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Article

Discovery of ZN-c5, an Orally Bioavailable Selective Estrogen Receptor Degrader (SERD) with Improved Pharmacokinetics

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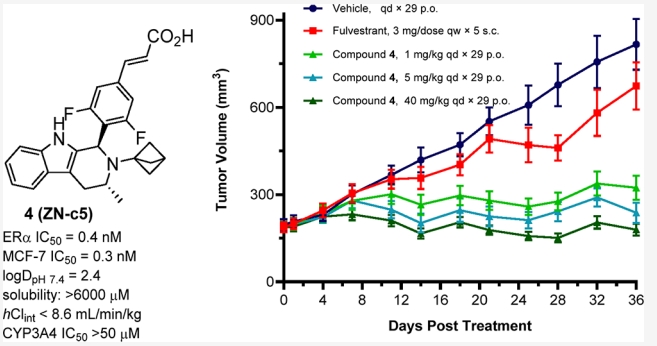
ABSTRACT: Here, we report our strategy to design an optimized oral selective estrogen receptor degrader (SERD), including human pharmacokinetics, by exploiting the bicyclo[1.1.1]pentane (BCP) ring system. The BCP has been shown to serve as a surrogate for phenyl rings and alkyl groups in drug candidates, reducing metabolism and improving physicochemical properties. It has not been extensively profiled in human clinical trials. We optimized a number of molecules and ultimately selected compound **4**, which showed excellent cell potency in breast cancer lines and displayed highly favorable in vitro ADME properties across multiple species. This translated into highly desired exposure in vivo across both rodent and nonrodent species exceeding that observed with other contemporary SERDs and downregulators. After fully profiling the compound, we nominated compound **4** (ZN-c5) for clinical development. Compound **4** advanced into Phase 1/2 clinical trials, which demonstrated high human exposure upon dosing patients with 50 mg once a day.



INTRODUCTION

Breast cancer (BC) is implicated in 12% of global cancer cases, with a significant impact on mortality in women (15% of all cancer-related deaths).[1] Most cases can be classified based on the higher expression levels of the estrogen receptor alpha (ERα), without expression of human epidermal growth factor (HER2), namely HR-positive/HER2-negative type breast cancer. Historically, there have been several treatment paradigms often administered sequentially. First, an aromatase inhibitor (AI) to block the biosynthesis of estradiol is used in combination with antihormonal agents. A second strategy employs selective estrogen receptor modulators (SERMs) such as tamoxifen, a mixed agonist/antagonist of the estrogen α- and β- receptors. This approach, while initially effective, can ultimately lead to resistance.[2] The more recent approach of using a dual ER degrader/antagonist[3−6] such as the clinically efficacious fulvestrant,[7] suffers from inherent limitations in delivery.[8] The compound must be administered via intramuscular (IM) injection which limits the maximum dose to 500 mg/month, and may be the cause of suboptimal receptor occupancy.[9−11] Therefore, in the past decade, there has been significant interest in the discovery of orally bioavailable small molecule antagonists/degraders, to enable improved systemic exposure leading to enhanced clinical benefit. Several molecules have been evaluated in the clinic and selected examples are depicted in Figure 1. Most SERDs possess either an acrylic acid or an amine tail group, directed toward helix H12 of the ER protein that promotes receptor destabilization and degradation by exposing hydrophobic H12 residues. E3 ligase-mediated ubiquitination of K302 and K303 residues in the hinge region of the ER receptor in turn leads to the downstream degradation of the ER protein by the ubiquitin proteasome system.[12−16] Some examples of SERDs bearing an acrylic acid tail group include GW7604,[17,18] GDC-0810,[19] AZD9496,[20] LSZ102,[21] D-0502 and G1T48 (Figure 1). Examples of SERDs bearing an amine tail group include SAR439859[22] which was halted in 2022, as well as a number of other molecules in late clinical development, including GDC-9545,[23] AZD9833,[24,25] LY3484356,[26] OP-1250[27] and the newly approved elacestrant[28] (also known as Orserdu). A recent review captures the current molecular landscape.[29]

RESULTS AND DISCUSSION

Early SERDs and Challenges with Achieving Human Exposure. The discovery of GDC-0810/ARN-810[19] was based

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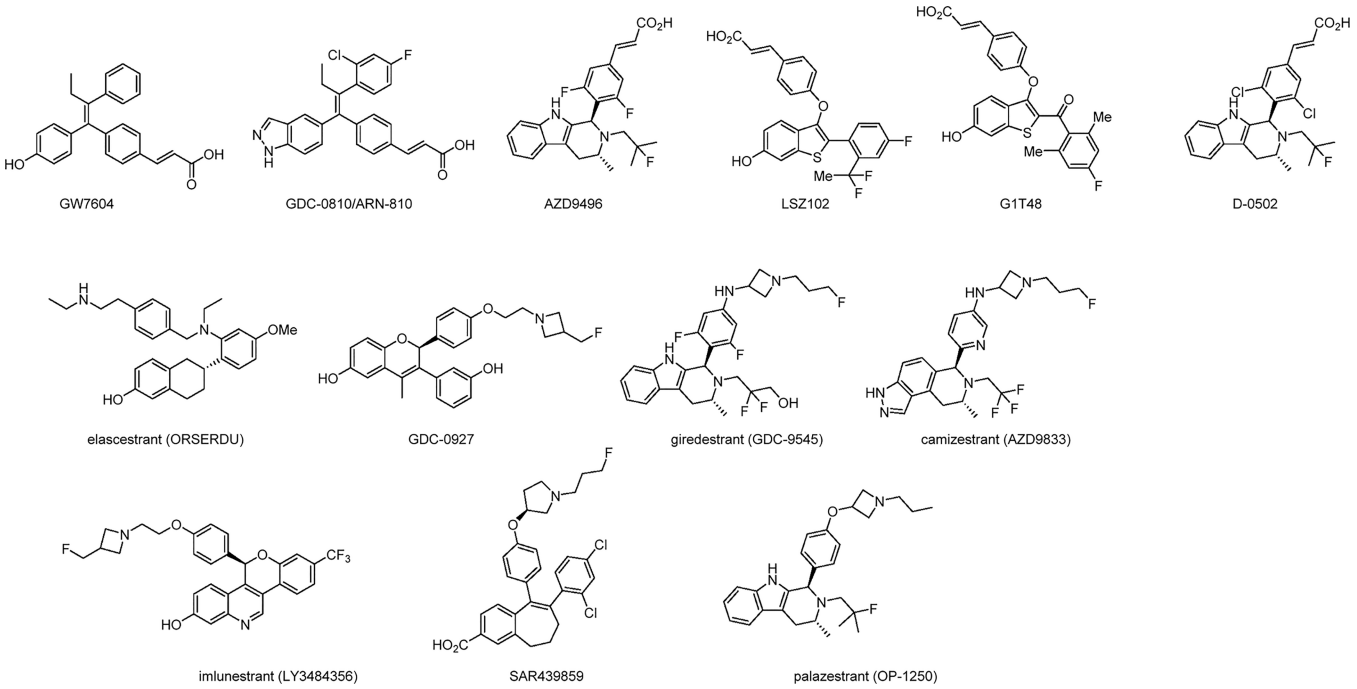

Figure 1. Selected examples of SERDs bearing acrylic acid or amine tails.

on the pioneering work of McDonnell and co-workers (Duke University), Glaxo Wellcome, and later Dupont that led to GW-5638.[17] GDC-0810 showed promise in early clinical trials but ultimately was halted in Phase 2 trials, partly due to the high doses needed for efficacy, adverse events (such as significant GI issues), and associated formulation challenges.[30] Meanwhile, AstraZeneca discovered the novel tetrahydrocarboline core[20] which was elegantly optimized to AZD9496, a potent antagonist and degrader with high selectivity and good oral exposure across multiple preclinical species, including both rodent and non-rodent. AZD9496 rapidly progressed into Phase 1 clinical studies in ER-positive BC patients as well as a study to assess the pharmacokinetics of different formulations in healthy patients (NCT02780713).[31] AZD9496 was ultimately replaced by AZD9833 which is currently in Phase 3, likely due to target coverage.[32,33] Our analysis of AZD9496 identified a few possible metabolic soft spots to explore and optimize, particularly the *N*-isobutyl substituent. In a series of aldosterone synthase inhibitors, the isobutyl functionality was identified as a metabolic soft spot and replacement with an oxetane moiety led to significantly reduced metabolic clearance.[34,35] We compared the binding poses of AZD9496 and estradiol, an endogenous ligand of ER, in the ER ligand binding domain (LBD) and speculated that alternative lipophilic groups could better occupy the lipophilic pocket defined by Leu525:Leu384 residues.[20] Therefore, we considered a handful of replacements, one of which was the bicyclo[1.1.1]pentane (BCP) ring system. The initial synthesis was reported by Wiberg and Connor in 1966.[36] More robust synthetic methods have emerged in only the past decade (e.g., see Bunker, Huang and co-workers,[37] Baran and co-workers in their seminal early demonstration of the scalability of preparation of BCP amines,[38,39] and Shire and Anderson for a recent review[40]). The BCP framework represents a nonclassical bioisostere of phenyl rings[41] but also alkynes[42] and other alkyl groups.[43] Pellicciari and co-workers demonstrated how a BCP could be a potent replacement for benzene rings in a new class of glutamate receptor antagonists.[44] The pioneering work of Stepan and co-workers at Pfizer illustrated the true potential of the BCP moiety beyond serving as just a spacer.[41] The replacement of a central para-substituted fluorophenyl ring with the BCP ring in the γ-secretase inhibitor BMS-708163 improved passive permeability, solubility, and metabolic stability, resulting in a 4-fold increase in oral exposure in mouse. In vitro and in vivo data were quite compelling, demonstrating that the BCP ring could be utilized to overcome liabilities in drug design and provide the benefits of additional sp^3 character to enhance physicochemical and ADME properties.[45] Multiple groups have recently confirmed the positive benefits on in vivo exposure of the BCP ring.[46−48] We felt this would be a good opportunity to leverage the BCP moiety to address the less than desired human exposure associated with AZD9496. Furthermore, the goal of our SERD program was to discover and develop a small molecule that could act as both a degrader and a full antagonist of the estrogen receptor and to obviate risks associated with partial agonist activity such as increased risk of endometrial tissue thickening.[49] This, in combination with a clean safety profile could maximize clinical benefit in patients.

Introduction of Bicyclo[1.1.1]pentyl Group to Fill the Leu525-Leu384 Lipophilic Pocket. A series of compounds were designed and synthesized. Our initial screening cascade focused on testing the ability of synthesized compounds to inhibit the proliferation of the MCF-7 cancer cell line. At the onset we hypothesized that the structural features of the tetrahydrocarboline core would continue to provide selectivity against other nuclear hormone receptor off-targets similar to the exquisitely selective AZD9496.[20] Additionally, the cellular readouts would provide an early indication of permeability with the caveat that compounds of interest could also be tested in a separate biochemical binding assay. Select results are shown in Table 1. Based on our analysis of AZD9496 and the potential liability of the isobutyl alkyl side chain, we designed and synthesized compounds **1** and **2** using the Pictet-Spengler cyclization conditions which mostly afforded the *trans* isomer,

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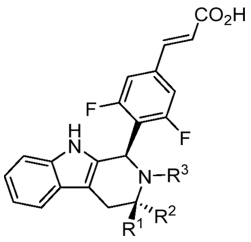
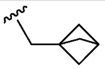
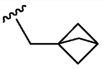
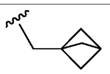
Table 1. Incorporation of BCP Ring to Occupy the Leu525:Leu384 Lipophilic Pocket



Compound	R^1	R^2	R^3	MCF-7[a] IC_{50} (nM)	Log *D*	Cl_{int}[b] (mL/min/kg)	
						mouse	*human*
AZD9496	H	CH_3		0.1	2.8	<38	15.9
1	H	CH_3		ND[c,d]	ND[d]	ND[d]	ND[d]
2[e]	H	CH_3		42	1.0	<38	<8.6
3	H	CH_3		1.0	3.3	111	66.7
4[e]	H	CH_3		0.3	2.4	< 38	<8.6
5	H	CH_2CH_3		11			
6	H	CH_3		23			
7[f]	H	CH_2CH_3		75			
8[g]	CH_3	CH_3		43			

[a]MCF-7 is a breast cancer cell line. [b]Cl_{int} refers to intrinsic microsomal clearance. [c]IC_{50} in LTED assay: 512 nM. [d]ND, not determined. [e]Chirality was confirmed by small molecule X-ray or protein-crystallization. [f]Isolated as mixture of isomers. [g]Isolated as mixture of enantiomers.

