-iodo-1-oxoisoindolin-2-yl)piperidine-2,6-dione, $Pd(PPh_3)Cl_2$, CuI, DMF/TEA, 80 °C, 1 h.

boxylic acid. Under the same conditions, compound **56** was deprotected, followed by amide coupling with commercial (*S*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid to afford compound **57**, which after acidic deprotection afforded compound **58**.

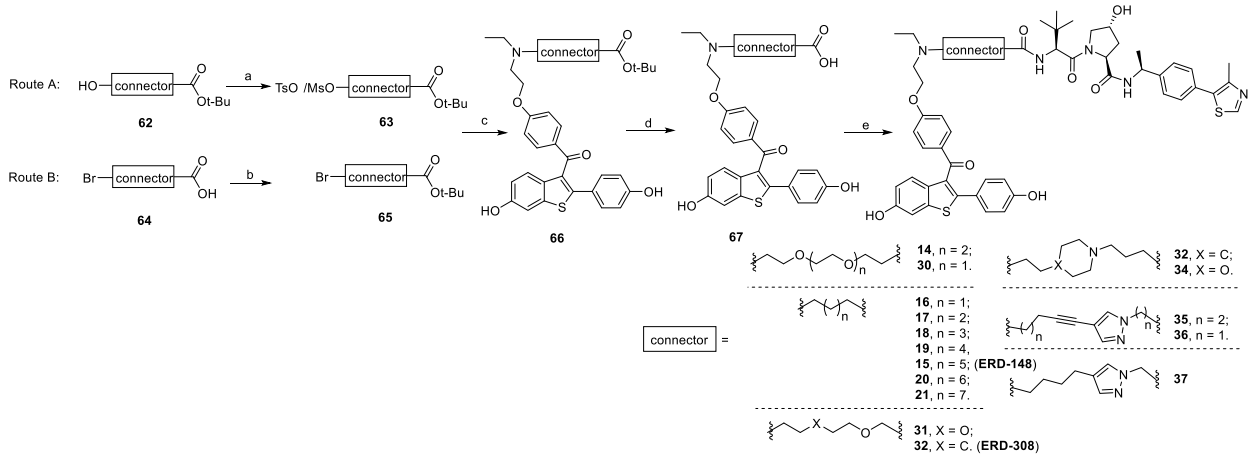
As shown in Scheme 3, synthesis of compound **12** commenced with the conversion of commercial 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethan-1-ol (**59a**) to compound **60a** using methanesulfonyl chloride with trimethylamine as base. Nucleophilic substitution of **60a** with compound **53** under mild basic conditions afforded the *N*-substituted compound (**61a**). Sonogashira coupling of compound **61a** with the previously published compound 3-(4-iodo-1-oxoisoindolin-2-yl)piperidine-2,6-dione[50] afforded the compound **12** in high yield. Compound **13** was synthesized using the procedure described for the synthesis of compound **12** with oct-7-yn-1-ol **59b** as the starting material.

As shown in Scheme 4, diversity of the linkers commenced with the preparation of compound **63** or **65**, which are commercially available or can be prepared from **62** or **64**, respectively. The substitution reaction of compound **63** or **65** with compound **53** furnished compound **66**, which underwent acidic deprotection to afford the acid (**67**). Amide coupling between compound **67** and **58** afforded the final compounds **14**−**21** and **30**−**37** in high yields.

As shown in Scheme 5, the common intermediate **51** was used for the SAR studies of the *N*-substituent groups. Compound **51** was first converted to the alkyl bromide, which underwent nucleophilic attack with excess primary amines furnishing compound **68**. Substitution reaction of compound **68** with *tert*-butyl 8-bromooctanoate (**65**) furnished the linker attached intermediate, which underwent boron tribromide-mediated demethylation and deprotection to afford the acid (**69**). Amide coupling between compounds **69**

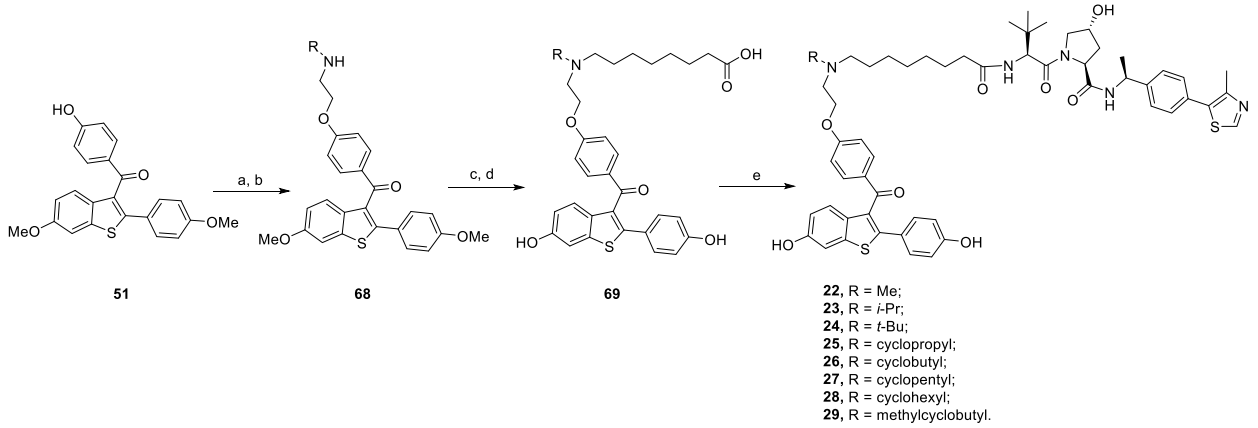
DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of Compounds 14−21, 30−37[a]



[a]Reagents and conditions: (a) TsCl or MsCl, TEA, DCM, 0 °C to RT, 1 h; (b) TFAA, *tert*-BuOH, DCM, 0 °C to RT, 12 h; (c) **53**, DIPEA, DMF, 80 °C, 12 h; (d) TFA/DCM, 0 °C to RT, 6 h; (e) **58**, HATU, DIPEA, DMF, RT, 12 h.

Scheme 5. Synthesis of Compounds 22−29[a]



[a]Reagents and conditions: (a) 1,2-dibromoethane, Cs_2CO_3, MeCN, reflux, 12 h; (b) RNH_2, K_2CO_3, MeCN, 80 °C; (c) **65**, K_2CO_3, DMF, 80 °C, 12 h; (d) BBr_3, DCM, 0 °C to RT, 1 h; (e) **58**, HATU, DIPEA, DMF, RT, 12 h.

and **58** afforded the final compounds **22−29** in high yields. Compounds **38-48** were synthesized using the general procedure used to prepare compound **15**.

CONCLUSIONS

We have designed, synthesized, and evaluated a series of PROTAC ER degraders. Through extensive optimization of three structural components in our designed ER degraders, we have successfully obtained a number of highly potent ER degraders, exemplified by **32** (ERD-308). With 4 h treatment, compound **32** achieves DC_{50} values of 0.17 and 0.43 nM in MCF-7 and T47D cells, respectively, and induces over 95% of ER degradation at concentrations as low as 5 nM. In direct comparison, compound **32** can achieve more complete degradation than the only approved SERD molecule fulvestrant. Consistently, compound **32** achieves more complete cell growth inhibition than fulvestrant in MCF-7 cells. Our data suggest that further optimization of PROTAC ER degraders may lead to a class of completely new and effective therapeutic agents for the treatment of advanced and metastatic ER+ breast cancer.

EXPERIMENTAL SECTION

General Chemical Methods. Unless otherwise noted, all purchased reagents were used as received without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer. ^{1}H NMR spectra are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). All ^{13}C NMR spectra are reported in ppm and obtained with ^{1}H decoupling. In the spectral data reported, the format (δ) chemical shift (multiplicity, J values in Hz, integration) was used with the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. MS analyses were carried out with a H2Os UPLC−mass spectrometer. The final compounds were all purified by C18 reverse phase preparative HPLC column with solvent A (0.1% TFA in H_2O) and solvent B (0.1% TFA in MeCN) as eluents. The purities of all the final compounds were determined to be over 95% by UPLC-MS.

*(2S,4R)-1-((S)-2-Acetamido-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**11**).* HATU (21 mg, 0.055 mmol, 1.1 equiv) was added to a mixture of compound **58** (23 mg, 0.05 mmol, 1.0 equiv), acetic acid (4 μL, 0.06 mmol, 1.2 equiv), and DIPEA (26 μL, 0.15 mmol, 3.0 equiv) in DMF (2 mL) at 0 °C under N_2. The mixture was stirred at ambient temperature for 1 h, then the crude mixture was purified by reversed-phase preparative HPLC to afford the title compound as a white solid (19 mg, 80% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.02 (s, 1H), 7.47−7.42 (m, 4H), 5.04−4.98 (m, 1H), 4.62−

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

4.55 (m, 2H), 4.43−4.41 (m, 1H), 3.88 (d, J = 1.08 Hz, 1H), 3.74 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 2.50 (s, 3H), 2.22−2.16 (m, 1H), 2.00 (s, 3H), 1.98−1.91 (m, 1H), 1.51 (d, J = 6.8 Hz, 3H), 1.05 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 173.26, 173.11, 172.28, 153.34, 148.20, 146.01, 133.91, 131.04, 130.51, 127.69, 127.52, 70.97, 60.55, 59.22, 57.97, 50.14, 38.77, 36.41, 26.99, 22.38, 22.29, 15.41. UPLC-MS (ESI^+) calcd for $C_{25}H_{35}N_4O_4S$ $[M + 1]^+$: 487.24, found 487.43. Purity 98.5% (HPLC).

*3-(4-(3-Ethyl-1-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]-thiophene-3-carbonyl)phenoxy)-6,9,12-trioxa-3-azapentadec-14-yn-15-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**12**).* Methanesulfonyl chloride (0.35 mL, 4.5 mmol, 1.5 equiv) and triethylamine (0.84 mL, 6.0 mmol, 2.0 equiv) were added sequentially to a solution of commercial compound 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)-ethoxy)ethan-1-ol (**59a**) (565 mg, 3.0 mmol, 1.0 equiv) in DCM (10 mL) at 0 °C. The mixture was warmed to rt and stirred for 1 h. After concentration, the residue was purified by silica gel flash column chromatography with hexane:EtOAc (2:1−1:2) to afford the title compound (**60a**) as a colorless oil (710 mg, 89% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.21−4.18 (m, 2H), 4.01 (d, J = 2.4 Hz, 2H), 3.61−3.58 (m, 2H), 3.51−3.46 (m, 8H), 2.92 (s, 3H), 2.41 (t, J = 2.4 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 79.71, 74.91, 70.33, 70.25, 70.09, 69.51, 68.90, 68.77, 58.09, 37.43.

DIPEA (0.09 mL, 0.5 mmol, 5.0 equiv) was added to a solution of compound **53** (44 mg, 0.1 mmol, 1.0 equiv) and **60a** (40 mg, 0.15 mmol, 1.5 equiv) in DMF (3.0 mL). The solution was stirred at 100 °C for 12 h. After cooling to rt, the residue was purified by reversed-phase preparative HPLC to afford the title compound (**61a**) as a white solid (30 mg, 50% yield). UPLC-MS (ESI^+) calcd for $C_{34}H_{38}NO_7S$ $[M + 1]^+$: 604.24, found 604.30.

3-(4-Iodo-1-oxoisoindolin-2-yl)piperidine-2,6-dione (19 mg, 0.05 mmol, 1.0 equiv) was added to a solution of compound **61a** (30 mg, 0.05 mmol, 1.0 equiv) in DMF (2.0 mL). The solution was purged and refilled with nitrogen three times with sonication then $Pd(PPh_3)_2Cl_2$ (3.5 mg, 0.005 mmol, 0.1 equiv), CuI (2.0 mg, 0.01 mmol, 0.2 equiv), and trimethylamine (2.0 mL) were added sequentially. The solution was purged and refilled with N_2 again. The solution was stirred at 80 °C for 1 h and was then cooled to rt. EtOAc and H_2O were added, and the aqueous layer was extracted with EtOAc twice. The combined organic layer was dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by reversed-phase preparative HPLC to afford the title compound (**12**) as a yellow solid (18 mg, 43% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.76−7.71 (m, 3H), 7.58 (d, J = 7.6 Hz, 1H), 7.46−7.40 (m, 2H), 7.25 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 6.85 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 6.60 (d, J = 8.8 Hz, 2H), 5.12 (dd, J = 13.2 Hz, J = 5.2 Hz, 1H), 4.42−4.34 (m, 6H), 3.81−3.78 (m, 2H), 3.70−3.59 (m, 10H), 3.43 (t, J = 4.8 Hz, 2H), 3.35 (q, J = 7.6 Hz, 2H), 2.90−2.81 (m, 1H), 2.74−2.68 (m, 1H), 2.37−2.30 (m, 1H), 2.11−2.05 (m, 1H), 1.32 (t, J = 7.6 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.30, 174.53, 172.14, 170.68, 163.12, 159.25, 156.85, 145.48, 144.12, 141.47, 135.99, 134.20, 133.51, 133.10, 132.62, 131.44, 130.98, 129.77, 126.01, 124.75, 124.71, 119.34, 116.50, 116.12, 115.49, 107.94, 91.94, 82.61, 71.34, 71.24, 70.43, 65.49, 63.61, 59.69, 54.01, 53.58, 53.13, 51.37, 32.30, 24.02, 9.07. UPLC-MS (ESI^+) calcd for $C_{47}H_{48}N_3O_{10}S$ $[M + 1]^+$: 846.31, found 846.52. Purity 99.1% (HPLC).

