Discovery of ERD-3111 as a Potent and Orally Efficacious Estrogen Receptor PROTAC Degrader with Strong Antitumor Activity

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Introduction

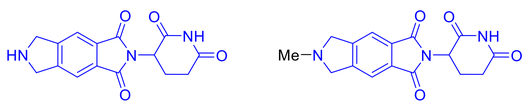
Estrogen receptor α (ERα) is a prime target for the treatment of ER+ breast cancer. Despite the development of several effective therapies targeting ERα signaling, clinical resistance remains a major challenge. In addition to oral selective estrogen receptor degraders (SERDs), another promising therapeutic strategy to overcome endocrine resistance in ER+ breast cancer is the development of ER degraders using the proteolysis targeting chimera (PROTAC) technology.

However, identifying highly potent and orally efficacious ERα PROTACs is difficult due to their relatively larger molecular weights and poorer physicochemical properties. Extensive research has led to the discovery of ARV-471 as an orally bioavailable ER PROTAC degrader. These preclinical and initial clinical data for ARV-471 suggested that oral ERα PROTACs may have a promising therapeutic potential for the treatment of ER+ human breast cancer.

Herein, we describe the design, synthesis, and biological evaluation of new classes of ERα PROTACs based on a new cereblon ligand and three classes of ER ligands. This work led to the discovery of **ERD-3111** as a potent, orally bioavailable, and highly efficacious ERα PROTAC that effectively inhibits the in vivo growth of breast cancers with either wild-type or mutated ER in mice.

Result

Table 1. Profiling of new CRBN ligand TX-16 and its analogue 9 (TX-16-Me)



9a (TX-16) **9** (TX-16-Me)

CRBN Binding affinity IC_{50} (μM)			Caco-2 Permeability of Compound **9**		Plasma Protein Binding of Compound **9** (%)
TX16 (**9a**)	Lenalidomide	Thalidomide	P_{app} (10^{-6} cm/s)	ER	human/dog/monkey/rat/mouse
2.6	3.6	2.9	7.4	1.1	12.5/17.0/14.4/36.5/26.3

Rat Pharmacokinetic Profile of Compound **9**						
IV/PO (mg/kg)	V_{ss} (L/kg)	Cl (mL/min/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	AUC (h*ng/mL)	F (%)
1/3	5.0	29.2	1.8	228.4	1147	70

Figure 1. Design of new classes of oral ER PROTACs based on CRBN ligand TX-16 and different classes of ER ligands

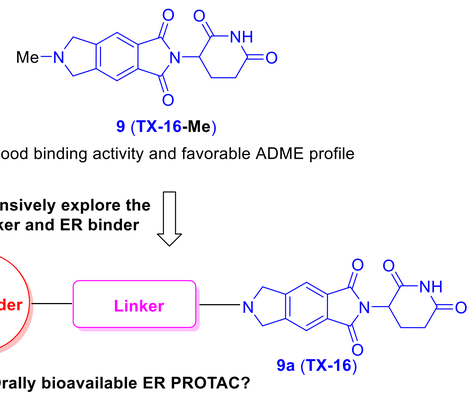
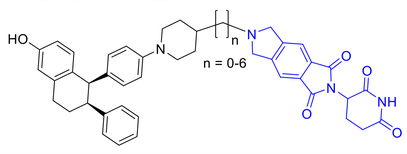
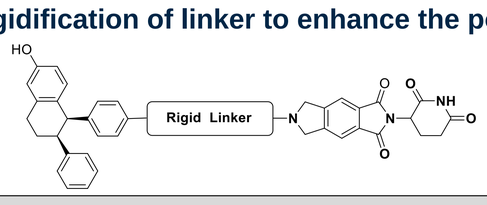


Table 2. Determination of optimal linker length based on ER binder in ARV-471 and TX16



Compound	Linker length (n)	ERα degradation	
		DC_{50} (nM)	D_{max} (%)
Fulvestrant	N.A.	0.9 ± 0.2	100 ± 3
ARV-471	N.A.	0.4 ± 0.04	89 ± 1
10	0	>1000	32 ± 5
11	1	415 ± 75	65 ± 8
12	2	>1000	50 ± 4
13	3	171 ± 48	79 ± 12
14	4	236 ± 40	67 ± 6
15	5	31 ± 4	106 ± 4
16	6	129 ± 15	101 ± 6