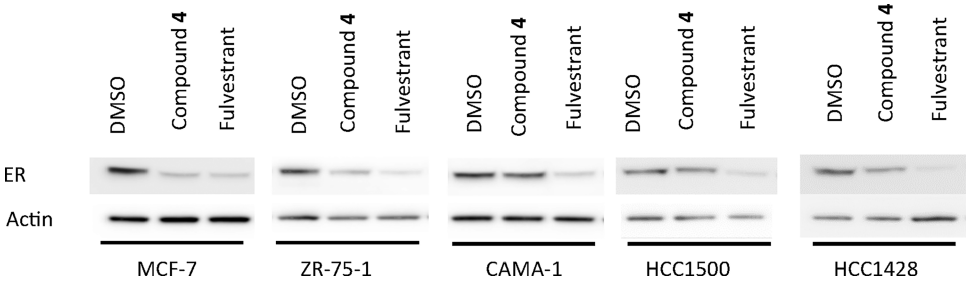


Figure 2. Compound **4** induces ERα degradation in breast cancer cell lines. ERα Western Blot Analysis. Cells were grown in phenol red free DMEM/F12 media supplemented with 8% charcoal-stripped FBS, nonessential amino acids and sodium pyruvate for 48 h before treatment with 100 nM of indicated ligands for 24 h. Immunoblotting performed to determine ERα expression levels using whole cell lysates.

in-line with earlier reports.[20] While compound **2** showed modest activity (MCF-7 IC_{50} = 42 nM), the simpler oxetane exhibited a significant drop in potency (IC_{50} = 512 nM in an orthogonal, long-term estrogen deprived (LTED) cell assay used earlier in our discovery cascade[50]). In parallel we also began exploring the incorporation of the BCP ring as a lipophilic, compact bioisostere which could occupy the lipophilic pocket. We were gratified when one of our first discoveries, compound **3**, showed excellent potency (MCF-7 IC_{50} = 1 nM). Furthermore, removal of the methylene spacer resulted in compound **4**, which was more potent in the cellular assay (MCF-7 IC_{50} = 0.3 nM) and bound with high affinity to ERα and ERβ (IC_{50} = 0.4 nM and 0.8 nM, respectively).[51] The enantiomer of compound **4** was not potent (IC_{50} > 1 μM, data not shown), consistent with modeling and literature.[20] Replacement of the methyl group at R^2 in compound **4** with an ethyl group (compound **5**) resulted in a 37-fold drop in potency (MCF-7 IC_{50} = 11 nM). To further probe the R^3 substituent pocket, we incorporated a sterically bulkier trifluoromethyl-BCP group (compound **6** vs compound **4**). We observed a 77-fold drop in cellular potency vs compound **4**.

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Table 2. Select ADME Properties of Compounds 3 and 4

compound	log D[a]	aqueous solubility[b] (μM)	Caco-2 (AB/BA, 10^{-6} cm s^{-1})	PPB[c] (%)		hERG[d] (μM)
				mouse	*human*	
3	3.3	434	1.9/1.8	99.9	99.9	>30
4	2.4	6237	8.1/5.7	99.7	99.8	>30

[a]Log D was measured in a pH 7.4 buffer. [b]Thermodynamic solubility. [c]Plasma protein binding. [d]Manual patch clamp assay.

Similar to that observed with compound **5**, replacement of the chiral methyl substituent of compound **3** with an ethyl group or with a *gem*-dimethyl substituent (compounds **7** and **8**, both tested as mixtures of isomers) resulted in a significant drop in cellular potency likely reflecting a poorer fit in the LBD of ER and again consistent with literature.[20] We further profiled the most potent compounds (**3** and **4**) in preclinical ADME assays and found that the metabolic stability correlated with lipophilicity (Table 1). Compound **4** showed low intrinsic clearance and well-balanced lipophilicity ($m\mathrm{Cl}_{int}$ < 38 mL/min/kg and $h\mathrm{Cl}_{int}$ < 8.6 mL/min/kg, log$D$ = 2.4) compared to the higher turnover associated with the more lipophilic compound **3** ($m\mathrm{Cl}_{int}$ = 111 mL/min/kg and $h\mathrm{Cl}_{int}$= 86.7 mL/min/kg, log$D$ = 3.3). Significantly, compound **4** exhibited much lower human microsomal clearance compared to **AZD9496** (Table 1, compound **4**: <8.6 mL/min kg vs **AZD9496**: 15.9 mL/min/kg).

In Vitro Pharmacology Profile of Compound 4. Detailed characterization of the ability of compound **4** to induce ER degradation was performed in breast cancer cell lines MCF-7, ZR-75−1, CAMA-1, HCC1500 and HCC1428 using fulvestrant as a reference with DMSO only as the control vehicle (Figure 2). Compound **4** demonstrated significant degradation of ER, comparable to fulvestrant in several cell lines, and warranted further profiling in the context of an in vivo tumor xenograft model. The poorer activity of compound **4** in both CAMA-1 and HCC1550 has been observed with other acidic side chain-containing SERDs such as **AZD9496** and has been attributed to a partial agonist phenotype and/or reduced antiproliferative activity in other cell lines.[52−55] Compound **4** also did not show any agonist or antagonist activity against other nuclear steroid receptors such as androgen receptor (AR), progesterone receptor (PR), mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR).[56] Additional mechanistic studies are described in a related publication by Ma, Samatar and co-workers.[56]

Physicochemical, Pharmacokinetic, Drug Metabolism, and Safety Profiling of Compound 4. The physicochemical properties of compound **4** as reflected in cellular permeability and thermodynamic solubility were excellent (Table 2). Efflux ratio was low (0.7) (Caco-2 AB/BA = 8.1/5.7 × 10^{-6} cm s^{-1}). Compound **3** also showed a relatively low efflux ratio but also much lower P_{app}. Solubility for compound **4** was high (6237 μM). Consistent with the presence of an acrylic acid functionality, both compounds **3** and **4** were highly plasma protein bound (>99.7% in human and mouse plasma, Table 2). The low turnover of compound **4** in mouse and rat microsomal preparations translated to low in vivo IV clearance (mouse: Cl_{obs} = 4.4 mL/min/kg and rat: Cl_{obs} = 1.2 mL/min/kg, dosed at 3 mg/kg) with good $T_{1/2}$ (mouse = 2.9 h and rat = 4.2 h) as shown in Table 3. Upon oral dosing (10 mg/kg) of compound **4** in both mouse and rat, we were gratified to observe the favorable physicochemical properties and metabolic stability were accompanied by excellent exposure and high bioavailability in both mouse (AUC = 65 μM·h, F%74 vs AUC = 30 μM·h, F%87 for **AZD9496**) and rat (AUC = 283 μM·h, F%88 vs AUC = 179 μM·h, F%74 for **AZD9496**). Significantly, the compound was rapidly absorbed (mouse T_{max} = 0.5 h and rat T_{max} = 1 h) and showed a high C_{max} (mouse = 10.8 μM and rat = 33 μM; see Figure 3a,b, respectively, for time-course versus concentration curves). Early in vitro safety profiling was performed to further support development. Compound **4** was tested in human liver microsomes and showed minimal inhibition in a panel of cytochrome P450 enzymes (IC_{50} ranged from 24 to 50 μM with the exception of 2C8 (IC_{50} = 0.158 μM[57]), which **AZD9496** also significantly inhibited.[32] Finally, compound **4** did not inhibit the *h*ERG channel (IC_{50} > 30 μM).

Table 3. Mouse and Rat PK Parameters. Formulation: DMSO/PEG400/150 mM Glycine (pH 9) for IV (5/10/85) and PEG400/PVP/Tween 80/0.5% CMC in Water for PO (9/0.5/0.5/90)

	mouse		rat	
	intravenous	PO (oral)	intravenous	PO (oral)
dose (mg/kg)	3	10	3	10
AUC (μM·h)	26	65	96	283
Cl_{obs} (mL/min/kg)	4.4		1.2	
C_{max} (μM)		10.8		33
$T_{1/2}$ (h)	2.9		4.2	
$V_{d,ss}$ (L/kg)	1.2		0.48	
F(%) bioavailability		74		88

X-ray Co-Crystal Structure of Compound 4 Complexed with ERα LBD. To further confirm the molecular details of ligand binding, the cocrystal structure of compound **4** with the estrogen receptor LBD was determined to 3.2 Å. Compound **4** occupies the estradiol hormone binding site located toward one end of the ligand binding domain comprised of the N-terminal half of helix-3, the C-terminal half of helix-5, N-terminal end of helix 7, and end of helix 11, with the bicyclo[1.1.1]pentane (BCP) substituted tetrahydrocarboline overlaying nicely with the estradiol steroid core (Figure 4). The indole of compound **4** occupies the same region as the estradiol benzoid ring, and the BCP moiety occupies the same space as the cyclopentyl portion of estradiol.[58] As in **AZD9496**,[20] the difluorophenyl acrylic acid moiety exits the binding pocket between helices 3 and 11 projecting toward solvent and the disordered helix 11/12 connecting loop, and participates in the previously described unusual acid−acid interaction with the helix 3 Asp351 side chain essential to confer a down regulator-antagonist ligand profile (Figure 5a).[20] The crystallography protein construct harbors the previously described L536S engineered mutation in the helix 11/12 interconnecting loop that biases the loop and helix 12 toward an antagonist conformation.[59]

Summary of Key Protein−Ligand Interactions. Several key interactions are evident from the crystal structure of compound **4** in complex with the ligand binding domain of the estrogen receptor α (ERα). Though moderate in resolution, ligand electron density in the compound **4** cocrystal structure is consistent with the assignment as a *trans* (*1R,3R*) stereoisomer

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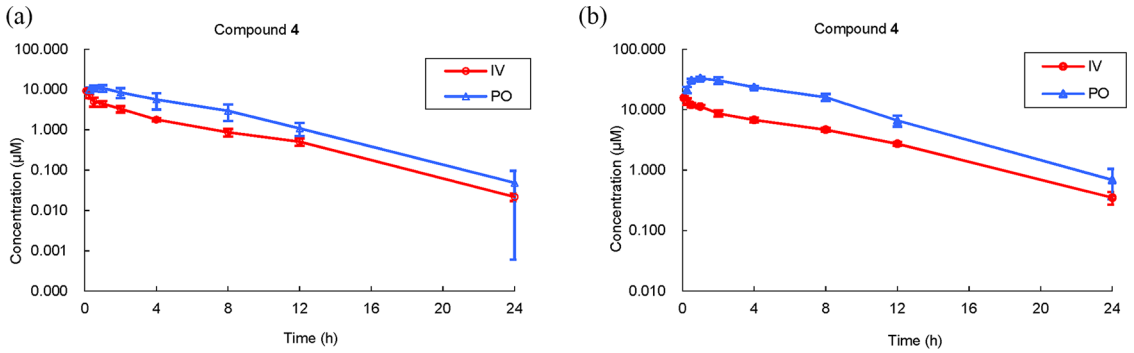

Figure 3. (a) Mouse PK profile of compound **4** (IV: 3 mg/kg, PO: 10 mg/kg). (b) Rat PK profile of compound **4** (IV: 3 mg/kg, PO: 10 mg/kg).

