Maximizing ER- α Degradation Maximizes Activity in a Tamoxifen-Resistant Breast Cancer Model: Identification of GDC-0927

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S Supporting Information

ABSTRACT: The further optimization of ER- α degradation efficacy of a series of ER modulators by refining side-chain substitution led to efficacious selective estrogen receptor degraders (SERDs). A fluoromethyl azetidine group was found to be preferred and resulted in the identification of bis-phenol chromene **17ha**. In a tamoxifen-resistant breast cancer xenograft model, **17ha** (ER- α degradation efficacy = 97%) demonstrated tumor regression, together with robust reduction of intratumoral ER- α levels. However, despite superior oral exposure, **5a** (ER- α degradation efficacy = 91%) had inferior activity. This result suggests that optimizing ER- α degradation efficacy leads to compounds with robust effects in a model of tamoxifen-resistant breast cancer. Compound **17ha** (GDC-0927) was evaluated in clinical trials in women with metastatic estrogen receptor-positive breast cancer.

KEYWORDS: Estrogen receptor, estrogen receptor degrader, SERD, antagonist, tamoxifen-resistant, breast cancer, chromene, GDC-0927, SRN-927

The estrogen receptor ER- α ¹ has been an important target in the pharmaceutical industry for many years, with ER antagonists such as tamoxifen (**1**; Figure 1) and aromatase inhibitors being key therapeutics in the management of ER-positive breast cancer.² Tamoxifen (**1**) and 4-hydroxytamoxifen (active metabolite) (**2**) are selective estrogen receptor modulators (SERMs), meaning they may act as an antagonist or agonist depending on the tissue in which they act.¹ Although women with ER- α positive breast cancer typically respond well to tamoxifen, resistance can emerge.³ However, ER- α has been shown to be involved in this resistant state,⁴ and fulvestrant (**3**), a selective estrogen receptor degrader (SERD) that antagonizes and degrades ER- α , is clinically beneficial in this patient population.⁵ However, fulvestrant, which was not designed prospectively as a SERD,⁵ has poor pharmaceutical properties and must be administered by an intramuscular injection. We sought to identify SERDs where ER- α degradation efficacy was a main driver for structure–

activity relationship (SAR) studies and that were orally bioavailable.

Much of the previous work on estrogen receptor ligands provides an understanding of the structural motifs required for potent ER- α antagonists, but there has been less disclosure on what is required to maximize ER- α degradation efficacy (i.e., SERD activity). To drive ER- α degradation SAR, we used an in-cell western assay that measured ER- α levels in MCF-7 breast cancer cells. This assay was used to develop the SAR of two chemical series on the program, including the clinical compound GDC-0810/ARN-810 (**4**),⁶ a carboxylic acid, and an early lead from an amine-based series (**5**).⁷ This Letter describes the further optimization of ER- α degradation efficacy

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Figure 1. Estrogen receptor ligands.

of the amine-based chromene series represented by **5**, leading to the clinical compound GDC-0927 (SRN-927). Following the completion of this work, other publications have been released that report SERDs.^{8,9}

The compounds described in this Letter consist of a central chromene core to which an amine-based side chain is appended. The side-chain was installed on the core by two methods: (i) Ullmann coupling between an amino alcohol side-chain and an aryl iodide on the chromene core (General Method 1, Scheme 1) or (ii) alkylation with the desired cyclic amine of a previously installed ethyl mesylate on the chromene core (General Method 2, Scheme 1). See Supporting Information for preparation of the amino alcohol side-chains **10a–c** and cyclic amines **6a–g**. Intermediate **15** was prepared

Scheme 1. Preparation of Chromene ER Ligands^a

^aReagents and conditions: (a) SOCl₂, DMF, DCM, 0 °C; (b) 1,4-dimethoxybenzene, AlCl₃, DCM, 0 °C, 55%; (c) BBr₃, DCM, –78 to 0 °C, 57%; (d) DHP, PPTS, DCM, 70%; (e) 4-iodobenzaldehyde, piperidine, DBU, *s*-butanol, reflux, 75%; (f) MeMgCl, THF, 0 °C to rt, 98%; (g) 80% AcOH/water, 90 °C, 67%; (h) DHP, PPTS, DCM, 55%; (i) ethane-1,2-diol, CuI, 1,10-phenanthroline, K₂CO₃, butyronitrile, 125 °C, 58%; (j) methanesulfonyl chloride, TEA, DCM, 0 °C, 88%; (k) 17h, 17j–k: CuI, K₂CO₃, butyronitrile, 125–135 °C, 2–4 d, 75–82%; (l) 17c–g, 17i: K₂CO₃, acetonitrile, 60 °C to reflux, 62–76%; (m) 80% AcOH/water, rt, 27–85%.