Table 3. Rigidification of linker to enhance the potency



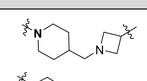
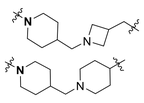
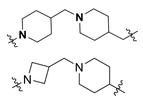
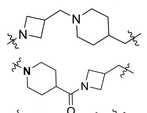
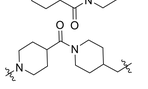
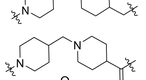
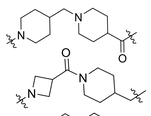
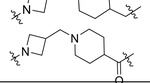
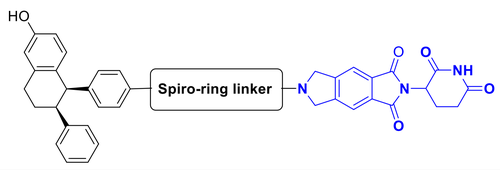
Compound	Linker	ERα degradation[a]	
		DC_{50} (nM)[b]	D_{max} (%)[c]
17		989 ± 237	50 ± 2
18		21 ± 7	59 ± 3
19		2.3 ± 0.7	84 ± 5
20		4.3 ± 1.0	79 ± 3
21		295 ± 94	57 ± 2
22		19 ± 7	64 ± 5
23		6.0 ± 1.4	84 ± 4
24		1.6 ± 0.3	95 ± 4
25		13 ± 3.2	76 ± 4
26		9.7 ± 3.4	63 ± 4
27		32 ± 6.3	87 ± 5
28		>1000	40 ± 8

Table 4. The PK profiles for compounds 19, 20, 24, 36 in rats and/or mice

Cpd	Species	IV/PO (mg/kg)	V_{ss} (L/kg)	Cl (mL/min/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	AUC (h*ng/mL)	F (%)
19	**Rat**	1/3	8.8	25.7	4.0	54.6	552	30
	mouse	1/3	16.2	14.2	9.8	93.3	1482	58
20	**Rat**	1/3	55.4	17.0	16.0	24.9	399	36
24	**Rat**	1/3	2.0	23.1	2.7	40.6	206	10
36	**Rat**	1/3	1.6	20.0	2.5	69.5	328	13
	Mouse	1/3	2.2	31	3.1	146	550	35

Table 5. Improvement of the PK profiles by employing more rigid spiro-ring-containing linkers



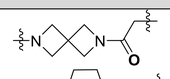
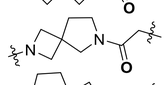
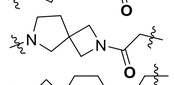
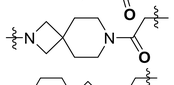
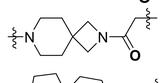
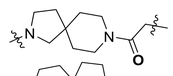
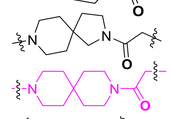
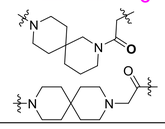
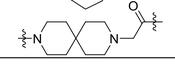
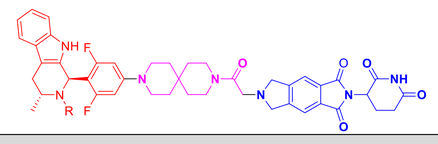
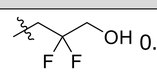
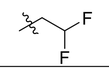
Cpd	Spiro-ring Linker	ERα degradation	
		DC_{50} (nM)	D_{max} (%)
29		11 ± 3.4	65 ± 3
30		>1000	23 ± 21
31		4.0 ± 1.5	63 ± 3
32		1.8 ± 0.6	68 ± 3
33		2.8 ± 0.9	71 ± 3
34		18 ± 6.3	58 ± 2
35		4.6 ± 1.2	75 ± 3
36		5.5 ± 1.2	90 ± 4
37		393 ± 165	58 ± 8
38		>1000	16 ± 8

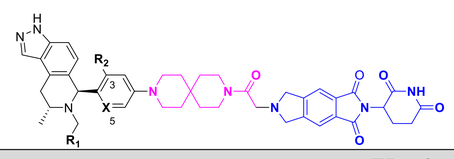
Table 6. Replacing the ER core of compound 36 with tricyclic indole cores



Cpd	R	ERα degradation		Oral plasma exposure in rat[a] (Drug concentration, ng/mL)			
		DC_{50} (nM)	D_{max} (%)	1 h	3 h	6 h	24 h
39		0.1 ± 0.02	97 ± 3	9.3 ± 4.1	6.6 ±3.2	3.0 ±1.0	N/A
40		0.8 ± 0.18	83 ± 3	7.9 ± 4.3	8.2 ± 3.4	8.3 ± 3.5	4.0 ± 0.2
41		0.8 ± 0.11	106 ± 2	8.3 ± 0.4	14.0 ± 1.5	10.3 ± 4.9	

[a]Dose: compounds **39-40** (3 mg/kg); compound **41** (5 mg/kg).