*3-(4-(8-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]-thiophene-3-carbonyl)-phenoxy)ethyl)amino)oct-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**13**).* This compound was prepared using a procedure similar to that used for compound **12**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.73−7.71 (m, 3H), 7.56 (d, J = 7.2 Hz, 1H), 7.47−7.42 (m, 2H), 7.26 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 6.91−6.86 (m, 3H), 6.60 (d, J = 8.8 Hz, 2H), 5.16 (dd, J = 13.6 Hz, J = 5.2 Hz, 1H), 4.50 (d, J = 17.2 Hz, 1H), 4.44 (d, J = 17.2 Hz, 1H), 4.35 (t, J = 4.4 Hz, 2H), 3.58 (t, J = 4.4 Hz, 2H), 3.32−3.30 (m, 2H), 3.21 (q, J = 7.6 Hz, 2H), 2.93−2.84 (m, 1H), 2.77−2.73 (m, 1H), 2.50−2.44 (m, 3H), 2.17−2.12 (m, 1H), 1.79−1.72 (m, 2H), 1.68−1.61 (m, 2H), 1.58−1.51 (m, 2H), 1.47−1.42 (m, 2H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.28, 174.58, 172.22, 170.98, 163.03, 159.27, 156.87, 145.24, 144.38, 141.48, 135.77, 134.20, 133.51, 132.92, 132.75, 131.47, 130.99, 129.62, 126.02, 124.72, 123.74, 120.90, 116.46, 116.12, 115.38, 107.92, 97.08, 77.41, 63.59, 54.42, 53.66, 52.53, 50.31, 32.33, 29.39, 27.05, 24.68, 24.08, 19.94, 9.10. UPLC-MS (ESI^+) calcd for $C_{46}H_{46}N_3O_7S$ $[M + 1]^+$: 784.31, found 784.27. Purity 98.9% (HPLC).

*(2S,4R)-1-((S)-17-(tert-Butyl)-3-ethyl-1-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)-15-oxo-6,9,12-trioxa-3,16-diazaoctadecan-18-oyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**14**).* This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.44−7.39 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.19 (d, J = 8.4 Hz, 2H), 6.89−6.85 (m, 3H), 6.63 (d, J = 8.4 Hz, 2H), 5.02−4.96 (m, 1H), 4.64−4.54 (m, 2H), 4.43−4.41 (m, 1H), 4.20 (t, J = 5.2 Hz, 2H), 3.85 (d, J = 11.2 Hz, 1H), 3.75−3.64 (m, 5H), 3.60−3.55 (m, 8H), 3.20−2.93 (m, 6H), 2.54−2.46 (m, 5H), 2.20−2.17 (m, 1H), 1.98−1.92 (m, 1H), 1.49 (d, J = 7.2 Hz, 3H), 1.67 (t, J = 7.2 Hz, 3H), 1.02 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{57}H_{70}N_5O_{11}S_2$ $[M + 1]^+$: 1064.45, found 1064.74. Purity 96.4% (HPLC).

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**15**, ERD-148).* Trifluoroacetic anhydride (3.80 mL, 27.34 mmol, 2.0 equiv) was added at 0 °C to a solution of commercial 8-bromooctanoic acid **64** (3.05 g, 13.67 mmol, 1.0 equiv) in 50 mL of DCM. The solution was stirred at rt for 2 h. Then *tert*-butanol (3.92 mL, 41.01 mmol, 3.0 equiv) was added. The solution was stirred at rt for 12 h. Saturated aqueous $NaHCO_3$ was added. The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by silica gel flash column chromatography with hexane:EtOAc (20:1−5:1) to afford *tert*-butyl 8-bromooctanoate (**65**) as a colorless oil (2.48 g, 65% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 3.34 (t, J = 6.8 Hz, 2H), 2.14 (t, J = 7.6 Hz, 2H), 1.83−1.75 (m, 2H), 1.54−1.49 (m, 2H), 1.40−1.36 (m, 11H), 1.29−1.25 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 173.08, 79.88, 35.47, 33.81, 32.73, 28.86, 28.44, 28.12, 27.99, 24.95;

Compound **15** (ERD-148) was prepared using a procedure similar to that used for compound **32** with intermediate **65** instead of compound **63** as the starting material. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.09 (s, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.47−7.41 (m, 5H), 7.27 (d, J = 2.4 Hz, 1H), 7.19−7.15 (m, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.63−6.59 (m, 2H), 5.02−4.90 (m, 1H), 4.64−4.54 (m, 2H), 4.43−4.41 (m, 1H), 4.35 (t, J = 4.4 Hz, 2H), 3.88 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.60 (t, J = 4.8 Hz, 2H), 3.31−3.17 (m, 4H), 2.50 (s, 3H), 2.32−2.17 (m, 3H), 1.98−1.91 (m, 1H), 1.75−1.65 (m, 2H), 1.65−1.55 (m, 2H), 1.50 (d, J = 6.8 Hz, 3H), 1.43−1.29 (m, 9H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 193.96, 174.53, 171.84, 170.93, 161.69, 157.88, 155.47, 152.13, 144.63, 142.99, 140.09, 132.82, 132.14, 131.39, 130.07, 129.62, 129.12, 126.33, 124.64, 123.31, 115.08, 114.74, 114.01, 106.51, 69.57, 62.17, 59.21, 57.61, 56.61, 53.15, 51.19, 48.85, 48.76, 37.41, 35.10, 28.54, 28.41, 25.93, 25.65, 25.33, 23.31, 20.96, 13.89, 7.67. UPLC-MS (ESI^+) calcd for $C_{56}H_{68}N_5O_8S_2$ $[M + 1]^+$: 1002.45, found 1002.51. Purity 97.5% (HPLC).

*(2S,4R)-1-((S)-2-(4-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**16**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.45−7.39 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 8.8 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.86 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.62 (d, J = 8.8 Hz, 2H), 5.00−4.95 (m, 1H), 4.56−4.50 (m, 2H), 4.38−4.36 (m, 1H), 4.26 (t, J = 4.8 Hz,

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

2H), 3.83 (d, J = 11.2 Hz, 1H), 3.66 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.06−2.99 (m, 4H), 2.47 (s, 3H), 2.43 (t, J = 6.4 Hz, 2H), 2.20−2.15 (m, 1H), 1.97−1.89 (m, 3H), 1.49 (d, J = 6.8 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H), 1.00 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{52}H_{60}N_5O_8S_2$ $[M + 1]^+$: 946.39, found 946.41. Purity 97.4% (HPLC);

(2S,4R)-1-((S)-2-(5-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3 carbonyl)phenoxy)ethyl)amino)-pentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(17)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.76−7.73 (m, 1H), 7.45−7.38 (m, 5H), 7.27 (d, J = 2.0 Hz, 1H), 7.20−7.14 (m, 2H), 6.94−6.92 (m, 2H), 6.89−6.85 (m, 1H), 6.62−6.59 (m, 2H), 5.01−4.97 (m, 1H), 4.59−4.52 (m, 2H), 4.43−4.41 (m, 1H), 4.36 (t, J = 4.8 Hz, 2H), 3.86 (d, J = 11.2 Hz, 1H), 3.72 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.59 (t, J = 4.8 Hz, 2H), 3.31−3.21 (m, 4H), 2.47 (s, 3H), 2.37 (t, J = 6.8 Hz, 2H), 2.21−2.16 (m, 1H), 1.98−1.91 (m, 1H), 1.79−1.68 (m, 4H), 1.48 (d, J = 7.2 Hz, 3H), 1.34 (t, J = 7.2 Hz, 3H), 1.04 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{53}H_{62}N_5O_8S_2$ $[M + 1]^+$: 960.40, found 960.84. Purity 96.9% (HPLC).

(2S,4R)-1-((S)-2-(6-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(18)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.73 (d, J = 9.2 Hz, 2H), 7.44−7.38 (m, 5H), 7.26 (d, J = 2.4 Hz, 1H), 7.16 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 9.2 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 5.03−4.96 (m, 1H), 4.61−4.54 (m, 2H), 4.41−4.39 (m, 1H), 4.35 (t, J = 4.8 Hz, 2H), 3.87−3.84 (m, 1H), 3.73−3.71 (m, 1H), 3.58 (t, J = 4.4 Hz, 2H), 3.31−3.17 (m, 4H),2.47 (s, 3H), 2.37−2.26 (m, 2H), 2.21−2.16 (m, 1H), 1.97−1.91 (m, 1H), 1.77−1.62 (m, 4H), 1.49 (d, J = 7.2 Hz, 3H), 1.45−1.30 (m, 5H), 1.02 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{54}H_{64}N_5O_8S_2$ $[M + 1]^+$: 974.42, found 974.63. Purity 99.6% (HPLC).

(2S,4R)-1-((S)-2-(7-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-heptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(19)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.86 (s, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.44−7.38 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 6.89−6.80 (m, 3H), 6.62 (d, J = 8.8 Hz, 2H), 5.02−4.97 (m, 1H), 4.63−4.55 (m, 2H), 4.43−4.41 (m, 1H), 4.24 (t, J = 4.8 Hz, 2H), 3.87 (d, J = 10.8 Hz, 1H), 3.74 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.32−3.30 (m, 2H), 3.04−2.90 (m, 4H), 2.47 (s, 3H), 2.34−2.16 (m, 3H), 1.98−1.91 (m, 1H), 1.63−1.60 (m, 4H), 1.49 (d, J = 7.2 Hz, 3H), 1.37−1.35 (m, 4H), 1.25−1.18 (m, 3H), 1.01 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{55}H_{66}N_5O_8S_2$ $[M + 1]^+$: 988.44, found 988.60. Purity 96.2% (HPLC).

(2S,4R)-1-((S)-2-(9-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-nonanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(20)***. This compound was prepared using a procedure similar to that for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.75−7.70 (m, 2H), 7.44−7.39 (m, 5H), 7.26 (d, J = 2.4 Hz, 1H), 7.18−7.15 (m, 2H), 6.90 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 5.04−4.88 (m, 1H), 4.64−4.55 (m, 2H), 4.45−4.40 (m, 1H), 4.35 (t, J = 4.8 Hz, 2H), 3.89−3.86 (m, 1H), 3.74 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.58 (t, J = 4.8 Hz, 2H), 3.31−3.28 (m, 2H), 3.21−3.15 (m, 2H), 2.47 (s, 3H), 2.31−2.17 (m, 3H), 1.98−1.92 (m, 1H), 1.75−1.55 (m, 4H), 1.50 (d, J = 7.2 Hz, 3H), 1.36−1.31 (m, 11H), 1.03 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{57}H_{70}N_5O_8S_2$ $[M + 1]^+$: 1016.47, found 1016.53. Purity 95.7% (HPLC).

(2S,4R)-1-((S)-2-(10-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-decanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(21)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.86 (s, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.43−7.36 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 6.89−6.84 (m, 3H), 6.61 (d, J = 8.8 Hz, 2H), 5.02−4.97 (m, 1H), 4.64−4.55 (m, 2H), 4.43−4.41 (m, 1H), 4.27 (t, J = 4.8 Hz, 2H), 3.88 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.39 (t, J = 4.4 Hz, 2H), 3.15−3.09 (m, 2H), 3.03−2.98 (m, 2H), 2.46 (s, 3H), 2.33−2.17 (m, 3H), 1.98−1.92 (m, 1H), 1.69−1.50 (m, 4H), 1.49 (d, J = 7.2 Hz, 3H), 1.33−1.23 (m, 13H), 1.03 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{58}H_{72}N_5O_8S_2$ $[M + 1]^+$: 1030.48, found 1030.46. Purity 96.4% (HPLC).

(2S,4R)-4-Hydroxy-1-((S)-2-(8-((2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-(methyl)amino)octanamido)-3,3-dimethylbutanoyl)-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(22)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.95 (s, 1H), 7.74 (d, J = 9.2 Hz, 2H), 7.45−7.42 (m, 5H), 7.27 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 5.02−4.97 (m, 1H), 4.64−4.54 (m, 2H), 4.42−4.37 (m, 3H), 3.87 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 11.2 Hz, J = 4.4 Hz, 1H), 3.66−3.48 (m, 2H), 3.23−3.13 (m, 2H), 2.93 (s, 3H), 2.48 (s, 3H), 2.34−2.17 (m, 3H), 1.98−1.91 (m, 1H), 1.80−1.70 (m, 2H), 1.63−1.55 (m, 2H), 1.50 (d, J = 7.2 Hz, 3H), 1.45−1.35 (m, 6H), 1.03 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{55}H_{66}N_5O_8S_2$ $[M+1]^+$: 988.44, found 988.54. Purity 95.0% (HPLC).

(2S,4R)-4-Hydroxy-1-((S)-2-(8-((2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-(isopropyl)amino)octanamido)-3,3-dimethylbutanoyl)-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(23)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.86 (s, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.43−7.38 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 6.88−6.84 (m, 3H), 6.62 (d, J = 8.8 Hz, 2H), 5.02−4.97 (m, 1H), 4.63−4.55 (m, 2H), 4.43−4.41 (m, 1H), 4.16 (t, J = 5.2 Hz, 2H), 3.87 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.37−3.35 (m, 1H), 2.86−2.82 (m, 2H), 2.46 (s, 3H), 2.31−2.16 (m, 3H), 1.98−1.92 (m, 1H), 1.59−1.56 (m, 4H), 1.49 (d, J = 6.8 Hz, 3H), 1.33−1.27 (m, 10H), 1.18 (d, J = 6.8 Hz, 6H), 1.03 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{57}H_{70}N_5O_8S_2$ $[M + 1]^+$: 1016.47, found 1016.55. Purity 96.0% (HPLC).

(2S,4R)-1-((S)-2-(8-(tert-Butyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(24)***. This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 8.97 (s, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.47−7.40 (m, 5H), 7.26 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.92−6.86 (m, 3H), 6.62−6.60 (m, 2H), 5.03−4.97 (m, 1H), 4.62 (s, 1H), 4.59−4.55 (m, 1H), 4.45−4.41 (m, 1H), 4.32 (t, J = 4.4 Hz, 2H), 3.91−3.83 (m, 2H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.49−3.38 (m, 2H), 3.14−3.10 (m, 1H), 2.48 (s, 3H), 2.33−2.17 (m, 3H), 1.99−1.92 (m, 1H), 1.83−1.73 (m, 1H), 1.70−1.55 (m, 4H), 1.51−1.47 (m, 3H), 1.47−1.45 (m, 9H), 1.39−1.29 (m, 6H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.34, 175.92, 173.23, 172.33, 163.09, 159.30, 156.89, 153.17, 148.54, 145.80, 144.45, 141.51, 134.22, 133.59, 132.77, 131.50, 131.25, 130.98, 130.51, 127.67, 127.45, 126.04, 124.72, 116.47, 116.15, 115.30, 107.91, 70.97, 67.43, 65.32, 60.63, 59.02, 58.00, 53.38, 51.70, 50.16, 49.71, 38.82, 36.50, 29.99, 29.86, 27.63, 27.53, 27.06, 26.76, 24.94, 22.37, 15.57. UPLC-MS (ESI^+) calcd for $C_{58}H_{72}N_5O_8S_2$ $[M + 1]^+$: 1030.48, found 1030.52.