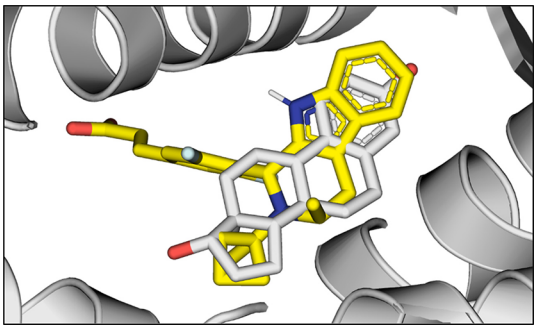

Figure 4. Superposition of compound **4** from the compound **4**/ERαLBD cocrystal structure (PDB code 9ECK) with estradiol (PDB 1A52). Ligands are depicted as sticks (compound **4**, yellow; estradiol, light gray). Rendering performed with PyMol.[60]

(Figure 5b). Similar to **AZD9496**, the tetrahydrocarboline core participates in a single hydrogen bond interaction within the hydrophobic binding site by donating a hydrogen from the indole NH to the backbone carbonyl oxygen of Leu-346 (helix-3; 2.2 Å as measured for monomer D) and the indole ring participates in an edge to face T-shaped $\pi-\pi$ interaction with Phe-404 of the strand of the β-turn. The core chiral methyl group is retained in compound **4** to maintain the aforementioned potency boost by occupying the 'Phe-404:Phe-425 lipophilic hole' that is occupied by the ethyl group of hydroxytamoxifen[20] (Figure 5a). The BCP moiety of compound **4** makes slightly increased van der Waals (VDW) interactions within the Leu525:Leu384 lipophilic hole at one end of the binding site occupied by the cognate 2-fluoro-2-methyl propyl substituent of **AZD9496**. The BCP and $-CH_2(CH_3)_2F$ moieties occupy 76.5 and 52.1 Å^3 of the lipophilic pocket, respectively, in the context of their cocrystal structures (BCP volume occupancy calculated from monomer D). Residues defining this region include Met343, Leu384, Met421, Ile424, Gly521, and Leu525.

In Vivo Efficacy Studies of Compound 4. The advanced lead compound **4** was evaluated in several xenograft mouse models (CDX and PDX models) to first understand how the cellular potency and mouse exposure would translate into an in vivo setting relevant for the clinic and second, to set the stage for follow up combination studies. Compound **4** was profiled in a MCF-7 mouse xenograft model (Figure 6a). Day 0 dosing was done in BALB/c nude mice when tumor volumes reached around 200 mm^3. Estradiol benzoate pellets were inserted at the beginning of the study to maintain the tumor growth driven by E2. Mice were dosed for 28 days with either vehicle (daily, p.o.), fulvestrant (3 mg/dose qw × 5, s.c.) or compound **4** (dose titration was done with parallel arms at 1, 5, and 40 mg/kg, p.o.). Even at 1 mg/kg, compound **4** (MCF-7 cell proliferation inhibition IC_{50} = 0.3 nM) showed significant tumor growth inhibition. Dosing at 5 mg/kg resulted in complete tumor growth inhibition. By comparison, fulvestrant at 3 mg/dose, qw × 5 gave only 20% tumor growth inhibition. In the WHIM20 xenograft model (Figure 6b), an *ESR1* mutant model (Y537S) without concomitant estrogen supplementation, fulvestrant (200 mg/kg) was compared to a 40 mg/kg dose of compound **4** over 90 days of once-a-day dosing. Compound **4** significantly induced tumor growth inhibition compared to vehicle or fulvestrant and was well-tolerated with no loss in body weight. Additional in vivo pharmacology studies with compound **4** are described in more detail in a related publication.[56]

Clinical Candidate Selection. BCP incorporation into the tetrahydrocarboline scaffold afforded active compounds that significantly inhibited tumor growth. Lead compound **4**, with the best drug-like properties, demonstrated potency in several cell lines, with significant degradation of ER. Furthermore, compound **4** exhibited excellent exposure and tumor growth inhibition in both endocrine sensitive and mutant ESR mouse xenograft models. The absence of *h*ERG signals, no observed in vitro cardiotoxicity, manageable CYP450 inhibition, clean profile in the Cerep panel as well as negative results for Ames and micronucleus tests further underscored the favorable safety profile of our lead candidate. Finally, the consistently high bioavailability across multiple preclinical species supported advancement into the clinic. As a point of reference, Figure 7 shows the time course vs concentration curve upon a single dose administration of compound **4** to dogs. Compound **4** shows very low clearance (0.18 mL/min/kg) and high oral bioavailability ($AUC_{0-24\,h}$ = 2004 μM·h, F%88) (Table 4) with a half-life of 21 h in the IV arm. Taken together with the low predicted human clearance based on liver microsome/hepatocyte studies, and a good safety profile in 28-day GLP toxicity studies, we nominated compound **4** for clinical development.

Human Pharmacokinetics. A Phase 1/2, open label, multicenter trial (NCT03560531) was conducted with compound **4** (later also named ZN-c5) in patients with ER-positive/HER2-negative advanced or metastatic breast cancer. The goal was to assess safety, tolerability, PK, PD, and antitumor activity as a monotherapy as well as in combination with the CDK4/6 inhibitor, palbociclib. The doses began at 50 mg/day and escalated higher using a 28-day cycle.[51,56] The exposures on Day 1 and Day 15 were 65.7 and 61.3 (μg*h/mL), respectively. Exposures even at this lowest dose exceeded the EC_{100}, the dose (10 mg/kg) which resulted in 100% TGI in the MCF-7-derived xenograft model. The estimated mean elimination half-life

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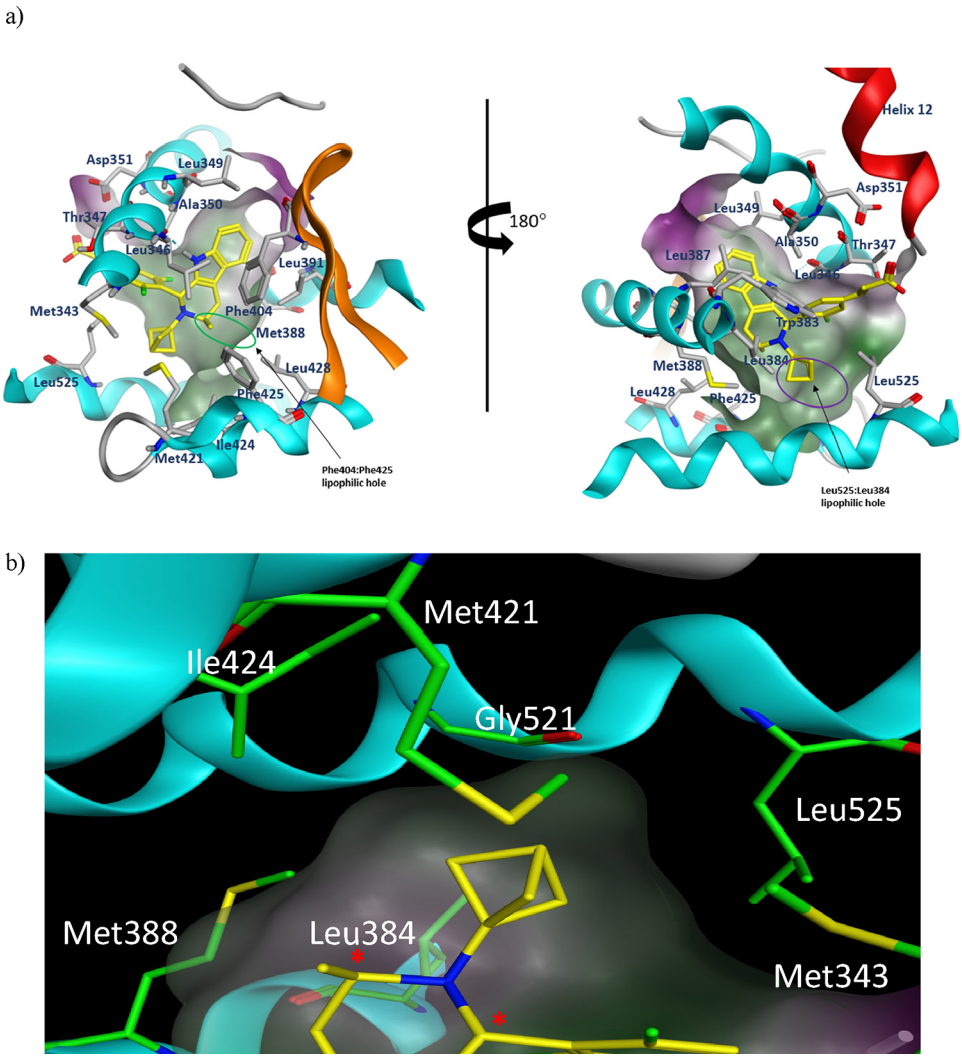

Figure 5. (a) Co-crystal structure of compound **4** complexed with ERα ligand binding domain. Two views of compound **4** bound to ERα ligand binding domain separated by 180° (PDB code 9ECK). The Phe404:Phe425 and Leu525:Leu384 lipophilic holes occupied by the chiral methyl and BCP are highlighted by green and purple ovals, respectively. The molecular surface of the pocket is colored according to its lipophilicity (green: hydrophobic, pink: hydrophilic). Rendering performed with MOE.[61] (b) Close-up view of the compound **4** BCP moiety (PDB code 9ECK) and fit into the Leu525:Leu384 lipophilic hole and other residues within 5 Å that define the subpocket. The 2 chiral centers of the tetrahydropyrido portion of the core are denoted with red asterisks. Rendering performed with MOE.[61]

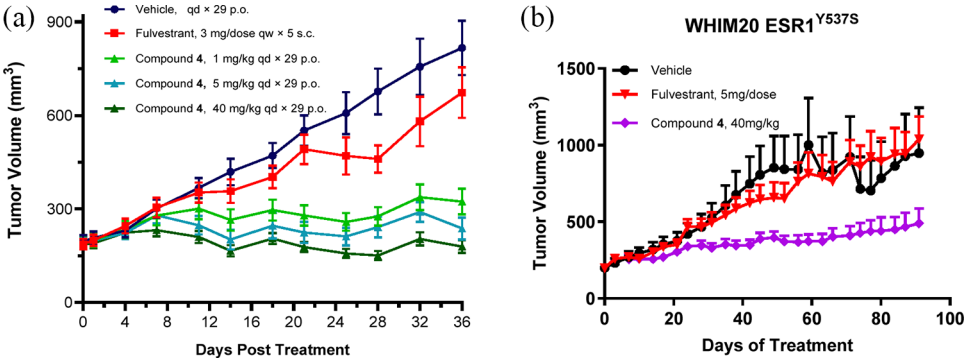

Figure 6. (a) In vivo efficacy of compound **4** in the MCF-7 CDX model. The BALB/c nude mice bearing MCF-7 tumor cells were dosed orally once a day (qd) with compound **4** for 28 days. Fulvestrant was dosed subcutaneously once a week (qw). All the animals in the study received estradiol benzoate injection subcutaneously twice a week. (b) In vivo efficacy of compound **4** in the WHIM20 ESR1^{Y537S} model. The athymic nude mice bearing WHIM20 PDX tumors were orally dosed once a day (qd) with compound **4** (40 mg/kg, qd) for 90 days. Fulvestrant was dosed subcutaneously once a week (qw). In both studies, Compound **4** was formulated in the following vehicle: 20% (2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD) at the proper concentration to dose as indicated. Fulvestrant was formulated in 5% DMSO/95% Kolliphor RH40.