in an eight-step sequence from 2-(3-methoxyphenyl)acetic acid (**12**), while mesylate **16** was prepared from **15** in two additional steps. Chromene intermediate **15** was prepared from 2-(3-methoxyphenyl)acetic acid **12**.⁷ Intermediate **16** was prepared from **15** via Ullmann coupling with ethylene glycol, followed by mesylation to afford **16** in 51% over two steps. The final compounds **17h** and **17j–k** were prepared from **15** using an Ullmann coupling with side-chains **10a–c** followed by deprotection of the THP groups using acetic acid. In turn, final compounds **17c–g** and **17i** were prepared by S_N2 displacement of mesylate **16** with the cyclic secondary amines **6a–g**, followed by THP deprotection.

The monophenol analogs **20c** and **20d** (Table 2) were prepared from the corresponding chromene intermediate using an Ullmann coupling with the appropriate amino-alcohol side chains.

Previously we demonstrated that subtle structural changes to the side-chain of the chromene scaffold could lead to large changes in ER- α degradation efficacy.⁷ For example, installation of an (*R*)-3-methyl-substituent on the pyrrolidine of **17a**, giving **17b**, led to a large increase in ER- α degradation efficacy (75% → 89%, Table 1). We continued to optimize ER- α degradation efficacy while maintaining potency by further exploring the substituent at this 3-position on the pyrrolidine, but found that the methoxymethyl (**17c**) and nitrile (**17d**) derivatives, although potent ER- α degraders, lost ER- α degradation efficacy. However, when a fluoromethyl-substituted pyrrolidine group was incorporated (**17e**), a substantial increase in ER- α degradation efficacy over **17b** was observed (89% → 97%). It was apparent that the monofluoromethyl at the 3-position of the pyrrolidine was optimal, as **17f** and **17g** had inferior ER- α degradation efficacy compared to **17e**. Addition of the fluoromethyl substituent to either the azetidine (**17h**) or piperidine (**17i**) side-chains again led to an increase in ER- α degradation efficacy compared to previously described, nonfluorinated methyl-substituted parent compounds⁷ (for azetidine **17h**, 86% → 97%; for piperidine **17i**, 63% → 80%). Previously we found that a (*S*)-methyl group on the linker ethyl chain led to a boost in ER- α degradation efficacy compared to the unsubstituted system,⁷ and so we installed a methyl in the linker of azetidine **17h** and pyrrolidine **17e**, resulting in **17j** and **17k**, respectively. However, no further increases in ER- α degradation efficacy were obtained, although **17k** was found to be another potent, high efficacy ER- α degrader compared to its nonfluorinated parent compound **5** (98% ER- α degradation efficacy versus 91%, respectively). Lead compounds **17e**, **17h**, and **17k** all displayed an MCF-7 proliferation IC₅₀ ≤ 0.2 nM.

Of the side-chains shown in Table 1, pyrrolidine **17e** was found to have inferior mouse PK compared to azetidine **17h** and was deprioritized as a lead side-chain (AUC at 10 mg/kg PO: 0.04 μ g·h/mL for **17e** versus 0.16 μ g·h/mL for **17h**). Compounds **17h** and **17k** and their single active stereoisomers (see below) were profiled further; the fluoromethyl azetidine side-chain of **17h** was ultimately selected over the fluoromethyl pyrrolidine of **17k** as the lead side-chain due to (i) reduced number of stereocenters (1 versus 3) and (ii) improved performance of the single stereoisomer in a tamoxifen-resistant breast cancer xenograft model (see below).