Table 7. Replacing the ER core of compound 36 with tricyclic indazole cores



Cpd	X	R_1	R_2	ERα degradation	
				DC_{50} (nM)	D_{max} (%)
42	N	CF_3	H	3.8 ± 0.6	107 ± 4
43	C-F	CF_3	F	2.0 ± 0.3	88 ± 2
44[a] (ERD-3111)	C-F	CHF_2	F	0.5 ± 0.04	91 ± 1
45	N	CHF_2	H	14 ± 3.0	112 ± 6
46	C-H	CHF_2	OMe	1.4 ± 0.3	82 ± 3
ARV-471[b]				0.4 ± 0.04	89 ± 1

[a]n = 21 experiments. [b]n = 16 experiments.

Table 8. Summary of the PK profiles for compounds 42, ERD-3111 and ARV-471

Cpd	Species	IV/PO (mg/kg)	V_{ss} (L/kg)	Cl (mL/min/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	AUC (h*ng/mL)	F (%)
42	Rat	1/3	3.7	27.0	2.3	51.8	243	14
44 (ERD-3111)	Rat	1/3	1.3	7.4	4.0	141.1	1317	20
	Mouse	1/3	3.2	5.7	6.4	260	3366	42
	Dog	0.5/1	5.2	11.0	7.9	87	937	66
ARV-471	Rat	1/3	2.4	18.6	4.0	46.5	244	10
	Mouse	1/3	1.8	21.9	2.5	156.3	684	31

Figure 3. Western blot analysis of the concentration-dependent ERα degradation by ERD-3111, ARV-471 and compound 36 in MCF7 and T47D cell lines.

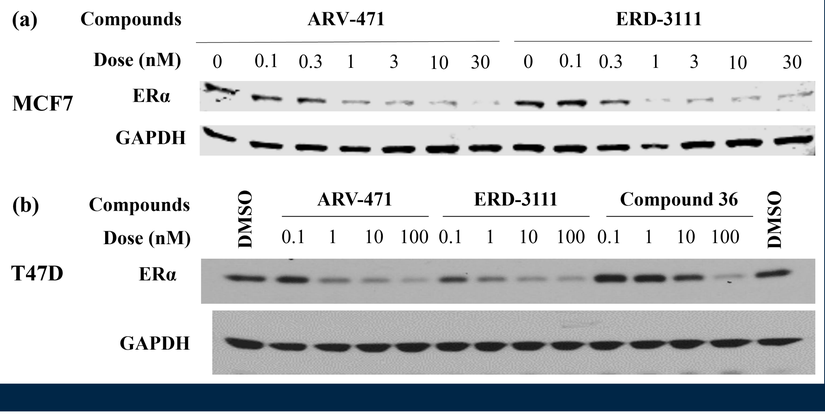
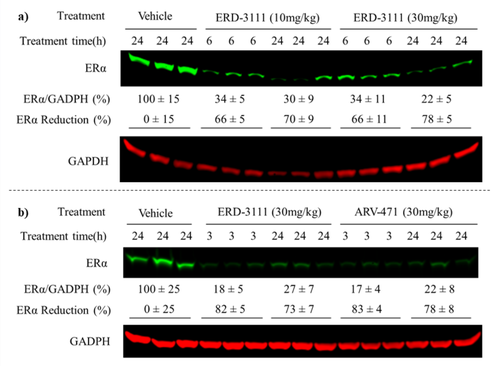


Table 9. Metabolic stability and safety profiling of ERD-3111

Liver microsomal stability $T_{1/2}$ (min)		hERG inhibition	CYP inhibition IC50 (μM)						
Human	Rat	IC_{50} (μM)	1A2	2C8	2C9	2C19	2D6	3A4 (Midazolam)	3A4 (Testosterone)
>60	>60	>30	>10	>10	>10	>10	>10	>10	>10

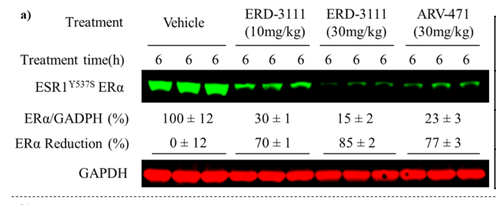
Figure 4. Pharmacodynamic and tissue distribution study of ERD-3111 in wide-type MCF7 tumor bearing mice after three continuous once-daily oral doses



Drug (Dose)	Time point (h)	Plasma Concentration (ng/mL)	Tumor Concentration (ng/mL)
ERD-3111 (10 mg/kg)	6	433 ± 116	385 ± 61
	24	18 ± 5	162 ± 50
ERD-3111 (30 mg/kg)	6	1437 ± 330	1466 ± 313
	24	80 ± 16	413 ± 84

Drug (Dose)	Time point (h)	Plasma Concentration (ng/mL)	Tumor Concentration (ng/mL)
ERD-3111 (30 mg/kg)	3	1977 ± 827	1407 ± 153
	24	167 ± 118	449 ± 269
ARV-471 (30 mg/kg)	3	921 ± 254	1708 ± 458
	24	56 ± 63	709 ± 512

Figure 5. Pharmacodynamic and tissue distribution study of ERD-3111 in $ESR1^{Y537S}$ (a) and $ESR1^{D538G}$ (b) mutant MCF7 tumor bearing mice after three continuous once-daily oral doses.