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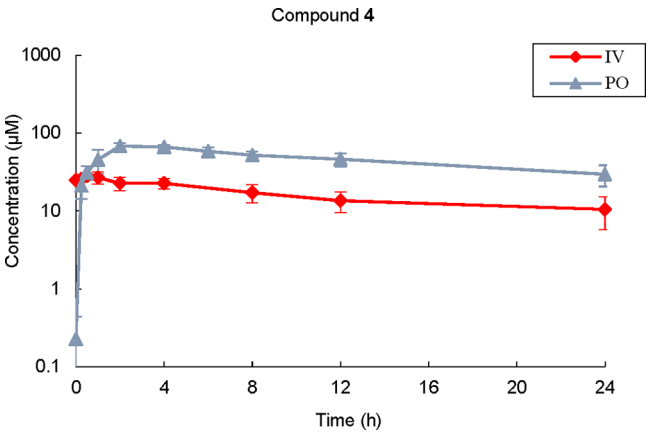


Figure 7. Dog PK profile of compound **4**.

Table 4. Dog PK Parameters of Compound 4[a]

	intravenous	PO (oral)
dose (mg/kg)	3	10
AUC (μM·h)	732	2004
Cl_{obs} (mL/min/kg)	0.18	
C_{max} (μM)		68
$T_{1/2}$ (h)	21	
$V_{d,ss}$ (L/kg)	0.33	
F (%) bioavailability		88

[a]Compound **4** was formulated in PEG400/PVP/Tween 80/0.5% CMC in water (9/0.5/0.5/90) for oral dosing and in EtOH/PEG400/30%HPβ-CD (10/40/50) for IV dosing. AUC refers to $AUC_{0-24\ h}$.

ranged from 11 to 18 h, which supported once a day dosing. Additional data is reported in a related publication by Ma, Samatar and co-workers.[56]

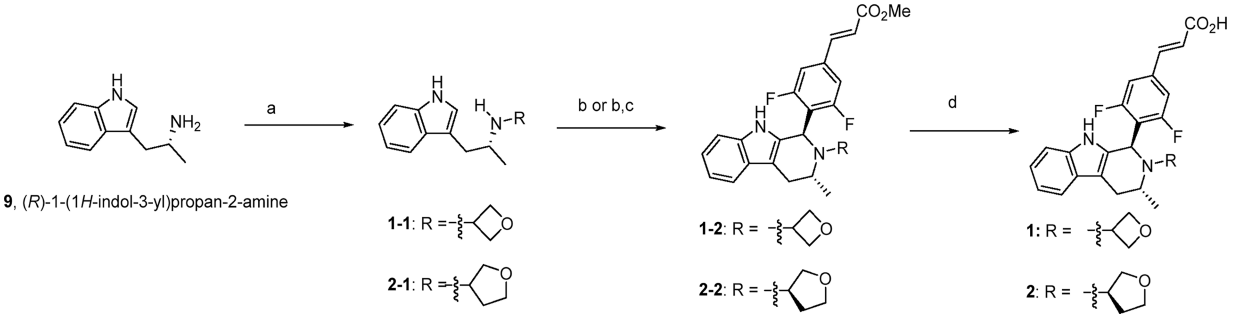
CHEMISTRY

Compounds **1** and **2** were prepared according to Scheme 1. Key starting materials were either commercially available or synthesized.[62] Compound **9** was treated with either oxetan-3-one or 3-dihydrofuran-3(2H)-one and sodium borohydride in methanol to afford amines **1** and **2** (as a mixture of isomers). The amines were then treated with methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate) and acetic acid in hot toluene (Pictet–Spengler cyclization) to afford mostly the *trans* isomeric esters **1–2** and **2–2**, similar to observations reported in the literature.[20] Ester hydrolysis under standard conditions using NaOH (aqueous) in MeOH mixtures, gave compounds **1** and **2** (56% yield for both analogs). Purification by chiral, supercritical fluid chromatography (SFC) was used to separate the diastereomers of compound **2**. The absolute configuration of compound **2** was verified by single crystal X-ray crystallography. Compounds **4** through **6** were prepared according to Scheme 2. Ketones **10** and **11** were similarly subjected to reductive amination conditions to afford the corresponding amines, **4–1**, **5–1** and **6–1**. The racemates were typically progressed (without chiral separation unless otherwise indicated) to the subsequent Pictet-Spengler cyclization followed by chiral separation where needed. Similar hydrolysis as in Scheme 1 afforded compounds **4–6** in 26–55% yield. For the methylene homologated *N*-substituted tetrahydrocarbolines shown in Scheme 3, the synthesis entailed reduction of intermediate amides prior to cyclization. The amines **9**, **12** and **13** were treated with either T3P or HATU and base in DCM or THF along with bicyclo[1.1.1]pentane-1-carboxylic acid to afford products **3–1**, **7–1** and **8–1** in 70–90% yield. Treatment of the amides with lithium aluminum hydride in refluxing THF afforded the corresponding amines **3–2**, **7–2** and **8–2**. Subsequent Pictet-Spengler cyclization afforded the tetrahydrocarboline esters as mixtures. In the case of **7–3**, an unexpected opening of the cyclopropyl ring afforded an ethyl substituted product as confirmed by ^{1}H NMR (data not shown). The esters were hydrolyzed to the carboxylic acids **3**, **7** (mixture of isomers) and **8** (racemic), of which **3** was purified by SFC chiral separation to afford the desired *trans* isomer. Crude products **7** and **8** were purified by rpHPLC to afford final compounds as a mixture of diastereomers or enantiomers, respectively.

CONCLUSIONS

The field of orally bioavailable selective estrogen receptor degraders has expanded greatly over the last 10 years. At the time of initiating our work, the acrylic acid containing clinical compounds suffered from poor human pharmacokinetics despite promising preclinical pharmacokinetics and efficacy. After analyzing the contemporary competitor landscape, we hypothesized that incorporation of the novel BCP ring system could improve the overall profile of SERDs through well-described improved physical properties and enhanced in vitro metabolic stability. This strategy led to a series of BCP-containing SERDs which were potent (including compounds **3** and **4**, <1 nM in MCF-7 assay). Optimization of key

Scheme 1. Synthesis of Compounds 1, 2[a]



[a]Reagents and conditions: (a) **9**, AcOH, sodium borohydride, MeOH, oxetan-3-one or 3-dihydrofuran-3(2H)-one, 28–40% yield; (b) methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate, acetic acid, toluene, 90 °C, 22–32% yield; (c) SFC chiral separation; (d) NaOH (aq) /MeOH, 56% yield.

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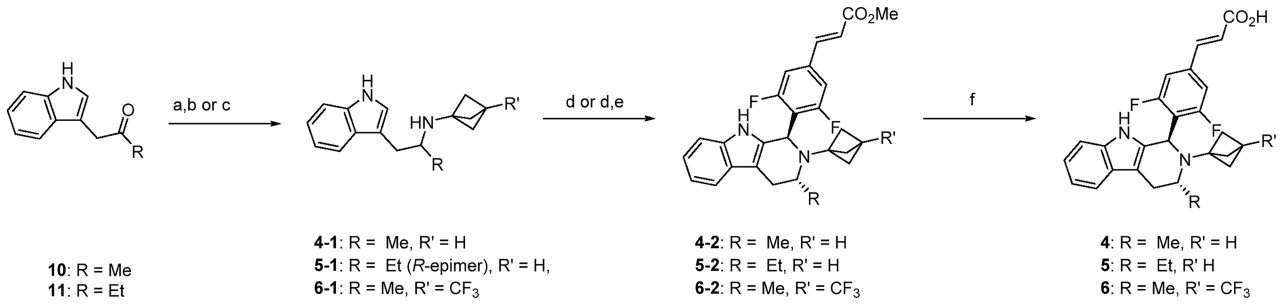
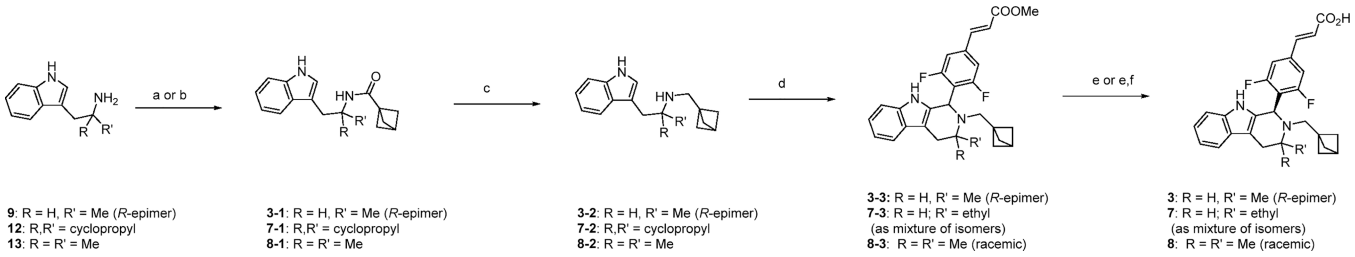
Scheme 2. Synthesis of Compounds 4−6[a]



[a]Reagents and conditions: (a) **10**, AcOH, $NaBH_3CN$, MeOH, bicyclo[1.1.1]pentan-1-amine, 58% yield; (b) **11**, AcOH, $NaBH_3CN$, MeOH, bicyclo[1.1.1]pentan-1-amine, then SFC chiral separation, 33% yield; (c) **10**, AcOH, $NaBH_3CN$, MeOH, 3-(trifluoromethyl)bicyclo[1.1.1]pentan-1-amine, 47% yield; (d) methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate, acetic acid, toluene, 90 °C, 9−41% yield; (e) SFC chiral separation; (f) NaOH or LiOH, THF/MeOH, 26−55% yield.

Scheme 3. Synthesis of Compounds 3, 7, 8[a]



[a]Reagents and conditions: (a) **9** or **12**, T3P, DIPEA, THF and bicyclo[1.1.1]pentane-1-carboxylic acid, 70−90% yield; (b) **13**, HATU, DIPEA, DCM and bicyclo[1.1.1]pentane-1-carboxylic acid, 77% yield; (c) LAH, THF, reflux, 41−91% yield; (d) methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate, acetic acid, toluene, 90 °C, 19−49% yield; (e) NaOH or LiOH, THF/MeOH, 31−41% yield; (f) SFC chiral separation.

physicochemical and ADME properties resulted in the clinical candidate compound **4** (now known as ZN-c5), one of the first clinical candidates containing a BCP moiety. Compound **4** has excellent in vivo exposure (mouse AUC = 65 μM·h, rat AUC = 283 μM·h and dog AUC = 2004 μM·h, resulting from a single dose at 10 mg/kg). More importantly, the human exposure of ZN-c5 (50 mg qd, AUC = 61.3 (μg·h/mL)) was high, supporting our initial hypothesis-driven design to utilize a BCP ring to mitigate metabolism. This resulted in a compound with higher oral exposure than many of the early clinical candidates at much lower doses[63−65] and represents significant advancement of our understanding of the potential of the BCP ring in an appropriate design space.