Previously we showed that replacement of one of the phenols of **5**, to give monophenol derivatives such as **20a** and **20b**, led to a loss of ER- α degradation efficacy (91% → 82–84%) and, in the case of **20a**, resulted in loss of antitumor

Table 1. Importance of a Monofluoromethyl Substituent to ER- α Degradation Efficacy

Cpd	R ¹	ER Degradation ^a		Proliferation ^b
		IC ₅₀ (nM)	Efficacy ^c	IC ₅₀ (nM)
5 ^d		0.2	91	0.2
17a ^d		0.4	75	0.3
17b ^d		0.4	89	0.2
17c		0.3	64	0.4
17d		0.8	79	3.6
17e		0.1	97	0.2
17f		0.2	90	0.4
17g		0.2	68	1.4
17h		0.3	97	0.2
17i		0.1	80	0.1
17j		0.2	92	0.3
17k		0.2	98	0.2

^aER- α in-cell western in MCF-7 cells ($n \geq 4$). ^bMCF-7 proliferation assay ($n \geq 3$). ^cEfficacy recorded as percent of efficacy of fulvestrant control. ^dSee ref 7.

activity in a tamoxifen-resistant breast cancer xenograft model (Table 2).⁷ Having selected the fluoromethyl azetidine as the preferred side-chain to maximize ER- α degradation on the bis-phenol chromene core, we revisited these monophenol chromenes and prepared 20c and 20d. In contrast to the methyl pyrrolidine monophenols 20a and 20b, fluoromethyl azetidines 20c and 20d have robust levels of ER- α degradation efficacy (98%) equal to that of the bis-phenol chromene 17h. Hence, the fluoromethyl azetidine side-chain not only increases the degradation efficacy of bis-phenol chromenes (compare 5 to 17h), it also maintains that level of ER- α degradation potency and efficacy for monophenol systems (compare 17h to 20c and 20d). As noted for the derivatives in Table 1, 20c and 20d are also potent in the MCF-7 proliferation assay (IC₅₀ < 0.5 nM).

Compounds were synthesized and tested in assays as a 50/50 mixture of stereoisomers at the chromene core. For the lead compounds 17h, 17k, and 20c (and 5) we confirmed that SERD activity was in one stereoisomer by separation by chiral supercritical fluid chromatography (SFC) and then characterized the stereoisomers. As shown in Table 3, the active

Table 2. Fluoromethyl Azetidine Side-Chain Significantly Improves ER- α Degradation Efficacy

Cpd	R ¹	R ²	ER Degradation ^a		Proliferation ^b
			IC ₅₀ (nM)	Efficacy ^c	IC ₅₀ (nM)
5		3'-OH	0.2	91	0.2
20a ^d		4'-F	0.4	82	0.4
20b ^d		4'-CN	1.5	84	10
17h		3'-OH	0.3	97	0.2
20c		4'-F	0.4	98	0.4
20d		4'-CN	0.2	98	0.3

^aER- α in-cell western in MCF-7 cells ($n \geq 4$). ^bMCF-7 proliferation assay ($n \geq 3$). ^cEfficacy recorded as percent of efficacy of fulvestrant control. ^dSee ref 7.

Table 3. SERD Activity Resides in One Chromene Stereoisomer

R ¹	R ²	ER- α Degradation ^a IC ₅₀ (Efficacy) ^b	
	3'-OH	5a : 0.1 nM (91%)	5b : 9.7 nM (90%) ^c
	3'-OH	17ha : 0.1 nM (97%)	17hb : 13 nM (96%) ^c
	3'-OH	17ka : 0.1 nM (97%)	17kb : 11 nM (97%) ^c
	4'-F	20ca : 0.1 nM (97%)	20cb : 15 nM (98%) ^c

^aER- α in-cell western in MCF-7 cells ($n \geq 4$). ^bEfficacy recorded as percent of efficacy of fulvestrant control. ^cActivity likely due to residual active stereoisomer (approximately 1% following SFC separation).

stereoisomers 17ha, 17ka, and 20ca (from 17h, 17k, and 20c, respectively), all had high potency (IC₅₀ = 0.1 nM) in the ER- α degradation assay compared to the inactive stereoisomers 17hb, 17kb, and 20cb (IC₅₀ > 10 nM). The activity seen in 17hb, 17kb, and 20cb is likely due to residual active stereoisomer (approximately 1% following SFC separation). Analogous results were obtained when these pairs of compounds were tested in an ER- α binding assay and MCF-7 breast cancer cell line antiproliferative assay, indicating that the profiling of enantiomeric or diastereoisomeric mixtures was acceptable during SAR studies.