(2S,4R)-1-((S)-2-(8-(Cyclopropyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(25)***. This compound was prepared using a procedure similar to that

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.04 (s, 1H), 7.73 (d, J = 9.2 Hz, 2H), 7.46−7.41 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 8.8 Hz, 2H), 6.91−6.86 (m, 3H), 6.61 (d, J = 8.8 Hz, 2H), 5.03−4.97 (m, 1H), 4.64−4.61 (m, 1H), 4.57 (t, J = 8.4 Hz, 1H), 4.42−4.29 (m, 3H), 3.87 (d, J = 11.2 Hz, 1H), 3.76−3.65 (m, 3H), 3.32−3.30 (m, 1H), 2.89−2.84 (m, 1H), 2.49 (s, 3H), 2.33−2.17 (m, 3H), 1.99−1.92 (m, 1H), 1.85−1.75 (m, 2H), 1.63−1.55 (m, 2H), 1.50 (d, J = 7.2 Hz, 3H), 1.45−1.35 (m, 6H), 1.03 (s, 9H), 1.00−0.90 (m, 4H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.30, 175.92, 173.23, 172.33, 162.98, 159.26, 156.84, 153.43, 145.96, 144.57, 141.46, 134.21, 133.53, 132.77, 131.50, 131.02, 130.49, 127.70, 126.01, 124.75, 116.45, 116.14, 115.38, 107.92, 70.95, 63.22, 60.61, 59.01, 57.98, 57.85, 55.33, 50.14, 38.78, 38.54, 36.47, 29.94, 29.81, 27.42, 27.04, 26.72, 24.72, 22.34, 15.31. UPLC-MS (ESI$^+$) calcd for $C_{57}H_{68}N_5O_8S_2$ [M + 1]$^+$: 1014.45, found 1014.61. Purity 96.1% (HPLC).

(2S,4R)-1-((S)-2-(8-(Cyclobutyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(26)***. This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.04 (s, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.44−7.42 (m, 5H), 7.26 (d, J = 2.4 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 6.91−6.85 (m, 3H), 6.62 (d, J = 8.8 Hz, 2H), 5.03−4.97 (m, 1H), 4.64−4.55 (m, 2H), 4.45−4.41 (m, 1H), 4.32−4.29 (m, 2H), 3.91−3.86 (m, 2H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.49 (t, J = 4.8 Hz, 2H), 3.09 (t, J = 8.8 Hz, 2H), 2.48 (s, 3H), 2.35−2.15 (m, 7H), 1.99−1.92 (m, 1H), 1.87−1.55 (m, 6H), 1.50 (d, J = 7.2 Hz, 3H), 1.40−1.30 (m, 6H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.32, 175.91, 173.22, 172.33, 163.01, 159.27, 156.85, 153.42, 145.95, 144.39, 141.46, 134.22, 133.54, 132.73, 131.45, 131.01, 130.49, 127.70, 125.99, 124.73, 116.49, 116.15, 115.37, 107.94, 70.95, 63.46, 60.60, 59.61, 59.01, 57.98, 52.11, 50.49, 50.14, 38.78, 36.49, 36.47, 29.94, 29.79, 27.40, 27.05, 26.71, 24.30, 22.35, 15.33, 14.18. UPLC-MS (ESI$^+$) calcd for $C_{58}H_{70}N_5O_8S_2$ [M + 1]$^+$: 1028.47, found 1029.18. Purity 97.7% (HPLC).

(2S,4R)-1-((S)-2-(8-(Cyclopentyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(27)***. This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.93 (s, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.45−7.41 (m, 5H), 7.26 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.92−6.85 (m, 3H), 6.61 (d, J = 8.8 Hz, 2H), 5.02−4.98 (m, 1H), 4.64−4.55 (m, 2H), 4.43−4.41 (m, 1H), 4.37−4.33 (m, 2H), 3.89−3.73 (m, 3H), 3.65−3.55 (m, 2H), 3.22 (t, J = 8.4 Hz, 2H), 2.48 (s, 3H), 2.30−2.15 (m, 5H), 2.03−1.94 (m, 1H), 1.84−1.57 (m, 10H), 1.50 (d, J = 7.2 Hz, 3H), 1.45−1.30 (m, 6H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.34, 175.89, 173.21, 172.34, 163.06, 159.29, 156.87, 153.06, 148.67, 145.73, 144.37, 141.49, 134.22, 133.55, 132.78, 131.46, 131.32, 131.01, 130.49, 127.65, 127.42, 126.03, 124.71, 116.48, 116.14, 115.36, 107.92, 70.96, 67.31, 63.94, 60.62, 59.00, 57.99, 54.01, 52.09, 38.80, 36.50, 29.97, 29.84, 29.20, 29.14, 27.39, 27.05, 26.72, 24.85, 24.81, 24.66, 22.34, 15.61. UPLC-MS (ESI$^+$) calcd for $C_{59}H_{72}N_5O_8S_2$ [M + 1]$^+$: 1042.48, found 1042.39. Purity >99.5% (HPLC).

(2S,4R)-1-((S)-2-(8-(Cyclohexyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(28)***. This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.45−7.39 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.91−6.84 (m, 3H), 6.62−6.60 (m, 2H), 5.02−4.97 (m, 1H), 4.64−4.54 (m, 2H), 4.45−4.41 (m, 1H), 4.32 (t, J = 4.4 Hz, 2H), 3.89−3.85 (m, 1H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.59−3.45 (m, 2H), 3.18−3.08 (m, 2H), 2.47 (s, 3H), 2.34−2.16 (m, 3H), 2.03−1.90 (m, 5H), 1.74−1.31 (m, 20H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.37, 175.94, 173.22, 172.33, 163.29, 159.29, 156.87, 152.87, 149.07, 145.61, 144.35, 141.49, 134.23, 133.56, 133.34, 131.53, 131.47, 131.03, 130.50, 127.62, 127.41, 126.04, 116.47, 116.13, 115.36, 107.91, 70.96, 60.62, 59.01, 58.00, 53.41, 51.06, 50.15, 49.28, 38.81, 37.63, 36.50, 30.00, 29.89, 27.58, 27.05, 26.20, 26.19, 26.18, 26.17, 26.15, 26.14, 22.36, 15.79. UPLC-MS (ESI$^+$) calcd for $C_{60}H_{74}N_5O_8S_2$ [M + 1]$^+$: 1056.50, found 1056.54.

(2S,4R)-1-((S)-2-(8-((Cyclobutylmethyl)(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo-[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(29)***. This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.98 (s, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.44−7.36 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.90−6.85 (m, 3H), 6.61(d, J = 8.4 Hz, 2H), 5.02−4.97 (m, 1H), 4.64−4.55 (m, 2H), 4.45−4.41 (m, 1H), 4.33 (t, J = 4.4 Hz, 2H), 3.87 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.54−3.52 (m, 2H), 3.28−3.25 (m, 2H), 3.16−3.11 (m, 2H), 2.80−2.73 (m, 1H), 2.48 (s, 3H), 2.32−2.14 (m, 5H), 2.04−1.84 (m, 5H), 1.75−1.65 (m, 2H), 1.65−1.55 (m, 2H), 1.49 (d, J = 7.2 Hz, 3H), 1.42−1.30 (m, 6H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.33, 175.89, 173.20, 172.32, 163.05, 159.27, 156.84, 153.25, 148.24, 145.84, 144.30, 141.45, 134.22, 133.52, 132.72, 131.43, 131.07, 131.01, 130.48, 127.67, 125.97, 124.72, 116.50, 116.14, 115.39, 107.95, 70.94, 63.50, 60.60, 60.11, 59.00, 57.98, 55.21, 51.06, 50.13, 38.78, 36.48, 31.78, 29.94, 29.81, 28.20, 28.09, 27.35, 27.05, 26.71, 24.52, 22.34, 19.42, 15.46. UPLC-MS (ESI$^+$) calcd for $C_{59}H_{72}N_5O_8S_2$ [M + 1]$^+$: 1042.48, found 1042.82. Purity >99.5% (HPLC).

(2S,4R)-1-((S)-14-(tert-Butyl)-3-ethyl-1-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]-thiophene-3-carbonyl)phenoxy)-12-oxo-6,9-dioxa-3,13-diazapentadecan-15-oyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(30)***. This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.86 (s, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.44−7.37 (m, 5H), 7.27 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.62 (d, J = 8.4 Hz, 2H), 5.02−4.95 (m, 1H), 4.57−4.53 (m, 2H), 4.40−4.37 (m, 3H), 3.85−3.80 (m, 3H), 3.73−3.58 (m, 9H), 3.45 (t, J = 8.8 Hz, 2H), 3.37 (q, J = 7.2 Hz, 2H), 2.55−2.44 (m, 5H), 2.22−2.17 (m, 1H), 1.97−1.91 (m, 1H), 1.48 (d, J = 7.2 Hz, 3H), 1.35 (t, J = 7.2 Hz, 3H), 1.01 (s, 9H). UPLC-MS (ESI$^+$) calcd for $C_{55}H_{66}N_5O_{10}S_2$ [M + 1]$^+$: 1020.43, found 1020.77. Purity 97.2% (HPLC).

(2S,4R)-1-((S)-2-(tert-Butyl)-12-ethyl-14-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]-thiophene-3-carbonyl)phenoxy)-4-oxo-6,9-dioxa-3,12-diazatetradecanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(31)***. This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.11 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.44−7.37 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 6.92−6.84 (m, 3H), 6.61 (d, J = 8.4 Hz, 2H), 4.97−4.91 (m, 1H), 4.71−4.68 (m, 1H), 4.57−4.54 (m, 1H), 4.41−4.38 (m, 3H), 4.02−3.40 (m, 16H), 2.48 (s, 3H), 2.36−2.20 (m, 1H), 1.95−1.89 (m, 1H), 1.45 (d, J = 7.2 Hz, 2H), 1.37 (t, J = 7.6 Hz, 3H), 1.01 (s, 9H). UPLC-MS (ESI$^+$) calcd for $C_{54}H_{64}N_5O_{10}S_2$ [M + 1]$^+$: 1006.41, found 1006.66. Purity 95.1% (HPLC).

(2S,4R)-1-((S)-2-(2-((5-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)pentyl)oxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide ***(32, ERD-308)***. Sodium hydroxide (4.0 g, 100.0 mmol, 10.0 equiv) and tetrabutyl ammonium chloride (2.78 g, 10.0 mmol, 1.0 equiv) were added sequentially to a solution of 5-(benzyloxy)-pentan-1-ol (1.94 g, 10.0 mmol, 1.0 equiv) and *tert*-butyl 2-bromoacetate (3.90 g, 20.0 mmol, 2.0 equiv) in H_2O (20 mL) and DCM (20 mL). The solution was stirred vigorously at rt overnight when TLC showed that the reaction was complete. The mixture was partitioned between DCM (100 mL) and H_2O (100 mL), and the organic layer was collected, washed with brine (100 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

to give a residue that was purified by silica gel flash column chromatography with hexane:EtOAc (10:1−5:1) to afford *tert*-butyl 2-((5-(benzyloxy)pentyl)oxy)acetate as a colorless oil (987 mg, 32% yield).

A mixture of *tert*-butyl 2-((5-(benzyloxy)pentyl)oxy)acetate (770 mg, 2.5 mmol, 1.0 equiv) and 10 wt % palladium on carbon (100 mg) in MeOH (20 mL) was stirred at rt overnight under a hydrogen atmosphere. TLC showed that the reaction was complete and the solution was filtered through Celite and washed with MeOH. The combined filtrate was concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with hexane:EtOAc (2:1−1:1) to afford *tert*-butyl 2-((5-hydroxypentyl)-oxy)acetate (**62**) as a colorless oil (671 mg, 95% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 3.58 (s, 2H), 3.21 (t, J = 6.8 Hz, 2H), 3.15 (t, J = 6.8 Hz, 2H), 1.31−1.14 (m, 4H), 1.12−1.05 (m, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 169.51, 80.94, 71.18, 68.20, 61.54, 31.96, 28.97, 27.67, 21.95.

4-Toluenesulfonyl chloride (879 mg, 4.6 mmol, 1.5 equiv) and Et_3N (0.86 mL, 6.14 mmol, 2.0 equiv) were added sequentially to a solution of *tert*-butyl 2-((5-hydroxypentyl)oxy)acetate (**62**) (671 mg, 3.07 mmol, 1.0 equiv) in DCM (10 mL) at 0 °C. The mixture was warmed to rt and stirred for 1 h. After concentration, the residue was purified by silica gel flash column chromatography with hexane:E-tOAc (5:1−2:1) to afford the intermediate *tert*-butyl 2-((5-(tosyloxy)-pentyl)oxy)acetate (**63**) as a colorless oil (1.02 g, 89% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.75 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 3.99 (t, J = 6.4 Hz, 2H), 3.88 (s, 2H), 3.43 (t, J = 6.4 Hz, 2H), 2.42 (s, 3H), 1.68−1.61 (m, 2H), 1.57−1.50 (m, 2H), 1.44 (s, 9H), 1.42−1.36 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 169.76, 144.75, 133.16, 129.89, 127.92, 81.54, 71.19, 70.55, 68.76, 28.99, 28.65, 28.15, 22.07, 21.67. UPLC-MS (ESI^+) calcd for $C_{18}H_{28}NaO_6S$ $[M + 23]^+$: 395.15, found 395.36.