Drug (Dose)	Time point (h)	Plasma Concentration (ng/mL)	Tumor Concentration (ng/mL)
ERD-3111 (10 mg/kg)	6	502 ± 44	692 ± 68
ERD-3111 (30 mg/kg)	6	2833 ± 379	3743 ± 1070
ARV-471 (30 mg/kg)	6	1494 ± 512	3345 ± 1084

Drug (Dose)	Time point (h)	Plasma Concentration (ng/mL)	Tumor Concentration (ng/mL)
ERD-3111 (10 mg/kg)	6	609 ± 34	1052 ± 262

Figure 6. Antitumor efficacy of ERD-3111 in wide-type MCF7 xenografts.

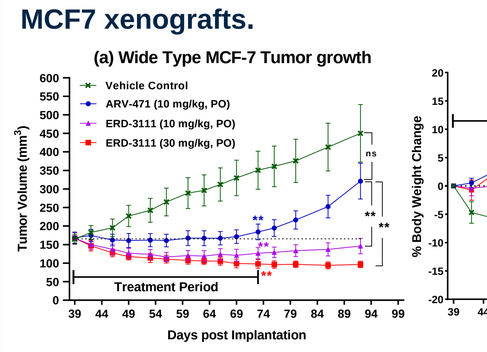
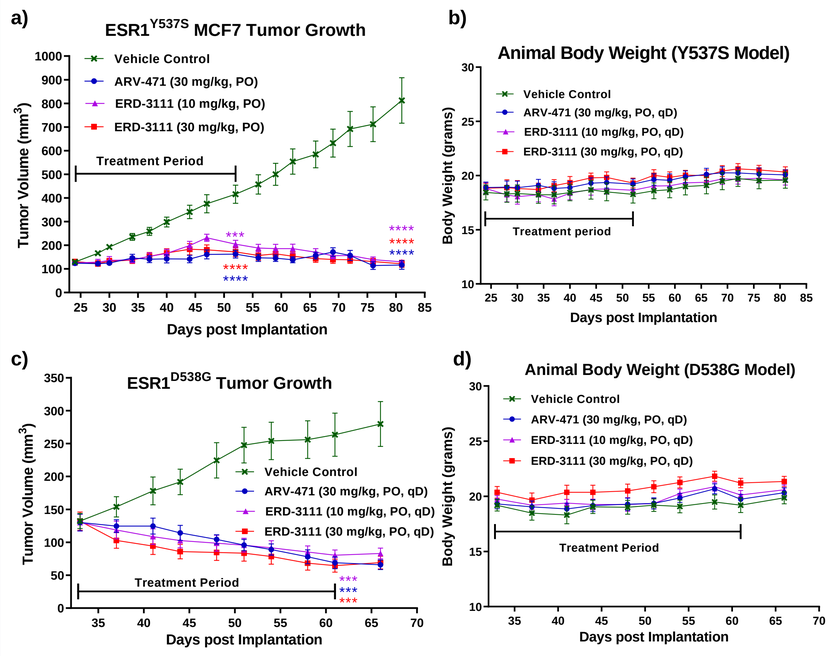


Figure 7. Antitumor efficacy of ERD-3111 in ESR1Y537S and ESR1D538G mutant MCF7 xenograft mouse models



Conclusion

In this study, we report the discovery of a new class of potent and orally efficacious ERα degraders using the PROTAC technology with **ERD-3111** being the most promising compound. **ERD-3111** exhibits potent *in vitro* degradation activity against ERα and demonstrates high oral bioavailability in mice, rats, and dogs. Oral administration of **ERD-3111** effectively reduces the levels of wild-type and mutated ERα proteins in tumor tissues. **ERD-3111** achieves tumor regression or complete tumor growth inhibition in the parental MCF-7 xenograft model with wild-type ER and two clinically relevant *ESR1* mutated models in mice. **ERD-3111** is a promising ERα degrader for further extensive evaluations for the treatment of ER+ breast cancer.

Reference

Chen, Z.; Hu, B.; Rej, R. K.; Wu, D.; Acharyya, R. K.; Wang, M.; Xu, T.; Lu, J.; Metwally, H.; Wang, Y.; et al. Discovery of ERD-3111 as a Potent and Orally Efficacious Estrogen Receptor PROTAC Degrader with Strong Antitumor Activity. *J. Med. Chem.* **2023**, *66*, 12559-12585.

Acknowledgement

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