Previously we described the use of the rat uterine wet weight assay to efficiently triage ER-based pharmacodynamics and to determine tissue-selectivity (breast versus uterine).^{6,7} Compounds **17ha** and **20ca** were examined in this assay in agonist mode (no added ethynyl estradiol) and, like **5a**, displayed an inverse agonist profile, reducing uterine weights below that of the vehicle treated animals (Figure 2). This is in contrast to tamoxifen (**1**), which showed partial agonist activity, increasing uterine weights above vehicle in the absence of added estradiol.¹

Figure 2. Compounds **17ha** and **20ca** are inverse agonists in a rat uterine wet weight assay: compounds administered orally for 3 days (**5a**, **17ha**, and **20ca** at 10 mg/kg po; tamoxifen (**1**) at 60 mg/kg).

As with other ER ligands that also contain two phenol groups, the mouse pharmacokinetics of **5a** and **17ha** are characterized by high clearance ($Cl > 60$ mL/min/kg) and low bioavailability ($\%F < 15$). In contrast to **17ha**, the monophenol **20ca** had improved clearance ($Cl = 36$ mL/min/kg) and bioavailability (42% F versus 10% F), leading to improved exposure following oral dosing (po AUC = $1.9 \mu\text{g}\cdot\text{h}/\text{mL}$ versus $0.16 \mu\text{g}\cdot\text{h}/\text{mL}$). Compounds **17ha** and **20ca** and the previous lead compound from the chromene series, **5a**, were profiled in a MCF-7 based tamoxifen-resistant xenograft model of breast cancer using subcutaneous estradiol pellets to deliver sufficient levels of estradiol to drive tumor growth (Figure 3).¹⁰ Importantly, **17ha** and **20ca** demonstrated superior activity to **5a** with 5 of 8 animals in each cohort showing tumor regressions. In contrast, only 1 of 8 animals showed tumor shrinkage in the cohort treated with **5a**.

The inferior profile of **5a** compared to **17ha** and **20ca** is not due to potency against the estrogen receptor (ER- α degradation IC_{50} : **5a** = 0.1 nM versus **17ha** = 0.1 nM, **20ca** = 0.1 nM; MCF-7 proliferation IC_{50} : **5a** = 0.1 nM versus **17ha** = 0.1 nM, **20ca** = 0.2 nM) or pharmacokinetics (day 28 xenograft free AUC = $0.19 \mu\text{g}\cdot\text{h}/\text{mL}$ for **5a** versus 0.13 and $0.08 \mu\text{g}\cdot\text{h}/\text{mL}$ for **17ha** and **20ca**, respectively). Importantly **5a** is a less efficacious ER- α degrader *in vitro* than **17ha** and **20ca** (ER- α degradation efficacy: **5a**, 91% versus **17ha** and **20ca**, 97%). As shown in Figure 4, this difference in *in vitro* ER- α degradation efficacy is reflected in the pharmacodynamic readout of tumor ER- α protein levels on day 28 of the xenograft study. Thus, while compound **5a** reduced ER- α levels to those of the vehicle plus estradiol cohort,¹¹ both **17ha** and **20ca** reduced levels substantially below that of **5a**. It is noteworthy that monophenol **20ca** had robust activity in this tamoxifen-resistant setting (equal to that of bis-phenol **17ha**) since as we previously described, monophenol **20a** was inferior to bis-phenol **5** in this same model.⁷ This result again

		Xenograft Day 28 PK parameters			
Cpd	Mouse free fraction	C _{max} (μg/mL)	AUC ₀₋₂₄ (μg·hr/mL)	Free C _{max} (μg/mL)	Free AUC ₀₋₂₄ (μg·hr/mL)
5a	0.016	1.3	12	0.021	0.19
17ha	0.019	0.95	6.9	0.018	0.13
20ca	0.004	2.3	21	0.009	0.08

Figure 3. Compounds **17ha** and **20ca** demonstrate superior activity to **5a** in an MCF-7 tamoxifen-resistant breast cancer xenograft model. MCF-7 tamoxifen-resistant xenograft in mice using subcutaneous 0.72 mg estradiol pellets. Plasma pharmacokinetics measured on day 28 of study during take down.

