Supporting Information

Optimization of a novel binding motif to (*E*)-3-(3,5-difluoro-4-((1*R*,3*R*)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylic acid (AZD9496), a potent and orally bioavailable selective estrogen receptor downregulator and antagonist

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Table 1. ER binding, ER downregulation, PR agonism, PR antagonism²⁹ and MCF7 antiproliferation^{40,41} data for **1-3, 6, 7b, 8, 9b, 15-16, 22b-24b, 27b-30b**

Entry	ER binding pIC ₅₀ ^a	ER downregulation pIC ₅₀ ^a	PR agonism pIC ₅₀ ^a	PR antagonism pIC ₅₀ ^a	MCF7 antiproliferation pIC ₅₀ ^a
1a	7.61 (±0.02)	5.0 (±0.04)	<4	6.89 (±0.03)	7.24 (±0.1)
1b	9.51 (±0.15)	5.31 (±0.07)	<4	9.1 (±0.06)	9.27 (±0.13)
2	9.09 (±0.18)	10.2 (±0.01)	<4	9.68 (±0.1)	10 (±0.06)
3a	8.49 (±0.02)	5.89 (±0.03)	<4	5.4 (±0.04)	6.53 (±0.05)
3b	9.84 (±0.06)	8.17 (±0.04)	<4.5	7.92 (±0.22) ^b	ND ^c
6	5.69 (±0.1)	5.93 (±0.21)	<4	5.57 (±0.05)	ND ^c
7b	4.86 (±0.16)	5.43 (±0.14)	<4.5	ND ^d	ND ^c
8	7.4 (±0.15)	7.07 (±0.04)	<4	6.14 (±0.04)	4.86 (±0.03)
9b	5.75 (±0.002)	6.11 (±0.04)	<4	5.82 (±0.05)	<4.6
15	7.65 (±0.18)	7.36 (±0.11)	7.48 (±0.36)	6.04 (±0.17)	<4.5
16	7.15 (±0.18)	7.52 (±0.1)	7.9 (±0.12)	5.68 (±0.27)	<4.5
22b	6.16 (±0.07)	5.88 (±0.05)	<4	5.64 (±0.06)	6.09 (±0.06)
23b	7.16 (±0.09)	7.33 (±0.03)	<4	7.31 (±0.05)	7.65 (±0.1)
24b	7.01 (±0.04)	7.34 (±0.07)	<4	7.3 (±0.07)	ND ^c
27a	8.19 (±0.04)	8.68 (±0.07)	<4	8.44 (±0.11)	9.11 (±0.07)
28b	9.15 (±0.26)	9.38 (±0.05)	<5.5	9.08 (±0.09)	9.64 ^e
29b	9 (±0.11)	9.07 (±0.08)	<5.5	8.46 (±0.12)	9.49 (±0.15)
30b	9.17 (±0.07)	9.86 (±0.03)	<5.5	9.55 (±0.06)	10.4 (±0.05)

^an ≥ 3; ^bn = 2; ^cNot determined; ^dUnable to determine PR antagonist pIC₅₀ due to inconsistent response across concentration range; ^en = 1.

(*E*)-Methyl 3-(4-((1*R*,3*R*)-2-(2-hydroxyethyl)-3-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylate (22a): ¹H NMR (400 MHz, CDCl₃) 1.15 (3H, d), 2.63 (2H, ddd), 2.77 - 2.98 (3H, m), 3.33 - 3.44 (1H, m), 3.61 (2H, td), 3.77 (3H, s), 4.85

(1H, s), 6.34 (1H, d), 7.1 - 7.19 (2H, m), 7.23 (2H, s), 7.45 (2H, d), 7.49 - 7.58 (3H, m); m/z = 391 $[M+H]^+$; RT = 2.32, 100% purity.