EXPERIMENTAL SECTION

Starting materials and other reagents were purchased from commercial suppliers and were used without further purification unless otherwise indicated. All the reactions were performed under a positive pressure of nitrogen or argon or with a drying tube, at ambient temperature (unless otherwise stated), in an anhydrous solvent, unless otherwise indicated. Analytical thin-layer chromatography (TLC) was performed on glass-backed silica gel and eluted with the appropriate solvent ratios (v/v). The reactions were assayed by high-performance liquid chromatography (HPLC), LC-MS or TLC and terminated as judged by the consumption of the starting material. The TLC plates were visualized by UV, phosphomolybdic acid stain, or iodine stain. Microwave-assisted reactions were run in a Biotage Initiator. ^{1}H NMR spectra were recorded on a Bruker instrument operating at 300 or 400 MHz. ^{1}H NMR spectra were obtained as DMSO-d_6 or $CDCl_3$ solutions as indicated (reported in parts per million, ppm), with chloroform as the reference standard (7.25 ppm) or DMSO (2.50 ppm). Other NMR solvents were used as needed. When peak multiplicities are reported, the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broadened, dd = doublet of doublets, and dt = doublet of triplets. Coupling constants, when given, are reported in hertz. The relative stereochemistry was confirmed by 2D-NMR. Mass spectra were obtained via liquid chromatography−mass spectrometry (LC−MS) on an Agilent instrument with atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). Reverse phase HPLC was used to assess purity. All key compounds showed >95% purity by HPLC except **5** (93.7% purity). Compounds **7** and **8** were purified to afford mixtures of isomers which were not further separated. All compounds tested in vivo were at least 95% pure. All the procedures related to animal handling, care, and the treatment in this study were performed according to guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Pharmaron, CrownBio or Horizon Biosciences.

(*R*)-*N*-(1-(1*H*-Indol-3-yl)propan-2-yl)oxetan-3-amine (1−1). To a stirred solution of (*R*)-1-(1*H*-indol-3-yl)propan-2-amine **9** (0.8 g, 4.60 mmol) in ethanol (3 mL) was added oxetane-3-one (0.397 g, 5.52 mmol) and the reaction mixture was stirred at 50 °C for 3 h. The resulting reaction mixture was cooled to 0 °C, treated with sodium borohydride (0.262 g, 6.90 mmol) and stirred for 5 h at rt. The mixture was diluted with ethyl acetate (10 mL) and washed with saturated aqueous NH_4Cl solution (10 mL). The combined organic layer was dried over sodium sulfate and concentrated. The crude residue was purified by column chromatography (SiO_2, 2−3% methanol in DCM) to afford compound **1−1** (0.3 g, 28% yield) as a colorless, gummy liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (br s 1H), 7.58 (d, J = 8 Hz, 1H), 7.38 (d, J = 8 Hz, 1H), 7.21 (t, J = 6.8 Hz, 1H), 7.12 (t, J = 6.8 Hz, 1H), 7.07 (d, J = 2 Hz, 1H), 4.82−4.77 (m, 1H), 4.71 (d, J = 6.8 Hz, 1H), 4.40 (t, J = 6.4 Hz, 1H), 4.23 (t, J = 6.4 Hz, 1H), 4.13−4.06 (m, 1H), 3.06−3.01 (m, 1H), 2.83 (t, J = 6.4 Hz, 2H), 1.09 (d, J = 6.4 Hz, 3H); MS (ESI) m/z 231.17 $[M + H]^+$.

Methyl (*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-3-methyl-2-(oxetan-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)-acrylate (1−2). To a stirred solution of (*R*)-*N*-(1-(1*H*-indol-3-yl)propan-2-yl)oxetan-3-amine **1−1** (0.300 g, 1.30 mmol) in toluene (3

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mL) was added methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate (0.353 g, 1.56 mmol) and acetic acid (0.156 g, 2.61 mmol). The resulting mixture was stirred at 90 °C for 5 h. The reaction mixture was diluted with EtOAc and washed with water. The combined organic layer was dried over sodium sulfate and concentrated. The residue was purified by reverse phase prep-HPLC to afford compound **1−2** (180 mg, 32% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.6 (br s 1H), 7.63 (d, J = 16.5 Hz, 1H), 7.52 (d, J = 10.8 Hz, 2H), 7.40 (d, J = 7.2 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.03−6.92 (m, 2H), 6.81 (d, J = 15.9 Hz, 1H), 5.13 (s 1H), 4.63 (t, J = 6.3 Hz, 1H), 4.51 (t, J = 6.9 Hz, 1H), 4.20 (t, J = 7.2, 1H), 3.97−3.89 (m, 2H), 3.73 (s, 3H), 3.31 (s, 1H), 2.93 (dd, J = 15 Hz, 4.5 Hz, 1H), 2.57 (d, J = 4.5 Hz, 1H), 0.99 (d, J = 6.6 Hz, 3H); MS (ESI) m/z 439.24 [M + H]$^+$.

(*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-3-methyl-2-(oxetan-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylic Acid (1). To a stirred solution of **1−2** (130 mg, 0.296 mmol) in methanol (1.5 mL) was added an aqueous solution of NaOH (35 mg, 0.888 mmol, 7.5 M) at 0 °C. The mixture was stirred at rt for 5 h. The reaction mixture was concentrated under reduced pressure to remove methanol. The obtained residue was acidified with 1 N hydrogen chloride solution at 0 °C and then diluted with EtOAc and washed with water. The combined organic layer was dried over sodium sulfate, filtered and concentrated. The residue was triturated with *n*-pentane to afford compound **1** (70 mg, 56% yield) as an off white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.6 (br s, 1H), 10.6 (br s, 1H), 7.57−7.39 (m, 4H), 7.19 (d, J = 7.2 Hz, 1H), 7.02−6.93 (m, J = 7.2 Hz, 2H), 6.68 (d, J = 16 Hz, 1H), 5.12 (s, 1H), 4.63 (br s, 1H), 4.52 (br s, 1H), 4.20 (br s, 1H), 3.93 (br s, 2H), 2.93 (d, J = 15.2 Hz, 1H), 2.56 (br s, 2H), 1.00 (br s, 3H); MS (ESI) m/z 425.29 [M + H]$^+$.

***N*-((*R*)-1-(1*H*-Indol-3-yl)propan-2-yl)tetrahydrofuran-3-amine (2−1).** To a stirred solution of compound **9** (0.8 g, 4.60 mmol) in ethanol (3 mL) was added dihydrofuran-3(2*H*) one (0.474 g, 5.52 mmol) and acetic acid (0.275 g, 4.60 mmol) at rt and stirred for 3 h. The resulting reaction mixture was cooled to 0 °C and treated with sodium borohydride (0.262 g, 6.90 mmol) and then stirred for 5 h at rt. The mixture was diluted with ethyl acetate (10 mL) and washed with saturated aqueous NH_4Cl solution (10 mL). The combined organic layer was dried over sodium sulfate and concentrated. The obtained residue was purified by column chromatography (SiO_2, 2−3% MeOH/DCM) to afford **2−1** (0.450 g, 40% yield) as a colorless gummy liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (br s 1H), 7.67−7.55 (m, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.20 (t, J = 7.2 Hz, 1H), 7.16−7.07 (m, 2H), 3.96−3.88 (m, 1H), 3.86−3.52 (m, 3H), 3.28−3.08 (m, 3H), 2.99−2.88 (m, 1H), 2.32−2.17 (m, 1H), 2.17−1.97 (m, 1H), 1.27 (d, J = 6.9 Hz, 3H); MS (ESI) m/z 245.21 [M + H]$^+$.

Methyl (*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-3-methyl-2-((*R*)-tetrahydrofuran-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylate (2−2). To a stirred solution of **2−1** (0.450 g, 1.84 mmol) in toluene (3 mL) was added methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate (0.501 g, 2.21 mmol) and acetic acid (0.221 g, 3.68 mmol). The resulting mixture was stirred at 90 °C for 5 h. The reaction mixture was diluted with EtOAc, washed with water, dried over sodium sulfate, filtered and concentrated. The residue was purified by chiral SFC to afford **2−2** (180 mg, 22% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.6 (br s, 1H), 7.62 (d, J = 15.9 Hz, 1H), 7.50 (d, J = 10.5 Hz, 2H), 7.39 (d, J = 7.5 Hz, 1H), 7.18 (d, J = 8.1 Hz, 1H), 7.03−6.92 (m, 2H), 6.79 (d, J = 15.9 Hz, 1H), 5.32 (s, 1H), 3.85−3.68 (m, 4H), 3.59−3.48 (m, 3H), 3.25 (t, J = 7.5 Hz, 1H), 3.08 (t, J = 7.5 Hz, 1H), 2.89 (dd, J = 15 Hz, 4.2 Hz, 1H) 2.55−2.49 (m, 1H), 2.24−1.96 (m, 2H), 1.14 (d, J = 6.6 Hz, 3H); MS (ESI) m/z 453.25 [M + H]$^+$. Compound **2−2** elutes as peak 1 and the undesired isomer elutes as peak 2.

(*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-3-methyl-2-((*R*)-tetrahydrofuran-3-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylic Acid (2). To a stirred solution of **2−2** (0.180 g, 0.398 mmol) in methanol (2 mL) was added an aqueous solution of NaOH (47 mg 1.19 mmol, 7.5 M) at 0 °C and stirred at rt for 5 h. The reaction mixture was concentrated under reduced pressure to remove solvent. The obtained residue was acidified with 1 N hydrogen chloride solution (aqueous) at 0 °C and extracted with ethyl acetate (3 × 50 mL). The combined organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford the crude product. The residue was purified by chiral SFC to afford **2** (80 mg, 46% yield) as an off white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.7 (br s, 1H), 10.6 (s, 1H), 7.58−7.32 (m, 4H), 7.18 (d, J = 8.1 Hz, 1H), 7.02−6.91 (m, 2H), 6.66 (d, J = 15.6 Hz, 1H), 5.31 (s, 1H), 3.88−3.75 (m, 1H), 3.68−3.48 (m, 3H), 3.31−3.22 (m, 1H), 3.08 (t, J = 8.1 Hz, 1H), 2.89 (dd, J = 15 Hz, 3.6 Hz, 1H), 2.54−2.51 (m, 1H), 2.14−1.96 (m, 2H), 1.14 (d, J = 6.3 Hz, 3H); MS (ESI): m/z 439.26 [M + H]$^+$; $[\alpha]_D$ = −70.4° (c 0.25, $CHCl_3$, 24 °C). Single crystal X-ray crystallography was used to assign absolute stereochemistry.

(*R*)-*N*-(1-(1*H*-Indol-3-yl)propan-2-yl)bicyclo[1.1.1]pentane-1-carboxamide (3−1). To a stirred solution of (*R*)-1-(1*H*-indol-3-yl)propan-2-amine **9** (0.500 g, 1.14 mmol) in THF (10 mL) was added propylphosphonic anhydride (2.25 mL, 50 wt %/wt in EtOAc) and DIPEA (1.5 mL), at rt. The solution was stirred for 10 min, and then a solution of bicyclo[1.1.1]pentane-1-carboxylic acid (0.375 g, 3.3 mmol) was added. The mixture was stirred at rt for an additional 16 h. Water (3 mL) was added and the reaction mixture was extracted with EtOAc (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was triturated with ether to obtain **3−1** (700 mg, 90% yield) as a pale yellow solid. MS (ESI) m/z 269.26 [M + H]$^+$.

