

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

Zhixiang Chen,^{†,‡} Biao Hu,^{†‡} Rohan Kalyan Rej,^{†,‡} Dimin Wu,^{†‡} Ranjan Kumar Acharyya,^{†‡} Mingliang Wang,^{†‡} Tianfeng Xu,^{†‡} Jianfeng Lu,[†] Hoda Metwally,[†] Yu Wang,[†] Donna McEachern,[†] Christina L. Gersch,^{II} Meilin Wang,[#] Wenjing Zhang,[#] Qiuxia Li,[#] Bo Wen,[#] Duxin Sun,[#] James M. Rae,^{II} Shaomeng Wang^{†*} [†]The Rogel Cancer Center, Department of Internal Medicine, Department of Pharmacology, and Department of Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States; #Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, United States; ^{ID}epartment of Internal Medicine, Department of Pharmacology, the Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan 48109, United States

Introduction

Estrogen receptor α (ER α) 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 ERa 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 ER α PROTAC that effectively inhibits the in vivo growth of breast cancers with either wild-type or mutated ER in mice.

rate	rats and/or mice														
Cpd	Species	IV/PO (mg/kg)	V _{ss} (L/kg)	CI (mL/min/kg)	T _{1/2} (h)	C _{max} (ng/mL)	AUC (h*ng/mL)	F (%							
10	Rat	1/3	8.8	25.7	4.0	54.6	552	3(
13	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	3							
24	Rat	1/3	2.0	23.1	2.7	40.6	206	1(
26	Rat	1/3	1.6	20.0	2.5	69.5	328	1:							
30	Mouse	1/3	2.2	31	3.1	146	550	3							

Table 4. The PK profiles for compounds 19, 20, 24, 36 in

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



Liver mic stability	rosomal T _{1/2} (min)	hERG inhibition		CYP inhibition IC50 (μM)						
Human	Rat	IC ₅₀ (μΜ)	1A2	2C8	2C9	2C19	2D6	3A4 (Midazolam)	3A4 (Testosterone	
>60	>60	>30	>10	>10	>10	>10	>10	>10	>10	

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

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

u)	venicie	ERD-311	1 (10mg/kg)	ERD-311	1 (30mg/kg)					
Treatment time(h) ERα	24 24 24	6 6 6	24 24 24	6 6 6	24 24 24	Drug (Dose)	Time point (h)	Plasma Concentration (ng/mL)	Tumor Concentration (ng/mL)	
ERα/GADPH (%)	100 ± 15	34 ± 5	30 ± 9	34 ± 11	22 ± 5	ERD-3111	6	433 ± 116	385 ± 61	
ERα Reduction (%)	0 ± 15	66 ± 5	70 ± 9	66 ± 11	78 ± 5	(10 mg/kg)	24	18 ± 5	162 ± 50	
						ERD-3111	6	1437 ± 330	1466 ± 313	
GAPDH						(30 mg/kg)	24	80±16	413 ± 84	
b) Treatment	Vehicle	ERD-311	1(30ma/ka)	ARV-471	(20ma/lea)					
Treatment time(h)	24 24 24	3 3 3	24 24 24	3 3 3	24 24 24	Drug (Dose)	Time point (h)	Plasma Concentration (ng/mL)	Tumor Concentration (ng/mL)	
Treatment time(h) ERα	24 24 24	3 3 3	24 24 24	3 3 3	24 24 24	Drug (Dose) ERD-3111	Time point (h) 3	Plasma Concentration (ng/mL) 1977±827	Tumor Concentration (ng/mL) 1407±153	
Treatment time(h) ERα ERα/GADPH (%)	$24 \ 24 \ 24 \ 24$ 100 ± 25 0 ± 25	$\frac{18\pm 5}{18\pm 5}$	$24 \ 24 \ 24 \ 24 \ 24 \ 27 \pm 7 \ 72 $	$\frac{17 \pm 4}{23 \pm 4}$	(30 mg/kg) 24 24 24 22 ± 8 78 + 8	Drug (Dose) ERD-3111 (30 mg/kg)	Time point (h) 3 24	Plasma Concentration (ng/mL) 1977±827 167±118	Tumor Concentration (ng/mL) 1407±153 449±269	
Treatment time(h) ERα ERα/GADPH (%) ERα Reduction (%)		$\frac{18\pm 5}{82\pm 5}$	$ \begin{array}{r} \hline 1 \ (30 \text{ mg/kg}) \\ 24 \ 24 \ 24 \\ 27 \pm 7 \\ 73 \pm 7 \\ \end{array} $	$\frac{17 \pm 4}{83 \pm 4}$	$\begin{array}{c} 24 & 24 & 24 \\ \hline 22 \pm 8 \\ \hline 78 \pm 8 \end{array}$	Drug (Dose) ERD-3111 (30 mg/kg) ARV-471	Time point (h) 3 24 3	Plasma Concentration (ng/mL) 1977 ± 827 167 ± 118 921 ± 254	Tumor Concentration (ng/mL) 1407±153 449±269 1708±458	