(E)-Methyl 3-(4-((1R,3R)-2-((R)-3-hydroxy-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (24a): ^1H NMR (400 MHz, DMSO d_6) 0.93 (3H, d), 1.05 (3H, d), 1.84 (1H, dt), 2.26 - 2.42 (2H, m), 2.48 - 2.58 (1H, m), 2.59 - 2.72 (1H, m), 3.09 - 3.15 (1H, m), 3.20 (1H, dt), 3.27 (1H, m), 3.71 (3H, s), 4.37 (1H, t), 4.82 (1H, s), 6.58 (1H, d), 6.92 - 6.99 (1H, m), 6.99 - 7.06 (1H, m), 7.22 - 7.29 (3H, m), 7.41 (1H, d), 7.59 - 7.68 (3H, m), 10.64 (1H, s); m/z = 419 $[M+H]^+$; RT = 2.85, 100% purity.

(E)-Methyl 3-(4-((1R,3R)-2-isobutyl-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (27a): ^1H NMR (400 MHz, DMSO d_6) 0.81 (3H, d), 0.97 (3H, d), 1.05 (3H, d), 1.83 (1H, dt), 2.14 (1H, dd), 2.35 (1H, dd), 2.51 - 2.57 (1H, m), 2.69 (1H, dd), 3.09 - 3.21 (1H, m), 3.72 (3H, s), 4.81 (1H, s), 6.60 (1H, d), 6.94 - 7.08 (2H, m), 7.28 (4H, dd), 7.43 (1H, d), 7.61 - 7.7 (2H, m), 10.61 (1H, s); m/z = 403 $[M+H]^+$; RT = 3.72, 100% purity.

(E)-Methyl 3-(3,5-difluoro-4-((1R,3R)-2-isobutyl-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (28a): ^1H NMR (400 MHz, DMSO d_6) 0.67 (3H, d), 0.81 (3H, d), 1.04 (3H, d), 1.62 - 1.77 (1H, m), 2.04 (1H, dd), 2.43 (1H, dd), 2.58 (1H, dd), 2.87 (1H, dd), 3.34 - 3.44 (1H, m), 3.74 (3H, s), 5.10 (1H, s), 6.79 (1H, d), 6.92 - 7.03 (2H, m), 7.17 - 7.21 (1H, m), 7.41 (1H, d), 7.50 (2H, d), 7.63 (1H, d), 10.54 (1H, s); m/z = 437 $[M-H]^-$; RT = 3.66, 100% purity.

(E)-Methyl 3-(4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (29a): ^1H NMR (400 MHz, CDCl_3) 1.09 (3H, d), 1.30 (3H, d), 1.47 (3H, d), 2.48 - 2.78 (4H, m), 3.30 (1H, s), 3.79 (3H, s), 5.09 (1H, s), 6.40 (1H, d), 7.12 (1H, td), 7.17 (1H, td), 7.29 (1H, d), 7.34 (2H, d), 7.43 (2H, d), 7.54 (1H, d), 7.66 (2H, m); $m/z = 421$ $[\text{M}+\text{H}]^+$; RT = 3.41, 95% purity.

Figure 1: HCC1428 long term estrogen deprived (LTED) model of aromatase inhibitor resistance. HCC1428LTED engrafts were grown in ovariectomised NSG mice and were dosed daily with Compound **30b** or once weekly with compound **2** at doses shown above. Tumor growth was measured by caliper at regular intervals.

Figure 2: ER- α down-regulation by Compound 2 and Compound 30b in the HCC1428 LTED in vivo model. HCC1428LTED xenografts were grown in ovariectomised NSG mice and were dosed daily with either PEG/captisol (vehicle), Compound 2 or Compound 30 at the doses shown above. Tumors from the treated mice were collected at 28 days and analysed by Western blot for ER and vinculin proteins. Protein levels were measured by chemiluminescent and quantified using Syngene software. ER protein levels were normalized to vinculin as a loading control and plotted as shown.

Table 2. Selected comparator pre-clinical data - Compound 30b and Compound 3c*

	Compound 30b	Compound 3c *
ER binding IC ₅₀ (μ M)	0.0008	0.0002
ER downregulation IC ₅₀ (μ M)	0.00011	0.0015
PR antagonism IC ₅₀ (μ M)	0.00028	0.0043
MCF7 antiproliferation IC ₅₀ (μ M)	0.00004	0.0007
LogD	2.8	>4.0
Human % free	0.2	0.08
Rat/dog bioavailability %	63/74	20/-
Human hep Clint μ L/min/10-6	32	31

*in-house data