(*R*)-*N*-(Bicyclo[1.1.1]pentan-1-ylmethyl)-1-(1*H*-indol-3-yl)-propan-2-amine (3−2). To a stirred solution of **3−1** (700 mg, 2.60 mmol) in THF (10 mL) was added LAH (600 mg, 15.8 mmol) in portions at 0 °C. The mixture was heated to reflux for 16 h, cooled to rt and then treated with ice-cold water (50 mL) slowly, and extracted with EtOAc (2 × 50 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was triturated with ether to obtain crude **3−2** (600 mg, 90% yield) as a pale yellow solid. MS (ESI) m/z 255.36 [M + H]$^+$.

Methyl (*E*)-3-(4-((1*R*,3*R*)-2-(Bicyclo[1.1.1]pentan-1-ylmethyl)-3-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylate (3−3). To a stirred solution of crude **3−2** (600 mg, 2.35 mmol) in toluene (12 mL), methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate (540 mg, 2.4 mmol) and acetic acid (285 mg, 4.75 mmol) were added and the mixture was stirred at 90 °C for 12 h. The reaction mixture was neutralized with a saturated, aqueous solution of sodium bicarbonate and extracted with EtOAc (2 × 50 mL). The combined organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford crude **3−3** (400 mg, 36% yield) as a mixture of *cis/trans* isomers. MS (ESI) m/z 463.38 [M + H]$^+$.

(*E*)-3-(4-((1*R*,3*R*)-2-(Bicyclo[1.1.1]pentan-1-ylmethyl)-3-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylic Acid (3). To a stirred solution of **3−3** (400 mg of a mixture of *cis/trans* isomers, 0.86 mmol) in a mixture of THF/MeOH (6 mL, 5:1 v/v) was added aqueous NaOH solution (0.5 mL, 1.5 M) at 0 °C and the mixture was stirred at rt for 16 h. Standard aqueous workup and SFC purification afforded **3** (140 mg, 37% yield) as pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (br s 1H), 10.50 (s, 1H), 7.55 (d, J = 16.0 Hz, 1H), 7.46 (d, J = 8 Hz, 2H), 7.39 (d, J = 8 Hz, 1H), 7.16 (d, J = 8 Hz, 1H), 6.99 (dd, J = 7.6 Hz, 6.8 Hz, 1H), 6.94 (dd, J = 7.6 Hz, 7.2 Hz, 1H), 6.67 (d, J = 16 Hz, 1H), 5.08 (s, 1H), 3.45−3.56 (m, 1H), 2.94 (dd, J = 12.8 Hz, 4 Hz, 1H), 2.68 (d, J = 14 Hz, 1H), 2.56 (dd, J = 12 Hz, 0.9 Hz, 1H), 2.37 (s, 1H), 2.24 (d, J = 14.8 Hz, 1H), 1.56 (d, J = 8.8 Hz, 3H), 1.46 (d, J = 9.2 Hz, 3H), 1.00 (d, J = 6.4 Hz, 3H); MS (ESI) m/z 449.39 [M + H]$^+$, $[\alpha]_D$ + 65.6° (c 0.25, MeOH, 24 °C).

***N*-(1-(1*H*-Indol-3-yl)propan-2-yl)bicyclo[1.1.1]pentan-1-amine (4−1).** To a solution of 1-(1*H*-indol-3-yl)propan-2-one **10** (0.062 g, 0.51 mmol) in MeOH (1 mL) was added glacial acetic acid to adjust the pH to 5−6. To this solution was added bicyclo[1.1.1]pentan-1-amine as the hydrochloride salt (0.075 g, 0.43 mmol) followed by sodium cyanoborohydride (0.054 g, 0.86 mmol). The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 16 h. The solvent was removed under reduced pressure, water (5 mL) was added and the mixture was extracted with ethyl acetate (2 × 5 mL). The combined organic layer was washed with saturated sodium bicarbonate solution, brine, dried over sodium sulfate, filtered and concentrated

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under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2, 3.5% MeOH/DCM) to afford **4−1** (0.060 g, 58% yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.22 (td, J = 8.0, 1.2 Hz, 1H), 7.14 (td, J = 8.0, 1.2 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 3.21−3.15 (m, 1H), 2.92−2.87 (m, 1H), 2.79−2.74 (m, 1H), 2.36 (s, 1H), 1.86−1.78 (m, 6H), 1.13 (d, J = 6.4 Hz, 3H); MS (ESI) m/z: 241.26 $[M + H]^+$.

Methyl (*E*)-3-(4-((1*R*,3*R*)-2-(Bicyclo[1.1.1]pentan-1-yl)-3-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylate (4−2). To a solution of **4−1** (1.3 g, 5.41 mmol) in toluene (15 mL) were added methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate (1.2 g, 4.87 mmol) and acetic acid (0.7 mL, 10.8 mmol). The resulting solution was stirred at 80 °C for 2 h. The reaction mixture was cooled to room temperature and poured into a solution of potassium carbonate (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layer was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 40% EtOAc/Hexanes) to provide 1.0 g of a racemic mixture of *trans* isomers. The isomers were separated by chiral SFC [Chiralpak AD-H, (250 × 21 mm), 70 mL per min, Liquid CO_2/IPA] to afford **4−2** which elutes as peak 1 (0.381 g, 17% yield) in >99% ee with a retention time of 6.41 min. ^{1}H NMR(400 MHz, DMSO) δ 10.5 (s, 1H), 7.6 (d, J = 16.0 Hz, 1H), 7.5 (d, J = 10.0 Hz, 2H), 7.4 (d, J = 7.6 Hz, 1H), 7.2 (d, J = 7.6 Hz, 1H), 7.0 (m, 2H), 6.8 (d, J = 16.0 Hz, 1H), 5.3 (s, 1H), 3.7 (s, 3H), 3.6 (m, 1H), 2.9 (m, 1H), 2.6 (m, 1H), 2.3 (s, 1H), 1.7 (d, J = 8.0 Hz, 3H), 1.6 (d, J = 1.2 Hz, 3H), 1.2 (d, J = 6.4 Hz, 3H); LC-MS m/z: 449.6 [M + H]. The undesired enantiomer elutes as Peak 2 (9.59 min).

(*E*)-3-(4-((1*R*,3*R*)-2-(Bicyclo[1.1.1]pentan-1-yl)-3-methyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylic Acid (4). To a solution of **4−2** (0.381 g, 0.850 mmol) in THF (4 mL) and MeOH (2 mL) was added an aqueous sodium hydroxide solution (1.2 mL, 7.5 M). The solution was stirred at room temperature for 4 h. Water (10 mL) was added to the reaction mixture and the pH of aqueous solution was adjusted to 5 by addition of 2 N HCl solution. The solution was extracted with diethyl ether (3 × 50 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by reverse phase prep HPLC [PURITAS PREP C_{18} (250 × 21.2 mm), 17 mL/min, (3 mM ammonium acetate +0.02% formic acid) in water/acetonitrile, retention time 4.47 min] to afford **4** (0.110 g, 29% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.4 (s, 1H), 10.5 (s, 1H), 7.6 (d, J = 16.0 Hz, 1H), 7.5 (d, J = 10.0 Hz, 2H), 7.4 (d, J = 8.0 Hz, 1H), 7.2 (d, J = 7.6 Hz, 1H), 6.9 (m, 2H), 6.7 (d, J = 16.0 Hz, 1H), 5.3 (s, 1H), 3.6 (m, 1H), 2.9 (m, 1H), 2.6 (m, 1H), 2.3 (s, 1H), 1.8 (d, J = 8.4 Hz, 3H), 1.6 (d, J = 1.2 Hz, 3H), 1.2 (m, 3H); MS (ESI) m/z: 435.4 $[M + H]^+$.

***N*-(1-(1*H*-Indol-3-yl)butan-2-yl)bicyclo[1.1.1]pentan-1-amine (5−1).** To a stirred solution of bicyclo[1.1.1]pentan-1-amine HCl salt (0.9 g, 7.69 mmol), in MeOH (180 mL) was added AcOH to adjust its pH to 5−6. Compound **11** (1.2 g, 6.41 mmol) was added at rt, followed by $NaCNBH_3$ (720 mg, 11.5 mmol) and the mixture was stirred for 12 h at rt under argon atmosphere. Solvent was removed in-vacuo and the resultant residue was poured into water and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with aq $NaHCO_3$, water, brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The resultant residue was first purified by column chromatography (3:7 ethyl acetate in hexanes) to afford *N*-(1-(1*H*-indol-3-yl)butan-2-yl)bicyclo[1.1.1]pentan-1-amine (0.90 g, 56% yield) as a yellow liquid. The enantiomers were further purified by SFC chromatography [*Lux Cellulose-2, 15% (0.5% DEA in MeOH)*] to afford peak 1 (0.160 g, 32.0% yield) in >99% ee and the desired peak 2 (0.160 g, 33.0% yield) in >99% ee. MS (ESI) m/z 255.0 $[M + H]^+$.

(*E*)-Methyl 3-(4-((1*R*,3*R*)-2-(Bicyclo[1.1.1]pentan-1-yl)-3-ethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylate (5−2). To a stirred solution of **5−1** (160 mg, 0.63 mmol) in toluene (8.0 mL) were added (*E*)-methyl-3-(3,5-difluoro-4-formylphenyl) acrylate (142.0 mg, 0.63 mmol) followed by AcOH (0.75 mL, 1.26 mmol) and stirred at 90 °C for 6 h. The reaction mixture was allowed to warm to room temperature, diluted with water and extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The resultant residue was purified by column chromatography (SiO_2, 2:8 ethyl acetate in hexanes) to afford **5−2** (80 mg, 27% yield). MS (ESI) m/z 463.7 $[M + H]^+$.

(*E*)-3-(4-((1*R*,3*R*)-2-(Bicyclo[1.1.1]pentan-1-yl)-3-ethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylic Acid (5). To a stirred solution of **5−2** (80 mg, 0.17 mmol) in THF/water (5.0 mL, 1:1) was added LiOH (42 mg, 1.03 mmol) at 0 °C and the mixture was stirred at rt for 6 h. The reaction mixture was washed with diethyl ether and the separated aqueous layer was acidified to pH 3−4 with 1.0 M HCl solution at 0 °C. The resulting solid was filtered, washed with water and dried to afford **5** (0.011 g, 14% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.56 (br s, 1H), 10.62 (br s, 1H), 7.56−7.40 (m, 4H), 7.18 (d, J = 5.7 Hz, 1H), 7.00−6.92(m, 2H), 6.66 (d, J = 11.7 Hz, 1H), 5.39 (s, 1H), 3.18 (s, 1H), 2.77 (s, 2H), 2.25 (s, 1H), 1.77−1.63 (m, 6H), 1.43 (s, 1H), 1.23 (s, 1H), 0.85 (m, 3H); MS (ESI) m/z 449.11 $[M + H]^+$, $[\alpha]^{25}_D$ − 279.1 (c 0.5, MeOH).

***N*-(1-(1*H*-Indol-3-yl)propan-2-yl)-3-(trifluoromethyl)bicyclo-[1.1.1]pentan-1-amine (6−1).** To a stirred solution of 1-(1*H*-indol-3-yl)propan-2-one (0.400 g, 2.31 mmol) in methanol (3 mL) was added 3-(trifluoromethyl)bicyclo[1.1.1]pentan-1-amine (0.518 g, 2.77 mmol) and acetic acid (14 mg, 0.231 mmol) and the mixture was stirred at rt for 16 h. The resulting solution was cooled to 0 °C and sodium cyanoborohydride (0.290 g, 4.62 mmol) was added followed by additional stirring for 5 h at rt. The reaction was diluted with ethyl acetate (10 mL) and washed with saturated, aqueous NH_4Cl solution (10 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated. The crude residue was purified by column chromatography using (SiO_2, 15% EtOAc/Hexane) to afford 0.4 g of *N*-(1-(1*H*-indol-3-yl)propan-2-yl)-3-(trifluoromethyl)bicyclo[1.1.1]-pentan-1-amine (**6−1**) which was used without further purification. MS (ESI) m/z: 309.18 $[M + H]^+$.