DIPEA (0.18 mL, 1.0 mmol, 5.0 equiv) was added to a solution of intermediate **53** (87 mg, 0.2 mmol, 1.0 equiv) and *tert*-butyl 2-((5-(tosyloxy)pentyl)oxy)acetate **63** (223 mg, 0.6 mmol, 3.0 equiv) in DMF (3.0 mL). The solution was stirred at 80 °C for 12 h. After cooling to temperature, the solution was diluted with EtOAc and H_2O. The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by silica gel flash column chromatography with DCM:MeOH (10:1) to afford the intermediate (**66**) as colorless oil (114 mg, 90% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.65 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 7.2 Hz, 1H), 7.19 (s, 1H), 7.08 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 9.2 Hz, 1H), 6.59−6.54 (m, 4H), 3.99−3.95 (m, 2H), 3.92 (s, 2H), 3.48−3.40 (m, 4H), 2.86−2.82 (m, 2H), 2.64 (q, J = 6.8 Hz, 2H), 2.55−2.51 (m, 2H), 1.59−1.52 (m, 2H), 1.44 (s, 9H), 1.31−1.25 (m, 2H), 1.01 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 194.16, 170.28, 162.73, 157.39, 154.83, 143.60, 140.09, 133.37, 132.59, 130.47, 129.90, 125.01, 124.06, 116.02, 115.57, 114.20, 107.67, 82.05, 71.67, 68.75, 53.54, 53.32, 51.72, 47.86, 29.78, 29.38, 28.18, 24.01, 10.64. UPLC-MS (ESI^+) calcd for $C_{36}H_{44}NO_7S$ $[M + 23]^+$: 634.28, found 634.18.

Trifluoroacetic acid (5.0 mL) was added to a solution of intermediate **66** (114 mg, 0.18 mmol) in DCM (10 mL) at 0 °C. The solution was stirred at rt for 6 h. After concentration, the residue was purified by reversed-phase preparative HPLC to afford the title compound (**67**) as a slightly yellow solid (81 mg, 78% yield). UPLC-MS (ESI^+) calcd for $C_{32}H_{36}NO_7S$ $[M + 23]^+$: 578.22, found 578.06.

HATU (53 mg, 0.14 mmol, 1.0 equiv) was added to a solution of intermediate **67** (81 mg, 0.14 mmol, 1.0 equiv), intermediate **58** (67 mg, 0.15 mmol, 1.1 equiv), and DIPEA (0.12 mL, 0.70 mmol, 5.0 equiv) in DMF (2 mL). The mixture was stirred at ambient temperature for 1 h then was purified by reversed-phase preparative HPLC to afford the title compound **32** (ERD-308) as a yellow solid (56 mg, 40% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.86 (s, 1H), 7.74 (d, J = 9.2 Hz, 2H), 7.43−7.35 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.91−6.85 (m, 3H), 6.62 (d, J = 8.4 Hz, 2H), 4.98−4.95 (m, 1H), 4.89 (s, 2H), 4.69−4.64 (m, 1H), 4.59−4.53 (m, 1H), 4.45−4.41 (m, 1H), 4.31 (t, J = 4.8 Hz, 2H), 4.02−3.92 (m, 2H), 3.84 (d, J = 11.2 Hz, 1H), 3.74 (dd, J = 10.8 Hz, J = 3.6 Hz, 1H), 3.56 (t, J = 6.8 Hz, 2H), 3.45 (t, J = 4.8 Hz, 2H), 3.11−3.07 (m, 2H), 2.47 (s, 3H), 2.22−2.19 (m, 1H), 1.98−1.92 (m, 1H), 1.76−1.66 (m, 4H), 1.57−1.46 (m, 5H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.36, 173.13, 173.05, 171.91, 171.80, 163.11, 159.29, 156.87, 152.90, 149.01, 145.60, 144.31, 141.47, 134.22, 133.55, 132.72, 131.45, 131.02, 130.49, 127.61, 127.38, 126.00, 124.72, 116.50, 116.13, 115.45, 107.93, 72.40, 70.90, 70.73, 69.08, 63.61, 60.68, 58.14, 54.33, 52.59, 50.26, 38.90, 37.80, 37.13, 29.90, 26.93, 24.56, 24.40, 22.43, 15.79, 9.18. UPLC-MS (ESI^+) calcd for $C_{55}H_{66}N_5O_9S_2$ $[M + 1]^+$: 1004.43, found 1004.11. Purity 97.4% (HPLC).

*(2S,4R)-1-((S)-2-(4-(4-(2-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)ethyl)piperidin-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**33**).* This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.95 (s, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.45−7.40 (m, 5H), 7.27 (d, J = 2.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 9.2 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.60 (d, J = 8.4 Hz, 2H), 5.03−4.90 (m, 1H), 4.59−4.53 (m, 2H), 4.43−4.41 (m, 1H), 4.37 (t, J = 4.8 Hz, 2H), 3.90 (d, J = 10.8 Hz, 1H), 3.74 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.61−3.56 (m, 4H), 3.35−3.27 (m, 4H), 3.10 (t, J = 6.8 Hz, 2H), 2.94−2.86 (m, 2H), 2.51 (t, J = 6.4 Hz, 2H), 2.48 (s, 3H), 2.22−2.17 (m, 1H), 2.01−1.95 (m, 5H), 1.78−1.68 (m, 3H), 1.59−1.57 (m, 2H), 1.50 (d, J = 7.2 Hz, 3H), 1.34 (t, J = 7.6 Hz, 3H), 1.06 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{59}H_{73}N_6O_8S_2$ $[M + 1]^+$: 1057.49, found 1057.90. Purity 99.1% (HPLC).

*(2S,4R)-1-((S)-2-(4-(4-(2-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)ethyl)piperazin-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**34**).* This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.94 (s, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.45−7.40 (m, 5H), 7.27 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 9.2 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.61 (d, J = 8.4 Hz, 2H), 5.03−4.99 (m, 1H), 4.58−4.54 (m, 2H), 4.44−4.39 (m, 3H), 3.90 (d, J = 10.8 Hz, 1H), 3.76−3.70 (m, 3H), 3.51−3.48 (m, 2H), 3.42−3.37 (m, 2H), 3.14−3.12 (m, 2H), 2.85 (t, J = 6.4 Hz, 2H), 2.53 (t, J = 6.4 Hz, 2H), 2.48 (s, 3H), 2.23−2.18 (m, 1H), 2.01−1.92 (m, 3H), 1.50 (d, J = 6.8 Hz, 3H), 1.36 (t, J = 7.2 Hz, 3H), 1.06 (s, 9H). UPLC-MS (ESI^+) calcd for $C_{58}H_{72}N_7O_8S_2$ $[M + 1]^+$: 1058.49, found 1058.72. Purity 99.3% (HPLC).

*(2S,4R)-1-((S)-2-(3-(4-(5-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)pent-1-yn-1-yl)-1H-pyrazol-1-yl)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)-ethyl)pyrrolidine-2-carboxamide (**35**).* This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.13 (s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.62 (s, 1H), 7.46−7.41 (m, 6H), 7.27 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.90−6.86 (m, 3H), 6.61 (d, J = 8.8 Hz, 2H), 5.01−4.96 (m, 1H), 4.59−4.55 (m, 2H), 4.42−4.26 (m, 5H), 3.86 (d, J = 10.8 Hz, 1H), 3.72 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.62−3.57 (m, 2H), 3.41−3.30 (m, 4H), 2.92−2.85 (m, 1H), 2.75−2.70 (m, 1H), 2.54 (t, J = 6.8 Hz, 2H), 2.50 (s, 3H), 2.22−2.17 (m, 1H), 2.01−1.91 (m, 3H), 1.50 (d, J = 6.8 Hz, 3H), 1.36 (t, J = 7.2 Hz, 3H), 0.95 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.31, 173.25, 172.24, 172.10, 163.01, 159.26, 156.86, 153.68, 146.13, 144.43, 142.84, 141.47, 134.21, 133.48, 132.76, 131.49, 131.00, 130.51, 127.74, 126.02, 124.77, 116.48, 116.15, 115.42, 107.92, 104.32, 88.60, 74.07, 70.96, 63.58, 60.60, 59.18, 57.88, 53.41, 52.83, 50.53, 50.45, 38.77, 36.74, 36.42, 26.98, 23.92, 22.36, 17.27, 15.15, 9.16. UPLC-MS (ESI^+) calcd for $C_{59}H_{66}N_7O_8S_2$ $[M + 1]^+$: 1064.44, found 1064.89. Purity 95.1% (HPLC).

*(2S,4R)-1-((S)-2-(2-(4-(4-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)-amino)but-1-yn-1-yl)-1H-pyrazol-1-yl)acetamido)-3,3-dimethyl-butanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)-ethyl)pyrrolidine-2-carboxamide (**36**).* This compound was prepared

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.87 (s, 1H), 7.74−7.70 (m, 3H), 7.52 (s, 1H), 7.44−7.41 (m, 5H), 7.26 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.88−6.85 (m, 3H), 6.63 (d, J = 8.8 Hz, 2H), 5.01−4.98 (m, 1H), 4.62−4.52 (m, 2H), 4.42−4.39 (m, 3H), 3.82 (d, J = 11.2 Hz, 1H), 3.76−3.64 (m, 3H), 3.50−3.39 (m, 6H), 2.96 (t, J = 7.2 Hz, 2H), 2.47 (s, 3H), 2.26−2.15 (m, 1H), 1.96−1.90 (m, 1H), 1.49 (d, J = 7.2 Hz, 3H), 1.38 (t, J = 7.2 Hz, 3H), 1.02 (s, 9H). UPLC-MS (ESI$^+$) calcd for $C_{57}H_{62}N_7O_8S_2$ [M + 1]$^+$: 1036.41, found 1035.92. Purity 98.8% (HPLC).

*(2S,4R)-1-((S)-2-(2-(4-(4-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)butyl)-1H-pyrazol-1-yl)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**37**).* This compound was prepared using a procedure similar to that used for compound **32**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.23 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.49 (s, 1H), 7.44−7.37 (m, 6H), 7.25 (d, J = 2.4 Hz, 1H), 7.14 (d, J = 8.8 Hz, 2H), 6.87−6.83 (m, 3H), 6.59 (d, J = 8.4 Hz, 2H), 4.99−4.83 (m, 3H), 4.61−4.52 (m, 2H), 4.40−4.37 (m, 1H), 4.30 (t, J = 4.4 Hz, 2H), 3.81 (d, J = 11.2 Hz, 1H), 3.69 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.54 (d, J = 4.0 Hz, 2H), 3.28−3.16 (m, 4H), 2.54−2.46 (m, 5H), 2.22−2.17 (m, 1H), 1.96−1.89 (m, 1H), 1.73−1.60 (m, 4H), 1.47 (d, J = 7.2 Hz, 3H), 1.30 (t, J = 7.2 Hz, 3H), 1.00 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.28, 173.08, 171.67, 169.31, 163.05, 159.21, 156.78, 153.34, 147.93, 145.88, 144.26, 141.40, 140.79, 134.19, 133.92, 133.49, 132.59, 131.61, 131.41, 130.96, 130.89, 130.45, 127.65, 125.90, 124.74, 122.64, 116.52, 116.16, 115.42, 108.00, 70.90, 63.51, 60.56, 59.13, 57.96, 54.70, 54.16, 52.44, 50.14, 38.80, 36.70, 28.55, 26.93, 26.82, 24.24, 24.05, 22.38, 15.39, 9.07. UPLC-MS (ESI$^+$) calcd for $C_{57}H_{66}N_7O_8S_2$ [M + 1]$^+$: 1040.44, found 1040.17. Purity 98.7% (HPLC).

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-((Z)-1-(4-hydroxyphenyl)-2-phenylbut-1-en-1-yl)-phenoxy)ethyl)amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)-ethyl)pyrrolidine-2-carboxamide (**38**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.98 (s, 1H), 7.47−7.36 (m, 4H), 7.13−7.01 (m, 7H), 6.84−6.75 (m, 3H), 6.66−6.60 (m, 2H), 5.03−4.98 (m, 1H), 4.64−4.62 (m, 1H), 4.56 (t, J = 8.4 Hz, 1H), 4.45−4.41 (m, 1H), 4.22−4.18 (m, 2H), 3.89−3.86 (m, 1H), 3.74 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.55−3.52 (m, 2H), 3.21−3.15 (m, 2H), 2.52−2.45 (m, 5H), 2.32−2.17 (m, 3H), 1.99−1.92 (m, 1H), 1.78−1.56 (m, 5H), 1.52−1.49 (m, 3H), 1.45−1.30 (m, 9H), 1.04 (s, 9H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 175.90, 173.22, 172.31, 157.41, 156.91, 153.20, 145.85, 144.03, 142.58, 139.49, 138.76, 136.07, 133.23, 131.55, 130.91, 130.51, 128.88, 127.67, 127.04, 115.92, 114.46, 70.96, 63.11, 60.60, 58.99, 58.00, 54.48, 52.76, 50.15, 38.82, 37.64, 36.51, 33.74, 29.95, 29.93, 29.86, 29.81, 27.36, 27.05, 26.73, 24.71, 22.37, 15.54, 13.86, 9.04. UPLC-MS (ESI$^+$) calcd for $C_{57}H_{74}N_5O_6S$ [M + 1]$^+$: 956.54, found 956.51.

*(2S,4R)-1-((S)-2-(8-((2-(4-(1,2-Diphenylbut-1-en-1-yl)phenoxy)-ethyl)(ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**39**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.08 (s, 1H), 7.47−7.42 (m, 4H), 7.36−7.32 (m, 1H), 7.28−7.22 (m, 0.5H), 7.21−7.07 (m, 7H), 7.03−6.95 (m, 2.5H), 6.86−6.82 (m, 2H), 6.66−6.64 (m, 1H), 5.03−4.98 (m, 1H), 4.64−4.62 (m, 1H), 4.57 (t, J = 8.4 Hz, 1H), 4.43−4.41 (m, 1H), 4.38 (t, J = 4.8 Hz, 1H), 4.21 (t, J = 4.8 Hz, 1H), 3.88 (d, J = 10.8 Hz, 1H), 3.74 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.64 (t, J = 4.8 Hz, 1H), 3.53 (t, J = 4.8 Hz, 1H), 3.38−3.34 (m, 1H), 3.31−3.17 (m, 3H), 2.49−2.42 (m, 5H), 2.30−2.19 (m, 3H), 1.99−1.95 (m, 1H), 1.70−1.56 (m, 4H), 1.50 (d, J = 7.2 Hz, 3H), 1.40−1.30 (m, 9H), 1.04 (s, 9H), 0.94−0.88 (m, 3H). UPLC-MS (ESI$^+$) calcd for $C_{57}H_{74}N_5O_5S$ [M + 1]$^+$: 940.54, found 940.82. Purity 97.0% (HPLC).