Result

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

	$HN \qquad \qquad$													
9a (TX-16) 9 (TX-16-Me)														
CRI	BN Binding a IC ₅₀ (µM)	ffinity	Caco-2 Pe of Comp	ermeability bound 9	P	lasma Protein Bin Compound 9 (%	ding of %)							
TX16 (9a)	Lenalidomid	e Thalidomide	P _{app} (10⁻ ⁶ cm/s) ER	hun	nan/dog/monkey/ra	at/mouse							
2.6	3.6	2.9	2.9 7.4 1.1 12.5/17.0/14.4/36.5/26.3											
		Rat Pharmacc	kinetic Prof	ile of Comp	ounc	9 b								
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



Cind	Spiro-ring Linker	ERα degradation							
opu		DC ₅₀ (nM)	D _{max} (%)						
29	$\frac{\xi}{\xi} N $ $N $ O	11 ± 3.4	65 ± 3						
30	Z N N T	>1000	23 ± 21						
31	ZN N F	4.0 ± 1.5	63 ± 3						
32		1.8 ± 0.6	68 ± 3						
33	$\frac{\xi}{\xi}$ N \sqrt{N} O	2.8 ± 0.9	71 ± 3						
34	ZZN N ZZ	18 ± 6.3	58 ± 2						
35	ZEN N ZE	4.6 ± 1.2	75 ± 3						
36	-{-{-}	5.5 ± 1.2	90 ± 4						
37	25°N N O N O	393 ± 165	58 ± 8						
38	$\frac{1}{\frac{1}{2}}$ N N $\frac{1}{\frac{1}{2}}$	>1000	16 ± 8						

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



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 oncedaily oral doses.

a)	E		EI	ERD-3111 ERD		2D-3	D-3111 ARV-471										
Treatment	Vehi	cle	(1	0mg/l	kg)	(30	0mg/	kg)	(3	0mg/	'kg)		Drug	Time point	Plası Concent	na ration	Tumor Concentration
Treatment time(h)	6	66	6	6	6	6	6	6 6 6 6 6			(Dose)	(h)	(ng/mL)		(ng/mL)		
ESR1 ^{Y537S} ERα				-			1	E (ERD-3111 10 mg/kg)	6	502±	44	692 ± 68				
ERα/GADPH (%) ERα Reduction (%)	100 0 ±	± 12 : 12		30 ± 1 70 ± 1	l 1	1 8	5 ± 2 85 ±	2 2		23 ± 77 ±	3 3	E ()	ERD-3111 30 mg/kg)	6	2833±	379	3743 ± 1070
GAPDH												ARV-471 30 mg/kg)	6	1494±512		3345 ± 1084	
b) Treatment	Veh	icle	E1 (1	RD-3 0mg/	111 'kg)												
Treatment time(h)	6	6 6	6	6	6			Dr	ug	T	ìme		Plasma	Tu	imor		
ESR1 ^{D538G} ERa				_				(Do	ose)	p	oint (h)	Col	ncentration (ng/mL)	Conce (ng	entration g/mL)		
ERα/GADPH (%)	100	± 10		32 ± 1	1			ERD-	3111		6		600 + 24	1052	2 - 262		
ERα Reduction (%)	0 =	± 10		$68 \pm$	1			(10 mg/kg) 0		(009±34	1052	2 = 202				
GAPDH																	