Methyl (*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-3-methyl-2-(3-(trifluoromethyl)bicyclo[1.1.1]pentan-1-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylate (6−2). To a stirred solution of **6−1** (1.2 g, 3.90 mmol) in toluene (6 mL) was added methyl (*E*)-3-(3,5-difluoro-4-formylphenyl)acrylate (0.880 g, 3.90 mmol) and acetic acid (0.467 g, 7.79 mmol). The resulting mixture was stirred at 90 °C for 5 h. The reaction mixture was diluted with EtOAc and washed with water. The combined organic phase was dried over sodium sulfate, filtered and concentrated. The residue was purified by chiral SFC (Chiralcel OD-H (5 μm, 250 × 21 mm) %CO_2: 85.0%, % Cosolvent (EtOH): 15.0%, to afford 180 mg (9% yield) of **6−2**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.6 (br s 1H), 7.74−7.52 (m, 3H), 7.41 (d, J = 6.6 Hz, 1H), 7.18 (d, J = 6.9 Hz, 1H), 7.02−6.92 (m, 2H), 6.82 (d, J = 15.9 Hz, 1H), 5.36 (s, 1H), 3.75 (s, 3H), 3.61 (br s 1H), 3.01−2.95 (m, 1H), 2.63−2.58 (m, 1H), 2.01 (d, J = 9.3 Hz, 3H) 1.83 (d, J = 9.3 Hz, 3H), 1.09 (d, J = 6.6 Hz, 3H); MS (ESI) m/z 517.18 [M + H]$^+$. Compound **6−2** elutes as peak 1 and the undesired enantiomer elutes as peak 2.

(*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-3-methyl-2-(3-(trifluoromethyl)bicyclo[1.1.1]pentan-1-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenyl)acrylic Acid (6). To a stirred solution of **6−2** (180 mg, 0.349 mmol) in methanol (1.5 mL) was added aqueous solution of NaOH (0.13 mL, 0.039 g, 0.988 mmol, 7.5 M) solution at 0 °C and the mixture was stirred at rt for 5 h. The reaction mixture was concentrated under reduced pressure to remove methanol. The residue was acidified with 1 N HCl solution at 0 °C, diluted with EtOAc and washed with water. The combined organic layer was dried over sodium sulfate, filtered and concentrated. The crude residue was purified by SFC (Chiralcel OJ-H (5 μm, 250 × 21 mm); %CO_2: 50.0%; % Cosolvent (EtOH): 50.0%, to afford **6** (90 mg, 55% yield) as an off white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.6 (br s, 1H), 7.62−7.39 (m, 4H), 7.18 (d, J = 7.5 Hz, 1H), 7.03−6.92 (m, J = 7.2 Hz, 2H), 6.68 (d, J = 16.2 Hz, 1H), 5.38 (s, 1H), 3.62 (br s, 1H), 3.05−2.97 (m, 1H), 2.63−2.58 (m, 1H), 2.08−1.79 (m 6H), 1.09 (d, J

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= 6 Hz, 3H); MS (ESI) m/z 503.26 $[M + H]^+$, $[\alpha]_D = -58.4°$ (c 0.25, $CHCl_3$, 24 °C).

***N*-(1-((1*H*-Indol-3-yl)methyl)cyclopropyl)bicyclo[1.1.1]-pentane-1-carboxamide (7−1).** To a stirred solution of **12** (0.19 g, 1.02 mmol) in dichloromethane (3.92 mL) was added HATU (0.312 g, 1.326 mmol) and *N*,*N*-Diisopropylethylamine (0.36 mL, 2.04 mmol) at rt and the mixture was stirred for 10 min. Bicyclo[1.1.1]pentane-1-carboxylic acid (0.13 g, 1.11 mmol) was added and the mixture was stirred at rt for 16 h. Ethyl acetate (15 mL) was added and the organic phase was washed with saturated, aqueous sodium bicarbonate solution. The organic layer was separated, dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography (SiO_2, 30−60% EtOAc in hexane) to afford **7−1** (0.20 g, 70% yield) as an off-white solid. MS (APCI) m/z 281.16 $[M + H]^+$.

1-((1*H*-Indol-3-yl)methyl)-*N*-(bicyclo[1.1.1]pentan-1-ylmethyl)cyclopropanamine (7−2). To a stirred solution of **7−1** (0.20 g, 0.71 mmol) in THF (2.85 mL) was added LAH (1.78 mL, 2 M LAH in THF solution, 3.57 mmol) dropwise at 0 °C and the mixture was refluxed for 18 h. The reaction mixture was quenched sequentially with cold (0−5 °C) water (0.15 mL), an aqueous solution of 15% NaOH (0.30 mL) and water (0.50 mL). The resulting mixture was extracted with EtOAc (3 × 10 mL). The separated organic phase was dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography [SiO_2, 0−50% (9:1 DCM:MeOH with ammonia) in DCM to afford **7−2** (0.13 g, 68% yield) as a white solid. MS (APCI) m/z 267.18 $[M + H]^+$.

Methyl (*E*)-3-(4-(2-(Bicyclo[1.1.1]pentan-1-ylmethyl)-3-ethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indol-1-yl)-3,5-difluorophenyl)acrylate (7−3). To a stirred solution of **7−2** (0.11, 0.41 mmol) in toluene were added (*E*)-ethyl 3-(3,5-difluoro-4-formylphenyl)acrylate (0.11 g, 0.11 mmol) and acetic acid (0.050 g, 0.83 mmol) and stirred at 80 °C for 3 h. The crude reaction products suggested that the cyclopropyl ring had been cleaved. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was separated, dried over sodium sulfate and concentrated. The resulting residue was purified by flash chromatography (SiO_2, 0−20% EtOAc in hexane) to afford **7−3** (0.10 g, 49% yield) as a yellow solid. MS (APCI) m/z 491.24 $[M + H]^+$.

(*E*)-3-(4-(2-(Bicyclo[1.1.1]pentan-1-ylmethyl)-3-ethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylic Acid (7). To a stirred solution of **7−3** (0.10 g, 0.20 mmol) in a mixture of methanol (1 mL), THF (1 mL) and water (1 mL) was added sodium hydroxide (0.024 g, 0.61 mmol) at 0 °C and stirred at rt for 2 h until starting material was completely consumed. The mixture was concentrated under reduced pressure and the residue was acidified to pH of 4 using aqueous citric acid solution (1 M) at 0 °C. The resulting precipitate was extracted with EtOAc and concentrated. The solid was dissolved in DMSO (1.5 mL) and purified by reverse-phase HPLC chromatography using 10−50% acetonitrile (contains 0.15% formic acid) in water (contains 0.1% formic acid) to afford **7** (0.03 g, 31% yield) as a mixture of isomers which were not further purified. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 10.57 (s, 1H), 7.54 (d, J = 16.0 Hz, 1H), 7.45 (d, J = 12 Hz, 2H), 7.41 (d, J = 7.8 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 7.03−6.91 (m, 2H), 6.67 (d, J = 16.0 Hz, 1H), 5.15 (s, 1H), 3.00 (qd, J = 4.6, 8.9 Hz, 1H), 2.81−2.62 (m, 3H), 2.40 (s, 1H), 2.29 (br d, J = 14.4 Hz, 1H), 1.64−1.50 (m, 7H), 1.36−1.21 (m, 1H), 0.84 (t, J = 7.3 Hz, 3H); MS (APCI) m/z 463.21 $[M + H]^+$.

***N*-(1-(1*H*-Indol-3-yl)-2-methylpropan-2-yl)bicyclo[1.1.1]-pentane-1-carboxamide (8−1).** To a stirred solution of **13** (0.31 g, 1.65 mmol) in dichloromethane (6.33 mL) were added HATU (0.50 g, 2.14 mmol) and *N*,*N*-Diisopropylethylamine (0.57 mL, 3.29 mmol) at rt and stirred for 10 min. Bicyclo[1.1.1]pentane-1-carboxylic acid (0.20 g, 1.81 mmol) was added and the mixture was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (15 mL) and washed with saturated sodium bicarbonate (aqueous) solution. The organic layer was separated, dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography (SiO_2, 30−60% EtOAc in hexane) to afford **8−1** (0.36 g, 77% yield). MS (APCI) m/z 283.17 $[M + H]^+$.

***N*-(Bicyclo[1.1.1]pentan-1-ylmethyl)-1-(1*H*-indol-3-yl)-2-methylpropan-2-amine (8−2).** To a stirred solution of **8−1** (0.36 g, 1.28 mmol) in THF was added LAH (5.19 mL, 2 M LAH in THF, 10.38 mmol) dropwise at 0 °C and the mixture was refluxed for 18 h. The reaction mixture was quenched sequentially with cold (0−5 °C) water (0.40 mL) slowly, then an aqueous solution of 15% NaOH (0.80 mL) and water (1.2 mL). The resulting mixture was extracted with EtOAc (2 × 20 mL). The separated organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (SiO_2, 0−50% (9:1 DCM:MeOH with ammonia) in DCM to afford **8−2** (0.14 g, 41% yield). MS (APCI) m/z 269.19 $[M + H]^+$.

Methyl (*E*)-3-(4-(2-(Bicyclo[1.1.1]pentan-1-ylmethyl)-3,3-dimethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylate (8−3). To a stirred solution of **8−2** (0.07 g, 0.26 mmol) in toluene (0.65 mL) was added (*E*)-ethyl 3-(3,5-difluoro-4-formylphenyl)acrylate (0.069 g, 0.29 mmol) and acetic acid (0.031 g, 0.52 mmol) and stirred at 90 °C for 5 h. The reaction mixture was diluted with EtOAc and washed with water. The separated organic phase was dried over sodium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography (SiO_2, 0−15% ethyl acetate in hexane) to afford **8−3** (0.025 g, 19% yield) as a pale yellow solid. MS (APCI) m/z 491.24 $[M + H]^+$.

(*E*)-3-(4-(2-(Bicyclo[1.1.1]pentan-1-ylmethyl)-3,3-dimethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)-3,5-difluorophenyl)acrylic Acid (8). To a stirred solution of **8−3** (0.02 g, 0.041 mmol) in a mixture of methanol (1 mL), THF (1 mL) and water (1 mL) was added sodium hydroxide (4.8 mg, 0.12 mmol) at 0 °C and stirred at rt for 2 h. The mixture was concentrated under reduced pressure and the residue was acidified with an aqueous solution of citric acid (1 M) at 0 °C to adjust pH to 4. The precipitate was extracted with EtOAc and concentrated. The resulting residue was dissolved in DMSO (1 mL) and purified by reversed-phase HPLC column chromatography using 10−60% acetonitrile (contains 0.1% formic acid) in water (contains 0.1% formic acid) to obtain **8** (0.008 g, 41% yield) as a mixture of enantiomers which were not further purified. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (br d, J = 16.0 Hz, 1H), 7.53−7.45 (m, 1H), 7.22−7.18 (m, 1H), 7.14−7.08 (m, 2H), 7.03 (d, J = 8 Hz, 2H), 6.43 (d, J = 16.0 Hz, 1H), 5.14 (br s, 1H), 3.02 (br d, J = 16.4 Hz, 1H), 2.91 (br d, J = 13.9 Hz, 1H), 2.63 (br d, J = 14.9 Hz, 1H), 2.28 (br d, J = 16.4 Hz, 1H), 2.19 (s, 1H), 1.33−1.22 (m, 6H), 1.18 (br d, J = 8 Hz, 3H), 1.03 (s, 3H) (missing carboxylic acid and NH protons); MS (APCI) m/z 463.21 $[M + H]^+$.