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-((5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1H-indol-1-yl)methyl)phenoxy)ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**40**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 9.08 (s, 1H), 7.45−7.40 (m, 4H), 7.12 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.8 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 6.84−6.75 (m, 6H), 6.63 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 5.11 (s, 2H), 5.02−4.97 (m, 1H), 4.64−4.62 (m, 1H), 4.56 (t, J = 8.4 Hz, 1H), 4.43−4.41 (m, 1H), 4.24 (t, J = 4.8 Hz, 1H), 3.87 (d, J = 11.2 Hz, 1H), 3.73 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.54 (t, J = 4.8 Hz, 1H), 2.49 (s, 3H), 2.31−2.15 (m, 6H), 1.98−1.92 (m, 1H), 1.73−1.65 (m, 2H), 1.59−1.55 (m, 2H), 1.50 (d, J = 7.2 Hz, 3H), 1.40−1.29 (m, 9H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 175.91, 173.21, 172.33, 158.52, 157.92, 153.66, 151.65, 146.06, 139.86, 133.87, 133.08, 132.79, 131.04, 130.50, 128.68, 127.72, 124.47, 116.23, 115.54, 112.17, 111.59, 108.66, 103.92, 70.94, 63.27, 60.60, 59.00, 57.98, 54.43, 52.72, 50.15, 47.58, 38.78, 36.47, 29.90, 29.80, 29.76, 27.04, 24.69, 22.35, 15.21, 9.62, 9.05. UPLC-MS (ESI$^+$) calcd for $C_{57}H_{73}N_6O_7S$ [M + 1]$^+$: 985.53, found 985.82. Purity >99.5% (HPLC).

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-((1R,2S)-6-hydroxy-2-phenyl-1,2,3,4-tetrahydronaphthalen-1-yl)phenoxy)ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**41**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.94 (s, 1H), 7.46−7.35 (m, 4H), 7.13−7.09 (m, 3H), 6.83−6.80 (m, 2H), 6.68−6.61 (m, 4H), 6.52 (dd, J = 8.4 Hz, J = 4.0 Hz 1H), 6.38 (d, J = 8.4 Hz, 2H), 5.03−4.98 (m, 1H), 4.64−4.54 (m, 2H), 4.43−4.41 (m, 1H), 4.25−4.20 (m, 3H), 3.89−3.86 (m, 1H), 3.75 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.56−3.53 (m, 2H), 3.37−3.35 (m, 1H), 3.23−3.16 (m, 2H), 3.06−2.99 (m, 2H), 2.49 (s, 3H), 2.34−2.14 (m, 4H), 1.99−1.92 (m, 1H), 1.79−1.50 (m, 8H), 1.38−1.29 (m, 9H), 1.03 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 175.89, 173.23, 172.31, 157.05, 156.64, 153.10, 148.68, 145.79, 138.82, 137.76, 132.43, 132.06, 131.33, 130.51, 129.21, 128.72, 127.66, 127.66, 127.44, 126.97, 126.63, 115.50, 114.68, 113.98, 70.96, 63.16, 61.04, 60.61, 58.98, 58.01, 54.48, 52.81, 52.24, 51.57, 50.15, 46.73, 38.82, 36.52, 36.49, 30.97, 29.97, 28.83, 27.38, 27.05, 26.74, 25.59, 24.73, 24.44, 23.24, 22.37, 15.62, 9.06. UPLC-MS (ESI$^+$) calcd for $C_{57}H_{74}N_5O_6S$ [M + 1]$^+$: 956.54, found 956.48.

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**42**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.86 (s, 1H), 7.74 (d, J = 9.2 Hz, 2H), 7.46−7.39 (m, 5H), 7.27 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 4.64 (d, J = 8.8 Hz, 1H), 4.58−4.49 (m, 3H), 4.38−4.33 (m, 3H), 3.90 (d, J = 11.2 Hz, 1H), 3.80 (dd, J = 10.8 Hz, J = 4.0 Hz, 1H), 3.59 (t, J = 4.8 Hz, 2H), 3.21−3.17 (m, 2H), 2.46 (s, 3H), 2.32−2.19 (m, 3H), 2.11−2.03 (m, 1H), 1.73−1.71 (m, 2H), 1.62−1.59 (m, 2H), 1.38−1.29 (m, 9H), 1.02 (s, 9H). UPLC-MS (ESI$^+$) calcd for $C_{55}H_{66}N_5O_8S_2$ [M + 1]$^+$: 988.44, found 988.98. Purity 97.8% (HPLC).

*(2S,4R)-N-((S)-1-(4-Chlorophenyl)ethyl)-1-((S)-2-(8-(ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)-phenoxy)ethyl)amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (**43**).* This compound was prepared using a procedure similar to that used for compound **15**. ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.75 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.8 Hz, 1H), 7.32−7.27 (m, 5H), 7.21−7.15 (m, 2H), 6.95−6.86 (m, 3H), 6.65−6.60 (m, 2H), 4.95−4.87 (m, 1H), 4.63−4.61 (m, 1H), 4.53 (t, J = 8.4 Hz, 2H), 3.87−3.84 (m, 1H), 3.73 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.64−3.54 (m, 3H), 3.26−3.16 (m, 3H), 3.07−2.96 (m, 1H), 2.33−2.13 (m, 3H), 1.95−1.88 (m, 1H), 1.78−1.58 (m, 5H), 1.51 (d, J = 7.2 Hz, 2H), 1.41−1.29 (m, 11H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.37, 175.91, 173.18, 172.29, 163.09, 159.28, 156.88, 144.38, 144.13, 141.49, 134.22, 133.71, 133.53, 132.77, 131.65, 131.47, 131.02, 129.56, 128.70, 128.70, 128.47, 126.04, 124.71, 116.59, 124.71, 116.59, 116.47, 116.13, 115.41, 107.91, 70.94, 63.57, 60.57, 58.99, 57.98, 54.55, 52.59, 50.24,

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

49.82, 38.78, 36.49, 34.62, 29.95, 29.81, 27.33, 27.03, 26.73, 25.75, 24.71, 22.28, 9.06. UPLC-MS (ESI^+) calcd for $C_{52}H_{64}ClN_4O_8S$ $[M + 1]^+$: 939.41, found 939.45.

*(2S,4R)-N-((S)-1-(4-Chlorophenyl)ethyl)-1-((S)-2-(8-(ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)-phenoxy)ethyl)amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (**44**).* This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 7.75 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.49−7.40 (m, 3H), 7.27 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 6.94−6.86 (m, 3H), 6.64−6.60 (m, 2H), 5.01−4.96 (m, 1H), 4.63−4.61 (m, 1H), 4.47−4.38 (m, 1H), 4.35 (t, J = 4.8 Hz, 2H), 3.88−3.85 (m, 1H), 3.72 (dd, J = 11.2 Hz, J = 4.0 Hz, 1H), 3.60−3.58 (m, 2H), 3.23−3.17 (m, 2H), 2.32−2.16 (m, 3H), 1.94−1.87 (m, 1H), 1.73−1.60 (m, 5H), 1.54−1.46 (m, 3H), 1.38−1.29 (m, 10H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.36, 175.91, 173.38, 172.30, 163.09, 159.28, 156.88, 151.22, 144.39, 141.49, 134.22, 131.47, 131.02, 128.06, 127.85, 126.04, 124.71, 119.67, 116.48, 116.13, 115.40, 111.78, 107.91, 70.95, 63.56, 60.51, 58.99, 57.99, 54.54, 52.58, 50.33, 50.22, 38.81, 36.49, 29.96, 29.82, 27.34, 27.02, 26.73, 24.72, 22.09, 9.06. UPLC-MS (ESI^+) calcd for $C_{53}H_{64}N_5O_8S$ $[M + 1]^+$: 930.45, found 930.48.

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-N-((S)-1-(4-ethynylphenyl)-ethyl)-4-hydroxypyrrolidine-2-carboxamide (**45**).* This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 7.75 (d, J = 8.8 Hz, 2H), 7.46−7.38 (m, 3H), 7.29−7.22 (m, 3H), 7.19−7.15 (m, 2H), 6.93−6.86 (m, 3H), 6.61(d, J = 8.8 Hz, 2H), 4.97−4.92 (m, 1H), 4.63−4.61 (m, 1H), 4.54 (t, J = 8.4 Hz, 2H), 4.42−4.39 (m, 1H), 4.36 (t, J = 4.8 Hz, 2H), 3.88−3.85 (m, 1H), 3.75−3.71 (m, 2H), 3.63−3.58 (m, 3H), 3.44 (s, 1H), 3.26−3.17 (m, 3H), 2.33−2.14 (m, 4H), 1.95−1.89 (m, 1H), 1.80−1.65 (m, 3H), 1.67−1.54 (m, 3H), 1.46−1.30 (m, 10H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.38, 175.91, 173.20, 172.29, 163.10, 161.00, 159.28, 156.88, 155.70, 146.12, 144.39, 141.49, 134.22, 133.53, 133.18, 132.77, 131.47, 131.02, 130.05, 129.93, 127.14, 126.04, 124.71, 122.43, 116.47, 116.12, 115.41, 107.90, 84.19, 78.50, 70.95, 64.27, 60.58, 59.62, 58.99, 57.98, 55.93, 55.83, 55.65, 54.56, 53.65, 52.59, 50.17, 36.49, 29.94, 29.81, 27.03, 26.73, 24.71, 22.23, 18.70, 17.26, 13.17, 9.07. UPLC-MS (ESI^+) calcd for $C_{53}H_{64}N_5O_8S$ $[M + 1]^+$: 929.45, found 929.49.

*(2S,4R)-N-((S)-1-(4-Cyclopropylphenyl)ethyl)-1-((S)-2-(8-(ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)-phenoxy)ethyl)amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (**46**).* This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 7.76 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 2.0 Hz, 2H), 7.20−7.10 (m, 4H), 7.02−6.98 (m, 2H), 6.94−6.86 (m, 3H), 6.63−6.59 (m, 2H), 4.95−4.91 (m, 1H), 4.63−4.61 (m, 1H), 4.54 (t, J = 8.4 Hz, 1H), 4.43−4.39 (m, 1H), 4.36−4.34 (m, 2H), 3.87−3.85 (m, 1H), 3.75−3.71 (m, 1H), 3.63−3.58 (m, 2H), 3.23−3.17 (m, 2H), 2.31−2.23 (m, 2H), 2.18−2.11 (m, 1H), 1.96−1.83 (m, 2H), 1.72−1.59 (m, 4H), 1.51−1.28 (m, 12H), 1.02 (s, 9H), 1.00−0.90 (m, 2H), 0.64−0.60 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.36, 175.88, 172.98, 172.30, 163.07, 159.29, 156.90, 144.39, 144.17, 142.11, 141.50, 134.22, 133.54, 131.48, 131.02, 126.95, 126.72, 126.06, 124.70, 116.47, 116.13, 115.40, 107.90, 70.94, 63.55, 60.64, 58.98, 57.98, 55.90, 54.57, 53.58, 52.62, 51.85, 51.29, 38.76, 36.51, 29.93, 29.83, 27.36, 27.04, 26.72, 24.72, 15.77, 9.51, 9.06. UPLC-MS (ESI^+) calcd for $C_{55}H_{69}N_4O_8S$ $[M + 1]^+$: 945.48, found 945.51.

*(2S,4R)-1-((S)-2-(8-(Ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)-benzo[b]thiophene-3-carbonyl)phenoxy)ethyl)amino)-octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-isopropylphenyl)ethyl)pyrrolidine-2-carboxamide (**47**).* This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 7.75 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 2.0 Hz, 1H), 7.22−7.15 (m, 6H), 6.93−6.86 (m, 3H), 6.63−6.59 (m, 2H), 4.96−4.91 (m, 1H), 4.62 (d, J = 8.8 Hz, 1H), 4.53 (d, J = 8.4 Hz, 1H), 4.43−4.36 (m, 1H), 4.35−4.29 (m, 2H), 3.87−3.84 (m, 1H), 3.75−3.71 (m, 1H), 3.60−3.53 (m, 2H), 3.24−3.15 (m, 2H), 2.89−2.81 (m, 1H), 2.34−2.21 (m, 2H), 2.18−2.12 (m, 1H), 1.98−1.91 (m, 1H), 1.73−1.70 (m, 2H), 1.63−1.58 (m, 2H), 1.52−1.29 (m, 12H), 1.22 (d, J = 7.2 Hz, 6H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.35, 175.89, 173.00, 172.30, 163.08, 159.29, 156.88, 148.92, 144.37, 142.57, 141.49, 134.22, 133.53, 132.79, 131.47, 131.01, 127.50, 126.76, 126.03, 124.71, 116.13, 115.40, 107.91, 70.94, 63.55, 60.63, 58.98, 57.98, 54.53, 52.57, 50.20, 50.10, 38.76, 36.51, 35.06, 29.94, 29.81, 27.34, 27.04, 26.72, 24.70, 24.45, 22.49, 9.06. UPLC-MS (ESI^+) calcd for $C_{55}H_{71}N_4O_8S$ $[M + 1]^+$: 947.50, found 947.53.