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



				Ö							
pd R	ERα de	egradation	Or (D	al plasma exp rug concentra	osure in rat ition, ng/mL	a)					
	DC ₅₀ (nl	M) D _{max} (%	5) 1h	3 h	centration, ng/mL) h 6 h ± 3.2 3.0 ± 1.0 ± 3.4 8.3 ± 3.5 ± 1.5 10.3 ± 4.9 mpound 36 w						
S9 Z OF	⁺ 0.1 \pm 0.	.02 97 ± 3	9.3 ± 4.7	6.6 ±3.2	3.0 ±1.0	N//					
₩ Me Me	$0.8\pm 0.$.18 83 ± 3	3 7.9 ± 4.	3 8.2 ± 3.4	8.3 ± 3.5	4.0 0.2					
1 → F F	$0.8\pm0.$.11 106 ±	2 8.3 \pm 0.4	4 14.0 \pm 1.5	10.3 ± 4.9	9					
Dose: compounds	s 39-40 (3 n	ng/kg); comp	ound 41 (5 r	ng/kg).							
Table 7. Retricyclic ind	eplacing lazole c	g the E ores		o o	una 30 v	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,					
Table 7. Retricyclic ind		g the E cores R_2 $\sqrt{3}$ X = -N		or compo							
Table 7. Re tricyclic ind	eplacing lazole c \downarrow^{H}_{N} \downarrow^{N}_{N} R_{1}	g the E cores R_2 X_5 X_5		$\frac{1}{\sqrt{n}} = \frac{1}{\sqrt{n}} = \frac{1}{\sqrt{n}}$ ERa deg	gradation						
Table 7. Re tricyclic ind	eplacing lazole c \downarrow_{I} \downarrow_{I} \downarrow_{I} \downarrow_{I} \downarrow_{I} R_1	g the E cores R_{2}	R Core R_2 –	or compo $ \frac{1}{\sqrt{n}} + \frac{1}{$	gradation D _{max} (%)					
Table 7. Retricyclic ind Cpd 42	eplacing lazole c \downarrow_{I} \downarrow_{I} \downarrow_{I} \downarrow_{I} \downarrow_{I} R_{1}	g the E cores R_{2}	R core R_2 - H	$\sqrt{100000000000000000000000000000000000$	gradation D _{max} (% 107 ±	5)					
Table 7. Retricyclic ind Cpd 42 43	eplacing lazole c \downarrow_{I} \downarrow_{I} \downarrow_{I} \downarrow_{I} \downarrow_{I} R_{1} R_{1} R_{1}	g the E cores R_2 x_3 x_5 R_1 CF_3 CF_3 CF_3	R Core $R_2 - H$ F	$\frac{compo}{ERa deg}$ $\frac{DC_{50} (nM)}{3.8 \pm 0.6}$ 2.0 ± 0.3	gradation D _{max} (% 107 土 88 土 2	5) 4					
Table 7. Retricyclic ind Cpd 42 43 44 ^a (ERD-3111)	eplacing lazole c $\int_{R_1}^{H}$ R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_2 R_1 R_2 R_1 R_2 R_1 R_2 R_1 R_2 R_2 R_1 R_2 R_2 R_2 R_3 R_1 R_2 R_2 R_3 R_2 R_3 R_2 R_3 R_2 R_3 R_3 R_2 R_3 R_3 R_3 R_4 R_2 R_3 R_3 R_4 R_2 R_3 R_3 R_4 R_2 R_3 R_3 R_4 R_2 R_3 R_3 R_4 R_4 R_3 R_4 $R_$	g the E cores R_{1} R_{1} CF_{3} CF_{3} CF_{3} CHF_{2}	R Core $R_2 - H$ F	or compo COMPO $COMPO ERadeg DC50 (nM) 3.8 ± 0.6 2.0 ± 0.3 0.5 ± 0.04$	$ \begin{array}{r} and 30 \\ and 30 \\ \hline and 30 \\ and 30 \\ $	b) 4 2					
Table 7. R tricyclic ind Cpd 42 43 44 ^a (ERD-3111) 45	eplacing lazole c \downarrow_{I} \downarrow	g the E cores R_{1} R_{1} CF_{3} CF_{3} CF_{3} CF_{2} CHF_{2}	R Core \mathbf{R}_2 - \mathbf{R}_2 - \mathbf{H} \mathbf{F} \mathbf{F} \mathbf{H}	Of Compo f(x) = 0 f(x) =	gradation D_{max} (% 107 ± 88 ± 2 91 ± 1 112 ± 0	b) 4 2 1 6					
Table 7. R tricyclic ind Cpd 42 43 44 ^a (ERD-3111) 45 46	eplacing lazole c $\int_{R_1}^{H}$ R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_1 R_2 R_1 R_2 R_1 R_2 R_1 R_2 R_2 R_3 R_1 R_2 R_3 R_1 R_2 R_3 R_1 R_2 R_3 R_2 R_3 R_3 R_1 R_2 R_3 R_3 R_3 R_4 R_1 R_2 R_3 R_3 R_4 R_1 R_2 R_3 R_3 R_4 R_3 R_4 R_3 R_4 $R_$	g the E cores R_2 r_3 r_4 R_1 CF_3 CF_3 CF_3 CF_3 CF_2 CHF_2 CHF_2 CHF_2 CHF_2 CHF_2 CHF_2	R Core R $(-)$ R $(-)$	Of Compo Compo ERadeg DC ₅₀ (nM) 3.8 ± 0.6 2.0 ± 0.3 0.5 ± 0.04 14 ± 3.0 1.4 ± 0.3	gradation D_{max} (% 107 ± 4 88 ± 2 91 ± 1 112 ± 4 82 ± 3	b) 4 2 1 6 3					