Construct Generation. The ESR1 gene (UniProt P03372) encoding amino acid residues 298−553 of the ligand binding domain was codon optimized, synthesized (Integrated DNA Technologies), cloned into the pET28a vector, which contains a TEV protease cleavable N-terminal 6xHis fusion tag. Three engineered amino acid mutations corresponding to C381S, C417S, and L536S were incorporated into the DNA construct compared to the ESR1 protein sequence.

Protein Expression and Purification. The plasmid expression construct was transformed and expressed in *E. coli* BL21 Rosetta (DE3) cells in TB media with IPTG induction (0.2 mM) at an OD600 at 1.0. Cultures were incubated at 17 °C postinduction and harvested via centrifugation overnight. Cells were resuspended in lysis buffer (25 mM tris, pH 8.0, 500 mM NaCl, 10 mM imidazole, 10% glycerol, 1 mM PMSF, 1 mM Benzamidine, 5 mM beta-mercaptoethanol) before sonication and clarification via high-speed centrifugation at 13 krpm. The supernatant was applied using a AKTA Purifier FPLC to HisTrap HP columns (GE Healthcare), before the columns were washed with lysis buffer supplemented with increasing imidazole concentrations (up to 40 mM) and the bound protein was eluted with lysis buffer supplemented with 250 mM imidazole. Following overnight dialysis, the ERα-LBD protein was incubated with TEV at room temperature for 4 h. Cleaved ERα-LBD was passed though Ni-NTA beads and collected in the flowthrough. Gel filtration using a Superdex 75 10/300 column pre-equilibrated with 25 mM tris, pH 8.0, 500 mM NaCl, 10% glycerol, 5 mM beta-mercaptoethanol on AKTA Purifier FPLC was performed to further purify the protein. The final protein for crystallization trials

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was concentrated to 1 mg/mL and flash frozen in liquid nitrogen in aliquots then stored at −80 °C.

Protein Co-Crystallization and Harvesting. Initially, the compound **4** was added to purified protein at 1 mg/mL to a final concentration of 0.2 mM from a 40 mM DMSO stock and incubated for 30 min at 4 °C before concentration of the protein to ∼10 mg/mL. The protein was subjected to extensive crystallization trials using commercially available screens as well as literature ERα-LBD crystallization conditions to identify initial crystallization conditions. Initial crystals appeared in 0.1 M tris pH 8.0, 20% PEG 3350, 200 mM $MgCl_2$. Further optimization of initial crystallization conditions produced suitable cocrystals affording diffraction data from which a costructure was solved and refined to 3.2 Å resolution. Crystals were harvested by brief transfer to a drop of reservoir solution supplemented with 50% glycerol prior to plunging in liquid nitrogen.

Crystal Screening and Data Collection. Over a hundred crystals were screened using the beam light sources at SSRL and ALS. Multiple data sets were collected and indexed. The best data set was collected at the beamline measured with a PILATUS detector. Data were collected from a single crystal maintained at 100 K and 360° of data were collected using 1-degree oscillations. Data Reduction and Co-structure Determination Reflections were indexed, integrated, and scaled using HKL3000.[66] The space group was P1 with 4 molecules per asymmetric unit. The structure of ERα- LBD complexed with Compound **4** was solved by molecular replacement using the program Phenix[67] with protein coordinates from PDB 6DFN as a search model. The final model was obtained by iterative rounds of refinement (Phenix) and building (Coot[68]) to a final Rcrys/Rfree of 0.2497/0.2893. Non-crystallographic symmetry (NCS) restraints were imposed in refinement. Coordinates and restraints for Compound **4** were generated using Phenix Elbow and added to the model after several rounds of refinement.

Cell Culture and Reagents. MCF-7 cell line was licensed from Duke University (Dr. Donald McDonnell lab) and maintained in DMEM/F12 (Gibco 11330−032), supplemented with Non-Essential Amino Acids (NEAA) (Gibco 11140−050), Na-pyruvate (Gibco 11360−070) and Fetal bovine serum (FBS) (Hyclone SH30084). CAMA-1, T47D, ZR-75−1, HCC1428 and HCC1500 were purchased from ATCC and cultured according to ATCC recommendation in RPMI-1640 complete medium. Cell culture medium and supplements, unless otherwise indicated, were purchased from either ATCC or Gibico. 4OH-tamoxifen, elacestrant, palbociclib, ribociclib, abemaciclib and alpelisib were purchased from Selleckchem. Fulvestrant and 17β-estradiol was purchased from Sigma-Aldrich. Antibodies for ERα, PR, and anti-β actin were from cell signaling technology (Sigma-Aldrich, V9131) as an internal control were used. Cells and tissues were lysed with RIPA buffer (Boston BioProducts) or with tissue protein extraction reagent with Halt protease inhibitors and EDTA (Thermo Fisher Scientific), respectively.

Cell Proliferation Assay. MCF-7 cells were seeded at a density of 3000 cells per well into flat clear bottom tissue cultured-treated 96-well plates (Corning) in assay medium. Assay medium components: phenol red free DMEM/F12 (Hyclone SH30272.01) 500 mL, NEAA (Gibco11140−050) 5 mL, Na-pyruvate (Gibco 11360−070) 5 mL, restripped Charcoal stripped FBS (Gemini 100−119) 45 mL (8%) (charcoal stripped (CSS) FBS. The Charcoal stripped FBS should be restripped as follows: Add 10 mg/mL activated charcoal Norit SA 2, Dextran 70 (1 mg/mL) to FBS, Incubate at 56 °C for 45 min with shaking. Centrifuge at 4 °C ∼ 3500 rpm for 30 min. Filter supernatant through 0.22 μm filter. Cell viability was assessed after a 6-day incubation with different compounds as indicated in the presence of and 0.1 nM 17β-estradiol (Sigma) using CellTiter-Glo (Promega) according to the manufacturer's protocol and relative luminescence units (RLU) were measured using an Envision Multilabel Reader (PerkinElmer). The RLUs of the treated samples were normalized to that of the untreated samples and cell viability was expressed as a percentage of the value of the untreated cells.

Western Blot Assay. For SERDs treatment, cells were seeded in phenol-red free media supplemented with charcoal-stripped FBS. Forty-eight h later, cells were treated with SERDs at the indicated concentrations. For other drug treatments, cells were seeded in regular culture medium. Cells were lysed in RIPA buffer protease and phosphoprotease inhibitors (Thermo Fisher Scientific) after 24 or 48 h of treatment, and total proteins were separated by SDS-PAGE and immunoblotted using antibodies as indicated. For pharmacodynamic studies using tumor samples from xenograft studies, flash-frozen tumors were pulverized and tissue was lysed in RIPA buffer. Total protein was analyzed by Western blot.

Mouse and Rat Pharmacokinetic Studies (PH-DMPK-KAP-16−035 and PH-DMPK-KAP-16−036, Respectively). Female CD1 mice (20−30 g) or female Sprague−Dawley rats (200−300 g) were randomly grouped. A group of 3 animals was administered the test compound at an indicated dose. The IV arm was dosed at 3 mg/kg using a formulation of DMSO/PEG400/150 mM glycine (pH 9) (5/10/85). The oral arm was dosed at 10 mg/kg using a formulation of PEG400/PVP/Tween 80/0.5% CMC in water (9/0.5/0.5/90). The blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h time points. The concentrations of test compounds in plasma samples were determined by using LC−MS/MS. Standard curves were plotted based on concentrations of the samples in a suitable range. Pharmacokinetic parameters were calculated according to a drug concentration−time curve using a noncompartmental method by WinNonLin (Phoenix, version 6.1) or similar software.

Dog Pharmacokinetic Study (Pharmaron Study: 66704−16−033). Female beagle dogs were randomly grouped. A group of three animals was administered the test compound at an indicated dose. The IV arm was dosed at 3 mg/kg using a formulation of EtOH/PEG400/30%HPβ-CD (10/40/50). The oral arm was dosed at 10 mg/kg using a formulation of PEG400/PVP/Tween 80/0.5% CMC in water (9/0.5/0.5/90). The blood samples for the IV arm were collected at predose, 5, 15, 30 min, 1, 2, 4, 8, 12, and 24 h time points. The blood samples for the PO arm were collected at predose, 15, 30 min, 1, 2, 4, 6, 8, 12, and 24 h time points. The concentrations of the test compound in plasma samples were determined by using LC−MS/MS. Standard curves were plotted based on concentrations of the samples in a suitable range. Pharmacokinetic parameters were calculated according to a drug concentration−time curve using a noncompartmental method by WinNonLin (Phoenix, version 6.1) or similar software.

In Vivo Efficacy Studies. Animal studies were performed according to guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of CrownBio or Horizon following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). For MCF-7 model (CrownBio study#: E1664-U1701), mice were inoculated subcutaneously on the second right mammary fat pad with the single cell suspension of 95% viable tumor cells (1.5×10^7) in 200 μL medium Matrigel mixture (1:1 ratio) without serum for the tumor development. The treatments were started when mean tumor size reached ∼200 mm^3. Mice were randomized into 10 groups. In addition, estradiol benzoate injection was delivered by s.c. (40 μg/20 μL, twice weekly). For WHIM20 PDX study (Horizon Study #0454), female athymic nude mice were implanted with a single cell suspension of 1.5×10^6 WHIM20 cells, passage 10. The cells were mixed 1:1 with PBS:Matrigel (Corning ref 354234, lot#0090009) in a total volume of 100 μL/mouse. Once tumors reached approximately 100−300 mm^3, mice were randomized by tumor volume into 1 of 10 treatment groups (8 mice/group) using Biopticon's Tumor Manager software.

Human Clinical Trials. The ZN-c5−001 clinical trial (NCT03560531) was conducted in accordance with United States ethical guidelines (i.e., U.S. Common Rule) and the Declaration of Helsinki, Good Clinical Practice, and all federal, state regulatory guidelines and approved by Western Institutional Review Board and NYSDOH Institutional Review Board (IRB). In the ZN-c5−001 study, ZN-c5 was administered once daily or twice daily with dose ranging from 25 mg to 300 mg per day in 28-day cycles to patients enrolled under the protocol. Plasma samples were collected at cycle 1, day 1 (single dose) or cycle 1, day 15 (steady state) for PK analysis (MicroConstant). All patients provided written informed consent prior to study enrollment.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.5c00887.

Characterization: HPLC traces of compounds **1**−**6** and ^{1}H-NMR spectra of compounds **1**−**6** (PDF)

SMILES molecular formula strings with compound data information (CSV)

Accession Codes

Atomic coordinates and structure factors for the ERα LBD complexed with compound **4** (PDB code: 9ECK) have been deposited in the Protein Data Bank with accession code 9ECK. Authors will release the atomic coordinates upon article publication.

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Notes

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■ ABBREVIATIONS USED

ADME:absorption, distribution, metabolism, and excretion; AUC:area under the curve; CDX:cell-line-derived xenograft; Cl_{int}:intrinsic clearance; CYP:cytochrome P450; HP-β-CD:(2-hydroxypropyl)-β-cyclodextrin; IC_{50}:50% inhibitory concentration; log *D*:octanol/buffer (pH 7.4) distribution coefficient; PDB:protein data bank; PK:pharmacokinetic; PDX:patient-derived xenograft; PPB:plasma protein binding; SAR:structure−activity relationship; SFC:supercritical fluid chromatography

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