*: $P < 0.01$ (t-test) versus **5a**

Figure 4. Compared to **5a**, ER- α levels are lower in tumors of animals treated with **17ha** and **20ca**.

demonstrates the effectiveness of the optimized fluoromethyl side-chain in maximizing *in vitro* ER- α degradation efficacy and *in vivo* xenograft performance.

The robust performance of **17ha** and **20ca** in this and other preclinical xenograft models of breast cancer led to consideration of both compounds as potential clinical candidates. Compound **17ha** was ultimately selected in part due to results from an assay probing the conformation of ER- α induced by these ligands. In this assay, ligand-induced ER- α conformation was monitored by the interaction of ligand-selective peptide probes with the ligand-bound receptor.¹² The peptide probes utilized in this study interact differentially with protein surfaces exposed on ER- α in response to estradiol, 4-hydroxytamoxifen, or fulvestrant binding.^{13,14} It was found that **17ha** and **20ca** induce ER- α conformations distinct from fulvestrant and 4-hydroxytamoxifen (see Supporting Information). However, **20ca**-bound ER- α interacted with the α III peptide probe, suggesting that aspects of the **20ca** induced ER- α conformation are shared with that induced by the SERM 4-hydroxytamoxifen. In contrast, the ER- α conformations in response to **17ha** did not show appreciable interaction with α III (see Supporting Information). As we were looking to

identify a candidate with a profile distinct from tamoxifen, we selected **17ha** for development.

Selectivity of **17ha** against nuclear hormone receptors was good. In transcriptional reporter assays for the glucocorticoid, mineralocorticoid, progesterone-A, and progesterone-B receptors, **17ha** had no significant agonist or antagonist activity. Cytochrome P450 inhibition profiling of **17ha** indicated it had little to no competitive inhibitory activity against CYP1A2, CYP2C19, CYP2D6, or CYP3A4 ($IC_{50} > 10 \mu M$) and modest inhibitory effect on CYP2C8 and CYP2C9 ($IC_{50} = 3.0$ and $3.2 \mu M$, respectively). In a CEREP panel of radioligand binding assays for 55 targets (protein-free conditions), **17ha** at $10 \mu M$ displayed >75% binding to six targets. However, in follow-up cell-based functional assays, the interactions with most of these six targets were weak with IC_{50} values $>10 \mu M$. The only interaction with an $IC_{50} < 1 \mu M$ was with the dopamine transporter ($IC_{50} = 0.39 \mu M$). When tested for its effect on the hERG channel in a patch clamp assay, **17ha** was found to be a moderate inhibitor ($IC_{50} = 4.6 \mu M$). This activity on the dopamine transporter and hERG channel was not considered an issue as selectivity for ER is >1000-fold (MCF-7 proliferation = 0.1 nM ; ER- α degradation = 0.1 nM). Compound **17ha** was negative in an Ames assay using the TA-98 and TA-100 tester strains.

In summary, we have further maximized ER- α degradation efficacy of a series of ER modulators resulting in highly potent and efficacious chromene SERDs. A fluoromethyl substituent on either a pyrrolidine or azetidine ring at the end of the side-chain was found to be optimal for maximizing ER- α degradation. Fluoromethyl azetidine was determined to be the preferred ring system on the side-chain, leading to the identification of bis-phenol chromene **17ha**. In contrast to previous work, when applied to a monophenol chromene core, the fluoromethyl azetidine side-chain gave highly efficacious ER- α degraders such as **20ca**. In a tamoxifen-resistant breast cancer xenograft model, **17ha** and **20ca** (ER- α degradation efficacy = 97%) demonstrated tumor regression, together with robust reduction intratumoral ER- α levels. However, despite higher drug levels, **5a** (ER- α degradation efficacy = 91%) had inferior activity. This data suggests optimizing ER- α degradation *in vitro* maximizes activity in a tamoxifen-resistant breast cancer xenograft derived from the MCF-7 breast cancer cell line. Compound **17ha** (GDC-0927 or SRN-927) was selected for development and was evaluated in clinical trials in women with metastatic ER-positive breast cancer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00414.

Experimental information for synthesis of compounds; ligand-induced ER- α conformation; biological assays (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

E2, estradiol; EE, ethynyl estradiol; ER, estrogen receptor; ERE, estrogen response element; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulator; UWW, uterine wet weight

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