*(2S,4R)-N-((S)-1-(4-(tert-Butyl)phenyl)ethyl)-1-((S)-2-(8-(ethyl(2-(4-(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophene-3-carbonyl)-phenoxy)ethyl)amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide (**48**).* This compound was prepared using a procedure similar to that used for compound **15**. ^1H NMR (CD_3OD, 400 MHz) δ (ppm) 7.75 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.8 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 2.0 Hz, 1H), 7.22−7.16 (m, 4H), 6.93−6.86 (m, 3H), 6.63−6.59 (m, 2H), 4.95−4.91 (m, 1H), 4.65−4.60 (m, 1H), 4.53 (t, J = 8.4 Hz, 1H), 4.42−4.40 (m, 1H), 4.35 (t, J = 4.4 Hz, 2H), 3.87−3.84 (m, 1H), 3.75−3.71 (m, 1H), 3.60−3.56 (m, 2H), 3.25−3.16 (m, 2H), 2.34−2.14 (m, 3H), 1.98−1.91 (m, 1H), 1.74−1.71 (m, 2H), 1.64−1.55 (m, 2H), 1.45−1.32 (m, 1H), 1.29 (s, 9H), 1.02 (s, 9H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 193.36, 173.88, 171.01, 170.31, 161.08, 157.29, 154.88, 149.06, 142.37, 140.13, 139.49, 132.22, 131.53, 130.80, 129.47, 129.02, 124.71, 124.48, 124.39, 124.04, 122.70, 114.47, 114.13, 113.40, 105.91, 68.95, 61.56, 58.64, 56.98, 55.98, 52.54, 50.58, 48.21, 48.02, 36.77, 34.51, 33.26, 29.78, 27.94, 27.81, 25.34, 25.04, 24.72, 22.70, 20.45, 7.06. UPLC-MS (ESI^+) calcd for $C_{56}H_{73}N_4O_8S$ $[M + 1]^+$: 961.51, found 961.55.

*4-(6-Methoxy-2-(4-methoxyphenyl)benzo[b]thiophene-3-carbonyl)phenyl acetate (**50**).* Oxalyl chloride (9.70 mL, 120 mmol, 3.0 equiv) was added dropwise under N_2 to a solution of 4-acetoxybenzoic acid (**49**) (7.206 g, 40 mmol, 1.0 equiv) in anhydrous DCM (80 mL) at 0 °C. Then several drops of DMF were added. The solution was warmed to rt and stirred for 1 h. The solution was concentrated and dried to obtain the acyl chloride as a white solid. The intermediate was dissolved in anhydrous DCM (150 mL), then 6-methoxy-2-(4-methoxyphenyl)-benzo[*b*]thiophene (8.65 g, 32 mmol, 0.8 equiv) was added followed by addition of $AlCl_3$ (8.00 g, 60 mmol, 1.5 equiv) in three portions over a period of 5 min with vigorous stirring at 0 °C under N_2. The mixture was warmed to rt and stirred for 1 h. The reaction was quenched by slow addition of ice−H_2O followed by 1 N HCl (aq). The layers were separated, and the aqueous layer was extracted twice with DCM. The combined organic layer was dried by anhydrous Na_2SO_4. After filtration and concentration, the residue was purified on a silica gel flash column with hexane:DCM (100:1−1:100) to afford the intermediate (**50**) as a yellow solid (5.517 g, 40% yield). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.81 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 8.8 Hz, 1H), 7.32−7.29 (m, 3H), 7.02−6.99 (m, 3H), 6.74 (d, J = 8.8 Hz, 2H), 3.86 (s, 3H), 3.73 (s, 3H), 2.25 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 193.15, 168.63, 159.99, 157.78, 154.38, 144.16, 140.10, 135.03, 133.76, 131.52, 130.48, 130.02, 125.76, 124.16, 121.54, 114.99, 114.13, 104.54, 55.65, 55.28, 21.16. UPLC-MS (ESI^+) calcd for $C_{25}H_{21}O_5S$ $[M + 1]^+$: 433.11, found 433.37.

*(4-Hydroxyphenyl)(6-methoxy-2-(4-methoxyphenyl)benzo[b]-thiophen-3-yl)methanone (**51**).* Compound **50** (5.517 g, 12.76 mmol, 1.0 equiv) was dissolved in EtOH (70 mL) and H_2O (30 mL). Then sodium acetate (5.23 g, 63.8 mmol, 5.0 equiv) was added. The solution was heated to 90−100 °C and stirred for 12 h. The solution was cooled to rt and concentrated. The residue was diluted in EtOAc and H_2O. The organic layer was separated, and the aqueous layer was extracted twice with EtOAc. The combined organic layer was dried by anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by silica gel flash column chromatography with hexane:EtOAc (5:1−2:1) to afford intermediate **51** as yellow oil (4.7 g, 95%

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.64 (d, J = 9.2 Hz, 2H), 7.43 (d, J = 8.8 Hz, 1H), 7.30 (d, J = 2.4 Hz, 1H), 7.24 (d, J = 8.8 Hz, 2H), 6.89 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.69−6.64 (m, 4H), 3.73 (s, 3H), 3.59 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 193.95, 162.85, 159.92, 157.81, 142.33, 140.08, 133.78, 132.55, 130.34, 129.91, 129.06, 125.78, 123.43, 115.04, 114.63, 113.79, 104.34, 54.78, 54.39. UPLC-MS (ESI$^+$) calcd for $C_{23}H_{19}O_4S$ [M + 1]$^+$: 391.10, found 391.42.

*(4-(2-(Ethylamino)ethoxy)phenyl)(6-methoxy-2-(4-methoxyphenyl)benzo[b]thiophen-3-yl)methanone (**52**).* 1,2-Dibromoethane (2.0 mL, 24.0 mmol, 2.0 equiv) and Cs_2CO_3 (5.86 g, 18.0 mmol, 1.5 equiv) were added sequentially to a solution of compound **51** (4.7 g, 12.0 mmol, 1.0 equiv) in 200 mL of MeCN. The solution was heated to reflux for 12 h. The solution was filtered and washed with MeCN. The concentrated residue was used in next step without further column purification. Ethylamine (2.0 M in THF) (60 mL, 120 mmol, 10.0 equiv) was added to a solution of the residue in DMF. The solution was heated to 80 °C and stirred for 12 h. After cooling to rt, the reaction mixture was diluted in EtOAc and saturated brine. The aqueous layer was extracted with EtOAc twice. The combined organic layer was dried and concentrated. The residue was purified by silica gel flash column chromatography with DCM:MeOH (10:1) to afford the compound **52** as a yellow solid (4.43 g, 80% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.63 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.8 Hz, 1H), 7.32 (d, J = 2.4 Hz, 1H), 7.20 (d, J = 8.8 Hz, 2H), 6.88 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.71 (d, J = 8.8 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 3.93 (t, J = 4.2 Hz, 2H), 3.75 (s, 3H), 3.59 (s, 3H), 2.83 (t, J = 5.2 Hz, 2H), 2.59 (q, J = 7.2 Hz, 2H), 1.06 (t, J = 7.2 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 194.88, 164.48, 161.27, 159.20, 143.95, 141.41, 135.02, 133.39, 131.61, 131.48, 131.26, 127.01, 124.76, 115.97, 115.30, 115.10, 105.67, 68.13, 56.10, 55.70, 49.65, 44.52, 14.68. UPLC-MS (ESI$^+$) calcd for $C_{27}H_{28}NO_4S$ [M + 1]$^+$: 462.17, found 462.27.

*(4-(2-(Ethylamino)ethoxy)phenyl)(6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thiophen-3-yl)methanone (**53**).* BBr_3 solution (8.0 mL, 1.0 M in DCM) (8.0 mmol, 4.0 equiv) was slowly added under N_2 to a solution of **52** (923 mg, 2.0 mmol, 1.0 equiv) in 30 mL of anhydrous DCM at 0 °C. The dark-red solution was stirred at rt for 2 h, then MeOH (1.0 mL) was added dropwise to quench the reaction. The solution was concentrated, and the residue was dissolved in EtOAc (50 mL) then aqueous saturated $NaHCO_3$ (50 mL) and EtOH (5 mL) were added. The organic layer was separated and dried over anhydrous Na_2SO_4. After filtration, the solution was concentrated and the residue was purified by silica gel flash column chromatography with DCM:MeOH (10:1−5:1) to afford the key intermediate (**53**) as a yellow solid (520 mg, 60% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 7.72 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 8.8 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H), 6.61 (d, J = 8.8 Hz, 2H), 4.27 (t, J = 4.8 Hz, 2H), 3.42 (t, J = 4.8 Hz, 2H), 3.14 (q, J = 7.2 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ (ppm) 195.41, 163.41, 159.22, 156.80, 144.30, 141.45, 134.21, 133.45, 132.55, 131.42, 131.00, 125.99, 124.68, 116.43, 116.08, 115.39, 107.90, 64.68, 47.41, 44.36, 11.40. UPLC-MS (ESI$^+$) calcd for $C_{25}H_{24}NO_4S$ [M + 1]$^+$: 434.14, found 434.11.

*tert-Butyl (2S,4R)-4-Hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)-phenyl)ethyl)carbamoyl)-pyrrolidine-1-carboxylate (**56**).* Compound **55**, synthesized using the reported procedure,[47] was dissolved in 4 N HCl in dioxane (25 mL, 100 mmol) and MeOH (25 mL), and the mixture was stirred at ambient temperature for 12 h. The mixture was concentrated, and the residue was dried under vacuum to afford the intermediate, which was used in next step without further purification.

HATU (14.51 g, 38.2 mmol, 1.2 equiv) was added to a solution of the intermediate (**55**) obtained above (6.95 g, 31.8 mmol, 1.0 equiv), (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid (7.36 g, 31.8 mmol, 1.0 equiv), and DIPEA (11.08 mL, 63.6 mmol, 2.0 equiv) in DMF (36 mL) at 0 °C under nitrogen. The mixture was stirred at ambient temperature for 12 h. TLC showed that the reaction was complete. The reaction mixture was quenched with H_2O (200 mL) and extracted with EtOAc (150 mL × 2). The combined organic layer was washed with brine (200 mL) and dried over Na_2SO_4. The organic solution was filtered and concentrated. The residue was purified by silica gel flash column chromatography with hexane:EtOAc (100:1−1:100), then DCM:MeOH (10:1) to afford the intermediate (**56**) as white solid (10.98 g, 80% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ (ppm) 8.84 (s, 1H), 7.43−7.37 (m, 4H), 5.11−5.07 (m, 1H), 4.44−4.37 (m, 2H), 3.60−3.46 (m, 2H), 2.44 (s, 3H), 2.27−2.22 (m, 1H), 1.98−1.91 (m, 1H), 1.50 (d, J = 7.2 Hz, 3H), 1.46 (s, 9H). UPLC-MS (ESI$^+$) calcd for $C_{22}H_{30}N_3O_4S$ [M + 1]$^+$: 432.20, found 432.20.

*tert-Butyl ((S)-1-((2S,4R)-4-Hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate (**57**).* This solid material (**56**), obtained as described above was dissolved in 4 N HCl in dioxane (25 mL, 100 mmol) and 25 mL of MeOH, and the mixture was stirred at ambient temperature for 12 h. The mixture was then concentrated, and the residue was dried under vacuum to afford intermediate, which was used in next step without further purification. UPLC-MS (ESI$^+$) calcd for $C_{17}H_{22}N_3O_2S$ [M + 1]$^+$: 332.14, found 332.11. HATU (1.37 g, 3.6 mmol, 1.2 equiv) was added to a solution of this intermediate (994 mg, 3.0 mmol, 1.0 equiv), (*S*)-2-((*tert*-butoxycarbonyl)amino)-3,3-dimethylbutanoic acid (694 mg, 3.0 mmol, 1.0 equiv), and DIPEA (1.57 mL, 9.0 mmol, 3.0 equiv) in DMF (10 mL) at 0 °C under N_2. The mixture was stirred at ambient temperature for 12 h when TLC showed that the reaction was complete. The reaction mixture was quenched with H_2O (100 mL) and extracted with EtOAc (75 mL × 2). The combined organic layer was washed with brine (100 mL) and dried over Na_2SO_4. The organic solution was filtered and concentrated. The residue was purified by silica gel flash column chromatography with hexane:EtOAc then DCM:MeOH to afford the desired compound (**57**) as white solid (1.31 g, 80% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.65 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.35−7.31 (m, 4H), 5.29 (d, J = 9.2 Hz, 1H), 5.06−5.01 (m, 1H), 4.67 (t, J = 8.0 Hz, 1H), 4.46−4.44 (m, 1H), 4.22−4.19 (m, 1H), 3.91 (d, J = 17.2 Hz, 1H), 3.61−3.58 (m, 1H), 2.46 (s, 3H), 2.37−2.30 (m, 1H), 2.04−1.99 (m, 1H), 1.44 (d, J = 7.2 Hz, 3H), 1.35 (s, 9H), 0.96 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 172.22, 170.13, 156.15, 150.56, 148.21, 143.43, 131.74, 130.59, 129.49, 126.46, 80.18, 69.91, 58.86, 56.58, 48.74, 38.60, 36.02, 35.48, 28.34, 26.39, 22.17, 15.95. UPLC-MS (ESI$^+$) calcd for $C_{28}H_{41}N_4O_5S$ [M + 1]$^+$: 545.28, found 545.35.

*(2S,4R)-1-((S)-2-Amino-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (**58**).* The solid material (**57**) obtained above was dissolved in 4 N HCl in dioxane (4 mL, 16 mmol) and MeOH (4.0 mL), and the mixture was stirred at ambient temperature for 12 h. The mixture was then concentrated and the residue was dried under vacuum to afford crude compound, which was purified by reversed-phase preparative HPLC to afford the pure final compound (**58**) as an off-white solid. UPLC-MS (ESI$^+$) calcd for $C_{23}H_{33}N_4O_3S$ [M + 1]$^+$: 445.23, found 445.44.

Cell Culture. Human breast cancer cell lines MCF-7 (ATCC HTB-22) and T47D (ATCC HTB-133) were purchased from the American Type Culture Collection (ATCC), Manassas, VA. They were maintained and cultured in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1 unit/mL of penicillin, and 1 μg/mL of streptomycin. Cells with 3−8 passages after purchasing were used in experiments as indicated.

Western Blot Analysis. Western blot analysis was performed essentially as described previously (Hu et al, 2015; PMID 26358219). Cells treated with indicated compounds were lysed in Radioimmunoprecipitation Assay Protein Lysis and Extraction Buffer (25 mmol/L Tris·HCl, pH 7.6, 150 mmol/L NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) containing proteinase inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). After determination of protein concentration by BCA assay (Fisher Scientific, Pittsburgh, PA), equal amounts of total protein were electrophoresed through 10% SDS-polyacrylamide gels. The separated protein bands were transferred onto PVDF membranes

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

(GE Healthcare Life Sciences, Marlborough, MA) and blotted against different antibodies as indicated. The human estrogen receptor α antibody (AB16460) were purchased from Abcam, Inc., Cambridge, MA. The membranes were reblotted with horseradish peroxidase-conjugated anti-glyceraldehyde-3-phosphate dehydrogenase antibody (G9295) from Sigma-Aldrich Corporation, St. Louis, MO. The blots were scanned, and the band intensities were quantified using GelQuant.NET software provided by biochemlabsolutions.com. The relative mean intensity of target proteins was expressed after normalization to the intensity of glyceraldehyde-3-phosphate dehydrogenase bands from individual repeats.