Table 8. Summary of the PK profiles for compounds 42,

Table 3. Rigidification of linker to enhance the potency



Compound	Linkor	ERα degra	dation ^a
Compound	LIIKCI	DC ₅₀ (nM) ^b	D _{max} (%) ^c
17	N N	989 ± 237	50 ± 2
18	Part N N N	21 ± 7	59 ± 3
19	St N N St	$\textbf{2.3}\pm\textbf{0.7}$	84 ± 5
20	N Z	$\textbf{4.3} \pm \textbf{1.0}$	79 ± 3
21	N N St	295 ± 94	57 ± 2
22	ZZN N ZZ	19 ± 7	64 ± 5
23	² ² N N N	$\textbf{6.0} \pm \textbf{1.4}$	84 ± 4
24	Provide the second seco	1.6 ± 0.3	95 ± 4
25	N N ZZ	13 ± 3.2	76 ± 4
26		9.7 ± 3.4	63 ± 4
27		32 ± 6.3	87 ± 5
28	ZN N YE	>1000	40 ± 8

ERD-3111 and ARV-471

Cpd	Cpd Species IV/PO (mg/kg)		V _{ss} (L/kg)	Cl (mL/min/kg)	T _{1/2} C _{max} (h) (ng/mL		AUC (h*ng/mL)	F (%)					
42	Rat	1/3	3.7	27.0	2.3	51.8	243	14					
	Rat	1/3	1.3	7.4	4.0	141.1	1317	20					
44 (ERD-3111)	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					
	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 depende compou	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.												

(a)	Compounds	ARV-471								ERD-3111						
	Dose (nM)	0	0.1	0.	3 1		3	10	30	0	0.1	0.3	1	3	10	30
MCF7	ERα	7	-	-	• -					_	-		a	-	10.00	
	GAPDH	5	_	_	• •		_	÷.,	-	_	_	_	~	_	-	-
(b)	Compounds		DCI		ARV	/-471	[ERI)-311	1		omp	ound	36	NSO
Г47D	Dose (nM) ERα			0.1	1	10	100	0.1	1	10	100	0.1	1	10	100	D
	GAPDH	-		-	_	-	-	-		-	-	-	_	-	_	_

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

This study was supported by funding from Oncopia Therapeutics, Inc., Proteovant Therapeutics, Inc. and Roivant Sciences, Inc, and the University of Michigan Comprehensive Cancer Center Core Grant from the National Cancer Institute, NIH (P30CA046592).