Cell Growth Assay. MCF-7 cancer cells or human mammary epithelial cells (HMEC) were seeded at 1500/well in 96-well plates overnight. One day after the seeding, they were treated with indicated doses of compounds, respectively. The growth of the cells was evaluated by colorimetric wst-8 assay 4 days after the compound treatment by following the instructions of the manufacturer, Cayman Chemical, Ann Arbor, MI.

Molecular Modeling. The binding pose of the *N*,*N*-diethylamino analogue of raloxifene with ER was modeled with the structure (PDB 1ERR)[49] cocrystallized with raloxifene using MOE program. Residues were rebuilt if atoms were missing based on the amber10 library in MOE, and protons were added using the "protonate 3D" module considered by setting pH at 7, temperature at 300 K, and salt concentration at 0.1 mol/L. Docking simulations were then performed using raloxifene to define the binding site with crystallized H_2O molecules preserved. The ligand was placed by "Triangle matcher" and evaluated by London dG scoring. DGVI/WSA dG scoring was then applied to rank the poses, and the top ranked pose was selected. Figures appeared in this paper were prepared using the PyMOL program (www.pymol.org).

Cloning and Purification of VHL-ElonginB/C Complex. The DNA sequence of VHL (coding for residues 54−213) was constructed by PCR and inserted into a His-TEV expression vector[58] using ligation-independent cloning. The DNA sequences of Elongin B (encoding residues 1−118) and Elongin C (encoding residues 1−96) were constructed by PCR and inserted into pCDFDuet 1 using Gibson assembly.[59] BL21(DE3) cells were transformed simultaneously with both plasmids and grown in Terrific broth at 37 °C until an OD600 of 1.2. The cells were induced overnight with 0.4 mM IPTG at 24 °C. Pelleted cells were freeze−thawed then resuspended in 20 mM Tris HCl pH 7.0, 200 mM NaCl, and 0.1% β-mercaptoethanol (bME) containing protease inhibitors. The cell suspension was lysed by sonication and debris removed via centrifugation. The supernatant was incubated at 4 °C for 1 h with Ni-NTA (Qiagen) prewashed in 20 mM Tris-HCl pH 7.0, 200 mM NaCl, and 10 mM imidazole. The protein complex was eluted in 20 mM Tris-HCl pH 7.0, 200 mM NaCl, and 300 mM imidazole, dialyzed into 20 mM Tris-HCl pH 7.0, 150 mM NaCl, and 0.01% bME, and incubated with TEV protease overnight at 4 °C. The protein sample was reapplied to the Ni-NTA column to remove the His-tag. The flow through containing the VHL complex was diluted to 75 mM NaCl and applied to a HiTrap Q column (GE Healthcare). The sample was eluted with a salt gradient (0.075−1 M NaCl), concentrated and further purified on a Superdex S75 column (GE Healthcare) pre-equilibrated with 20 mM Bis-Tris 7.0, 150 mM NaCl, and 1 mM DTT. Samples were aliquoted and stored at −80 °C.

Determination of Binding Affinities of Synthesized VHL Ligands to VHL-ElonginB/C Complex (VHL). We have developed a competitive, fluorescence polarization (FP) assay to determine the binding affinities of our synthesized VHL ligands to VHL-ElonginB/C complex (VHL) protein. For this purpose, we synthesized a fluorencently tagged VHL ligand (VHL-F1) (Figure S1(A) in Supporting Information) and determined its K_d value to be 2.3 ± 0.3 nM (Figure S1(B) in Supporting Information).

IC_{50} and K_i values of VHL ligands to VHL protein were then determined in competitive binding experiments. Mixtures of 5 μL of each compound in DMSO and 95 μL of preincubated VHL protein/VHL-F1 tracer complex solution were added into assay plates which were incubated at room temperature for 60 min with gentle shaking. Final concentrations of VHL protein and VHL-F1 fluorescent probe were both 5 nM. Negative controls containing protein/probe complex only (equivalent to 0% inhibition) and positive controls containing only the probe (equivalent to 100% inhibition) were included in each assay plate. FP values in millipolarization units (mP) were measured using the PHERAstar FSX HTS plate reader (BMG Labtech, 13000 Weston Parkway, Suite 109, Cary, NC 27513, USA) in 96-well, black, flat bottom microplates (Greiner Bio-One North America, Inc. 4238 Capital Drive Monroe, NC, 28110, USA) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. IC_{50} values were determined by nonlinear regression fitting of the competition curves. K_i values of competitive inhibitors were obtained directly by nonlinear regression fitting, based upon the K_D values of the probe and concentrations of the protein and probe in the competitive assays. All the FP competitive experiments were performed in duplicate in three independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b01572.

Structure characterization for compound **11**, VH032, **43a**−**48a**; ^{1}H NMR, ^{13}C NMR, and UPLC-MS analysis of compounds **15** (ERD-148) and **32** (ERD-308 (PDF)
Molecular string files for all the final target compounds (CSV)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (734)615-0362. Fax: (734)647-9647. E-mail: shaomeng@umich.edu.

ORCID

Biao Hu: 0000-0002-4691-6490

Shaomeng Wang: 0000-0002-8782-6950

Author Contributions

#Jiantao Hu, Biao Hu, Mingliang Wang, Fuming Xu, and Bukeyan Miao contributed equally.

Notes

The authors declare the following competing financial interest(s): The University of Michigan has filed a patent application on these ER degraders, which has been licensed by Oncopia Therapeutics LLC. S. Wang, J. Hu, B. Hu, M. Wang, F. Xu, and B. Miao are co-inventors on this patent application. The University of Michigan has received a research grant from Oncopia and owns equity in Oncipia. S. Wang is a co-founder of Oncopia, owns equity in Oncopia and is a paid consultant for Oncopia.

■ ACKNOWLEDGMENTS

This study is supported in part by funding from Oncopia Therapeutics, LLC, the University of Michigan Comprehensive Cancer Center Strategic Fund for Breast Cancer (to S.W.), the University of Michigan Comprehensive Cancer Center Core Grant from the National Cancer Institute, NIH (grant P30CA046592).

■ ABBREVIATIONS USED

BC, breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor 2; SERM, selective estrogen receptor modulator; SERD, selective estrogen receptor degrader; VHL, von Hippel-Lindau; CRBN, cereblon; FP, fluorescence polarization; GAPDH, glyceraldehyde 3-phosphate dehydrogenase;

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

PBS, phosphate buffered saline; SNIPER, specific and nongenetic IAP-dependent protein erasers; PROTAC, proteolysis targeting chimera; DMSO, dimethyl sulfoxide; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMA, *N,N*-dimethylacetamide; qPCR, quantitative polymerase chain reaction; pGR, progesterone receptor; GREB1, growth regulation by estrogen in breast cancer 1; HMEC, human mammary epithelial cells.

REFERENCES

(1) Tong, C. W. S.; Wu, M.; Cho, W. C. S.; To, K. K. W. Recent advances in the treatment of breast cancer. *Front. Oncol.* **2018**, *8*, 227.

(2) Anderson, W. F.; Katki, H. A.; Rosenberg, P. S. Incidence of breast cancer in the United States: current and future trends. *J. Natl. Cancer Inst.* **2011**, *103*, 1397−1402.

(3) Nilsson, S.; Koehler, K. F.; Gustafsson, J. A. Development of subtype-selective oestrogen receptor-based therapeutics. *Nat. Rev. Drug Discovery* **2011**, *10*, 778−792.

(4) Jordan, V. C. Tamoxifen: a most unlikely pioneering medicine. *Nat. Rev. Drug Discovery* **2003**, *2*, 205−213.

(5) Das, S.; Crockett, J. C. Osteoporosis - a current view of pharmacological prevention and treatment. *Drug Des., Dev. Ther.* **2013**, *7*, 435−448.

(6) De Marchi, T.; Foekens, J. A.; Umar, A.; Martens, J. W. Endocrine therapy resistance in estrogen receptor (ER)-positive breast cancer. *Drug Discovery Today* **2016**, *21*, 1181−1188.

(7) AlFakeeh, A.; Brezden-Masley, C. Overcoming endocrine resistance in hormone receptor-positive breast cancer. *Curr. Oncol.* **2018**, *25*, 18−27.

(8) Martin, L. A.; Ribas, R.; Simigdala, N.; Schuster, E.; Pancholi, S.; Tenev, T.; Gellert, P.; Buluwela, L.; Harrod, A.; Thornhill, A.; Nikitorowicz-Buniak, J.; Bhamra, A.; Turgeon, M. O.; Poulogiannis, G.; Gao, Q.; Martins, V.; Hills, M.; Garcia-Murillas, I.; Fribbens, C.; Patani, N.; Li, Z.; Sikora, M. J.; Turner, N.; Zwart, W.; Oesterreich, S.; Carroll, J.; Ali, S.; Dowsett, M. Discovery of naturally occurring ESR1 mutations in breast cancer cell lines modelling endocrine resistance. *Nat. Commun.* **2017**, *8*, 1865.

(9) Nardone, A.; De Angelis, C.; Trivedi, M. V.; Osborne, C. K.; Schiff, R. The changing role of ER in endocrine resistance. *Breast* **2015**, *24*, S60−S66.

(10) Robertson, J. F.; Harrison, M. Fulvestrant: pharmacokinetics and pharmacology. *Br. J. Cancer* **2004**, *90*, S7−S10.

(11) Osborne, C. K.; Wakeling, A.; Nicholson, R. I. Fulvestrant: an oestrogen receptor antagonist with a novel mechanism of action. *Br. J. Cancer* **2004**, *90*, S2−S6.

(12) Howell, A.; Sapunar, F. Fulvestrant revisited: efficacy and safety of the 500-mg dose. *Clin. Breast Cancer* **2011**, *11*, 204−210.

(13) Robertson, J. F.; Lindemann, J.; Garnett, S.; Anderson, E.; Nicholson, R. I.; Kuter, I.; Gee, J. M. A good drug made better: the fulvestrant dose-response story. *Clin. Breast Cancer* **2014**, *14*, 381−389.

(14) McDonnell, D. P.; Wardell, S. E.; Norris, J. D. Oral selective estrogen receptor downregulators (SERDs), a breakthrough endocrine therapy for breast cancer. *J. Med. Chem.* **2015**, *58*, 4883−4887.

(15) Abdel-Magid, A. F. Selective estrogen receptor degraders (SERDs): a promising treatment to overcome resistance to endocrine therapy in ERα-positive breast cancer. *ACS Med. Chem. Lett.* **2017**, *8*, 1129−1131.

(16) Weir, H. M.; Bradbury, R. H.; Lawson, M.; Rabow, A. A.; Buttar, D.; Callis, R. J.; Curwen, J. O.; de Almeida, C.; Ballard, P.; Hulse, M.; Donald, C. S.; Feron, L. J.; Karoutchi, G.; MacFaul, P.; Moss, T.; Norman, R. A.; Pearson, S. E.; Tonge, M.; Davies, G.; Walker, G. E.; Wilson, Z.; Rowlinson, R.; Powell, S.; Sadler, C.; Richmond, G.; Ladd, B.; Pazolli, E.; Mazzola, A. M.; D'Cruz, C.; De Savi, C. AZD9496: an oral estrogen receptor inhibitor that blocks the growth of ER-positive and ESR1-mutant breast tumors in preclinical models. *Cancer Res.* **2016**, *76*, 3307−3318.

(17) Joseph, J. D.; Darimont, B.; Zhou, W.; Arrazate, A.; Young, A.; Ingalla, E.; Walter, K.; Blake, R. A.; Nonomiya, J.; Guan, Z.; Kategaya, L.; Govek, S. P.; Lai, A. G.; Kahraman, M.; Brigham, D.; Sensintaffar, J.; Lu, N.; Shao, G.; Qian, J.; Grillot, K.; Moon, M.; Prudente, R.; Bischoff, E.; Lee, K. J.; Bonnefous, C.; Douglas, K. L.; Julien, J. D.; Nagasawa, J. Y.; Aparicio, A.; Kaufman, J.; Haley, B.; Giltnane, J. M.; Wertz, I. E.; Lackner, M. R.; Nannini, M. A.; Sampath, D.; Schwarz, L.; Manning, H. C.; Tantawy, M. N.; Arteaga, C. L.; Heyman, R. A.; Rix, P. J.; Friedman, L.; Smith, N. D.; Metcalfe, C.; Hager, J. H. The selective estrogen receptor downregulator GDC-0810 is efficacious in diverse models of ER+ breast cancer. *eLife* **2016**, *5*, No. e15828.

(18) Bihani, T.; Patel, H. K.; Arlt, H.; Tao, N.; Jiang, H.; Brown, J. L.; Purandare, D. M.; Hattersley, G.; Garner, F. Elacestrant (RAD1901), a selective estrogen receptor degrader (SERD), has antitumor activity in multiple ER+ breast cancer patient-derived xenograft models. *Clin. Cancer Res.* **2017**, *23*, 4793−4804.

(19) Tria, G. S.; Abrams, T.; Baird, J.; Burks, H. E.; Firestone, B.; Gaither, L. A.; Hamann, L. G.; He, G.; Kirby, C. A.; Kim, S.; Lombardo, F.; Macchi, K. J.; McDonnell, D. P.; Mishina, Y.; Norris, J. D.; Nunez, J.; Springer, C.; Sun, Y.; Thomsen, N. M.; Wang, C.; Wang, J.; Yu, B.; Tiong-Yip, C. L.; Peukert, S. Discovery of LSZ102, a potent, orally bioavailable selective estrogen receptor degrader (SERD) for the treatment of estrogen receptor positive breast cancer. *J. Med. Chem.* **2018**, *61*, 2837−2864.

(20) Carlson, R. W. The history and mechanism of action of fulvestrant. *Clin. Breast Cancer* **2005**, *6*, S5−S8.

(21) Marsaud, V.; Gougelet, A.; Maillard, S.; Renoir, J. M. Various phosphorylation pathways, depending on agonist and antagonist binding to endogenous estrogen receptor α (ERα), differentially affect ERα extractability, proteasome-mediated stability, and transcriptional activity in human breast cancer cells. *Mol. Endocrinol.* **2003**, *17*, 2013−2027.

(22) Wittmann, B. M.; Sherk, A.; McDonnell, D. P. Definition of functionally important mechanistic differences among selective estrogen receptor down-regulators. *Cancer Res.* **2007**, *67*, 9549−9560.

(23) Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; Mercurio, F.; Crews, C. M.; Deshaies, R. J. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 8554−8559.

(24) Toure, M.; Crews, C. M. Small-molecule PROTACS: new approaches to protein degradation. *Angew. Chem., Int. Ed.* **2016**, *55*, 1966−1973.

(25) Lai, A. C.; Crews, C. M. Induced protein degradation: an emerging drug discovery paradigm. *Nat. Rev. Drug Discovery* **2017**, *16*, 101−114.

(26) Burslem, G. M.; Crews, C. M. Small-molecule modulation of protein homeostasis. *Chem. Rev.* **2017**, *117*, 11269−11301.

(27) Cromm, P. M.; Crews, C. M. Targeted protein degradation: from chemical biology to drug discovery. *Cell Chem. Biol.* **2017**, *24*, 1181−1190.

(28) Ottis, P.; Crews, C. M. Proteolysis-targeting chimeras: induced protein degradation as a therapeutic strategy. *ACS Chem. Biol.* **2017**, *12*, 892−898.

(29) Rodriguez-Gonzalez, A.; Cyrus, K.; Salcius, M.; Kim, K.; Crews, C. M.; Deshaies, R. J.; Sakamoto, K. M. Targeting steroid hormone receptors for ubiquitination and degradation in breast and prostate cancer. *Oncogene* **2008**, *27*, 7201−7211.

(30) Jiang, Y.; Deng, Q.; Zhao, H.; Xie, M.; Chen, L.; Yin, F.; Qin, X.; Zheng, W.; Zhao, Y.; Li, Z. Development of stabilized peptide-based PROTACs against estrogen receptor α. *ACS Chem. Biol.* **2018**, *13*, 628−635.

(31) Winter, G. E. B. D. L.; Buckley, D. L.; Paulk, J.; Roberts, J. M.; Souza, A.; Dhe-Paganon, S.; Bradner, J. E. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* **2015**, *348*, 1376−1381.

(32) Lu, J.; Qian, Y.; Altieri, M.; Dong, H.; Wang, J.; Raina, K.; Hines, J.; Winkler, J. D.; Crew, A. P.; Coleman, K.; Crews, C. M. Hijacking the E3 ubiquitin ligase cereblon to efficiently target BRD4. *Chem. Biol.* **2015**, *22*, 755−763.

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX

(33) Bondeson, D. P.; Mares, A.; Smith, I. E.; Ko, E.; Campos, S.; Miah, A. H.; Mulholland, K. E.; Routly, N.; Buckley, D. L.; Gustafson, J. L.; Zinn, N.; Grandi, P.; Shimamura, S.; Bergamini, G.; Faelth-Savitski, M.; Bantscheff, M.; Cox, C.; Gordon, D. A.; Willard, R. R.; Flanagan, J. J.; Casillas, L. N.; Votta, B. J.; den Besten, W.; Famm, K.; Kruidenier, L.; Carter, P. S.; Harling, J. D.; Churcher, I.; Crews, C. M. Catalytic in vivo protein knockdown by small-molecule PROTACs. *Nat. Chem. Biol.* **2015**, *11*, 611−617.
(34) Lai, A. C.; Toure, M.; Hellerschmied, D.; Salami, J.; Jaime-Figueroa, S.; Ko, E.; Hines, J.; Crews, C. M. Modular PROTAC design for the degradation of oncogenic BCR-ABL. *Angew. Chem., Int. Ed.* **2016**, *55*, 807−810.
(35) Robb, C. M.; Contreras, J. I.; Kour, S.; Taylor, M. A.; Abid, M.; Sonawane, Y. A.; Zahid, M.; Murry, D. J.; Natarajan, A.; Rana, S. Chemically induced degradation of CDK9 by a proteolysis targeting chimera (PROTAC). *Chem. Commun.* **2017**, *53*, 7577−7580.
(36) Zhang, C.; Han, X. R.; Yang, X.; Jiang, B.; Liu, J.; Xiong, Y.; Jin, J. Proteolysis targeting chimeras (PROTACs) of anaplastic lymphoma kinase (ALK). *Eur. J. Med. Chem.* **2018**, *151*, 304−314.
(37) Lu, M.; Liu, T.; Jiao, Q.; Ji, J.; Tao, M.; Liu, Y.; You, Q.; Jiang, Z. Discovery of a Keap1-dependent peptide PROTAC to knockdown Tau by ubiquitination-proteasome degradation pathway. *Eur. J. Med. Chem.* **2018**, *146*, 251−259.
(38) Burslem, G. M.; Smith, B. E.; Lai, A. C.; Jaime-Figueroa, S.; McQuaid, D. C.; Bondeson, D. P.; Toure, M.; Dong, H.; Qian, Y.; Wang, J.; Crew, A. P.; Hines, J.; Crews, C. M. The advantages of targeted protein degradation over inhibition: an RTK case study. *Cell Chem. Biol.* **2018**, *25*, 67−77.
(39) Schiedel, M.; Herp, D.; Hammelmann, S.; Swyter, S.; Lehotzky, A.; Robaa, D.; Olah, J.; Ovadi, J.; Sippl, W.; Jung, M. Chemically induced degradation of sirtuin 2 (sirt2) by a proteolysis targeting chimera (PROTAC) based on sirtuin rearranging ligands (SirReals). *J. Med. Chem.* **2018**, *61*, 482−491.
(40) Shibata, N.; Nagai, K.; Morita, Y.; Ujikawa, O.; Ohoka, N.; Hattori, T.; Koyama, R.; Sano, O.; Imaeda, Y.; Nara, H.; Cho, N.; Naito, M. Development of protein degradation inducers of androgen receptor by conjugation of androgen receptor ligands and inhibitor of apoptosis protein ligands. *J. Med. Chem.* **2018**, *61*, 543−575.
(41) Crew, A. P.; Raina, K.; Dong, H.; Qian, Y.; Wang, J.; Vigil, D.; Serebrenik, Y. V.; Hamman, B. D.; Morgan, A.; Ferraro, C.; Siu, K.; Neklesa, T. K.; Winkler, J. D.; Coleman, K. G.; Crews, C. M. Identification and characterization of von Hippel-Lindau-recruiting proteolysis targeting chimeras (PROTACs) of TANK-binding kinase 1. *J. Med. Chem.* **2018**, *61*, 583−598.
(42) Sun, Y.; Zhao, X.; Ding, N.; Gao, H.; Wu, Y.; Yang, Y.; Zhao, M.; Hwang, J.; Song, Y.; Liu, W.; Rao, Y. PROTAC-induced BTK degradation as a novel therapy for mutated BTK C481S induced ibrutinib-resistant B-cell malignancies. *Cell Res.* **2018**, *28*, 779−781.
(43) Yang, K.; Song, Y.; Xie, H.; Wu, H.; Wu, Y. T.; Leisten, E. D.; Tang, W. Development of the first small molecule histone deacetylase 6 (HDAC6) degraders. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2493−2497.
(44) Ohoka, N.; Okuhira, K.; Ito, M.; Nagai, K.; Shibata, N.; Hattori, T.; Ujikawa, O.; Shimokawa, K.; Sano, O.; Koyama, R.; Fujita, H.; Teratani, M.; Matsumoto, H.; Imaeda, Y.; Nara, H.; Cho, N.; Naito, M. In vivo knockdown of pathogenic proteins via specific and nongenetic inhibitor of apoptosis protein (IAP)-dependent protein erasers (SNIPERs). *J. Biol. Chem.* **2017**, *292*, 4556−4570.
(45) Ohoka, N.; Morita, Y.; Nagai, K.; Shimokawa, K.; Ujikawa, O.; Fujimori, I.; Ito, M.; Hayase, Y.; Okuhira, K.; Shibata, N.; Hattori, T.; Sameshima, T.; Sano, O.; Koyama, R.; Imaeda, Y.; Nara, H.; Cho, N.; Naito, M. Derivatization of inhibitor of apoptosis protein (IAP) ligands yields improved inducers of estrogen receptor α degradation. *J. Biol. Chem.* **2018**, *293*, 6776−6790.
(46) Ottis, P.; Toure, M.; Cromm, P. M.; Ko, E.; Gustafson, J. L.; Crews, C. M. Assessing different E3 ligases for small molecule induced protein ubiquitination and degradation. *ACS Chem. Biol.* **2017**, *12*, 2570−2578.
(47) Raina, K.; Lu, J.; Qian, Y.; Altieri, M.; Gordon, D.; Rossi, A. M. K.; Wang, J.; Chen, X.; Dong, H.; Siu, K.; Winkler, J. D.; Crew, A. P.; Crews, C. M.; Coleman, K. G. PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 7124−7129.
(48) Zhou, B.; Hu, J.; Xu, F.; Chen, Z.; Bai, L.; Fernandez-Salas, E.; Lin, M.; Liu, L.; Yang, C. Y.; Zhao, Y.; McEachern, D.; Przybranowski, S.; Wen, B.; Sun, D.; Wang, S. Discovery of a small-molecule degrader of bromodomain and extra-terminal (BET) proteins with picomolar cellular potencies and capable of achieving tumor regression. *J. Med. Chem.* **2018**, *61*, 462−481.
(49) Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.-Å.; Carlquist, M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **1997**, *389*, 753−758.
(50) Qin, C.; Hu, Y.; Zhou, B.; Fernandez-Salas, E.; Yang, C. Y.; Liu, L.; McEachern, D.; Przybranowski, S.; Wang, M.; Stuckey, J.; Meagher, J.; Bai, L.; Chen, Z.; Lin, M.; Yang, J.; Ziazadeh, D. N.; Xu, F.; Hu, J.; Xiang, W.; Huang, L.; Li, S.; Wen, B.; Sun, D.; Wang, S. Discovery of QCA570 as an exceptionally potent and efficacious proteolysis targeting chimera (PROTAC) degrader of the bromodomain and extra-terminal (BET) proteins capable of inducing complete and durable tumor regression. *J. Med. Chem.* **2018**, *61*, 6685−6704.
(51) Bai, L.; Zhou, B.; Yang, C. Y.; Ji, J.; McEachern, D.; Przybranowski, S.; Jiang, H.; Hu, J.; Xu, F.; Zhao, Y.; Liu, L.; Fernandez-Salas, E.; Xu, J.; Dou, Y.; Wen, B.; Sun, D.; Meagher, J.; Stuckey, J.; Hayes, D. F.; Li, S.; Ellis, M. J.; Wang, S. Targeted degradation of BET proteins in triple-negative breast cancer. *Cancer Res.* **2017**, *77*, 2476−2487.
(52) Buckley, D. L.; Van Molle, I.; Gareiss, P. C.; Tae, H. S.; Michel, J.; Noblin, D. J.; Jorgensen, W. L.; Ciulli, A.; Crews, C. M. Targeting the von Hippel-Lindau E3 ubiquitin ligase using small molecules to disrupt the VHL/HIF-1α interaction. *J. Am. Chem. Soc.* **2012**, *134*, 4465−4468.
(53) Buckley, D. L.; Gustafson, J. L.; Van Molle, I.; Roth, A. G.; Tae, H. S.; Gareiss, P. C.; Jorgensen, W. L.; Ciulli, A.; Crews, C. M. Small-molecule inhibitors of the interaction between the E3 ligase VHL and HIF-1α. *Angew. Chem., Int. Ed.* **2012**, *51*, 11463−11467.
(54) Galdeano, C.; Gadd, M. S.; Soares, P.; Scaffidi, S.; Van Molle, I.; Birced, I.; Hewitt, S.; Dias, D. M.; Ciulli, A. Structure-guided design and optimization of small molecules targeting the protein-protein interaction between the von Hippel-Lindau (VHL) E3 ubiquitin ligase and the hypoxia inducible factor (HIF) α subunit with in vitro nanomolar affinities. *J. Med. Chem.* **2014**, *57*, 8657−8663.
(55) Soares, P.; Gadd, M. S.; Frost, J.; Galdeano, C.; Ellis, L.; Epemolu, O.; Rocha, S.; Read, K. D.; Ciulli, A. Group-based optimization of potent and cell-active inhibitors of the von Hippel-Lindau (VHL) E3 ubiquitin ligase: structure-activity relationships leading to the chemical probe (2S,4R)-1-((S)-2-(1-cyanocyclopropanecarboxamido)-3,3-dimethylbutanoyl)-4-hydroxy -N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (VH298). *J. Med. Chem.* **2018**, *61*, 599−618.
(56) Gadd, M. S.; Testa, A.; Lucas, X.; Chan, K. H.; Chen, W.; Lamont, D. J.; Zengerle, M.; Ciulli, A. Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat. Chem. Biol.* **2017**, *13*, 514−521.
(57) Long, M. J.; Poganik, J. R.; Aye, Y. On-demand targeting: investigating biology with proximity-directed chemistry. *J. Am. Chem. Soc.* **2016**, *138*, 3610−3622.
(58) Stols, L.; Gu, M.; Dieckman, L.; Raffen, R.; Collart, F. R.; Donnelly, M. I. A new vector for high-throughput, ligation-independent cloning encoding a tobacco etch virus protease cleavage site. *Protein Expression Purif.* **2002**, *25*, 8−15.
(59) Benoit, R. M.; Ostermeier, C.; Geiser, M.; Li, J. S.; Widmer, H.; Auer, M. Seamless insert-plasmid assembly at high efficiency and low cost. *PLoS One* **2016**, *11*, No. e0153158.

DOI: 10.1021/acs.jmedchem.8b01572
J. Med. Chem. XXXX, XXX, XXX−XXX