Journal of Medicinal Chemistry

Article

Optimization of a novel binding motif to (E)-3-(3,5-difluoro-4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-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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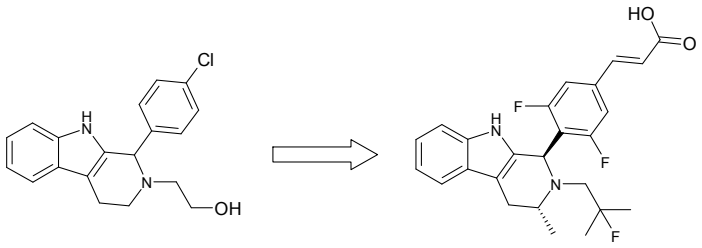


ER binding screen hit, pIC_{50} = 5.9

AZD9496, orally bioavailable SERD, pIC_{50} = 9.9

INTRODUCTION

Following the pioneering discovery of the estrogen receptor α (ERα) antagonist tamoxifen (Figure 1, **1a**) in the 1960s,[1,2] identification of the selective estrogen receptor downregulator (SERD) fulvestrant (**2**) represented a further step forward in treatment of advanced ER-positive breast cancer.[2-5]

Resistance to tamoxifen can develop due to a number of mechanisms that switch the mode of action from antagonism to agonism, thereby promoting tumour cell re-growth.[6a] The pure estrogen downregulator-antagonist fulvestrant offers the advantage of not only antagonising ERα-driven tumour cell growth but also degrading ERα *via* ubiquitinylation and thus diminishing growth due to reduced ERα content.[3b] Furthermore, the discovery of *ESR1* mutations in metastatic breast cancer patients that had received prior endocrine therapy, where the mutated receptor is active in the absence of estrogen, also highlights a further resistance mechanism.[6b,c]

Fulvestrant has very low oral bioavailability[7] and is administered as a monthly intramuscular implant at a dose of up to 500 mg.[8] Clinical efficacy of fulvestrant is believed to be limited by less than 50% ERα turnover detected in patient samples, compared to complete receptor downregulation seen in *in-vitro* breast cell line experiments.[9]

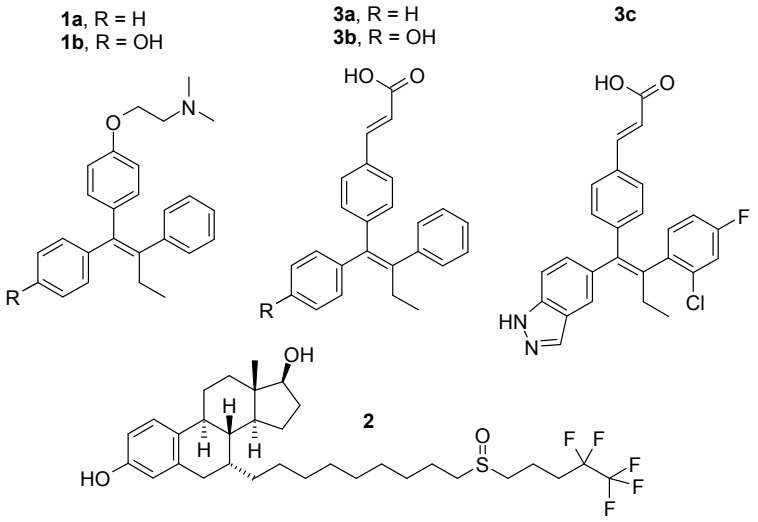


Figure 1. Chemical structures of tamoxifen **(1a)**, hydroxytamoxifen **(1b)**, fulvestrant **(2),** GW5638 (**3a**), GW7604 (**3b**) and ARN-810 (**3c**).

A potent, orally bioavailable SERD has potential for significantly higher receptor knockdown compared to fulvestrant due to higher exposure. A number of SERD agents with low to moderate rodent oral bioavailability have been reported, including tamoxifen analogs GW5638 (**3a**) and GW7604 (**3b**)[10-12] and various steroidal[13,14] and non-steroidal derivatives.[15-18] Other than ARN-810 (**3c**)[19] no oral SERD has yet entered clinical trials.

Although seldom made explicit in the medicinal chemistry literature,[20] achieving oral bioavailability is a key challenge in the design of ER modulators. Clinical efficacy of tamoxifen reflects formation of significantly more active hydroxytamoxifen (**1b**),[21,22] in which the phenolic moiety mimics the A-ring phenol group of the endogenous ligand estradiol. Tamoxifen is thus an orally bioavailable prodrug of more active phenolic metabolites.

Among the efforts of many research groups from the 1980s onwards in designing tissue-selective ER modulators for treatment of hormone-dependent breast cancer and osteoporosis,[2,22,23] lasofoxifene[20,24] provides a rare example of a phenolic ER modulator with low clearance and high oral bioavailability across species, a property attributed to conformational effects structurally remote from the phenolic moiety on the rate of gut wall glucuronidation.[20] We however concluded that, exacerbated by high lipophilicity, the presence of a phenol moiety in ER modulators is more generally associated with high clearance and/or poor oral absorption.

Rather than seeking to improve pharmacokinetic properties of known ER motifs, we chose to follow a less explored strategy of undertaking a directed ER binding screen to identify novel motifs with druglike properties that could then be optimized for cellular phenotype and potency. In this publication, we report the discovery of 1-aryl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indoles as a novel, orally bioavailable ER binding motif and outline the lead optimization program that led to SERD clinical candidate AZD9496 (**30b**).

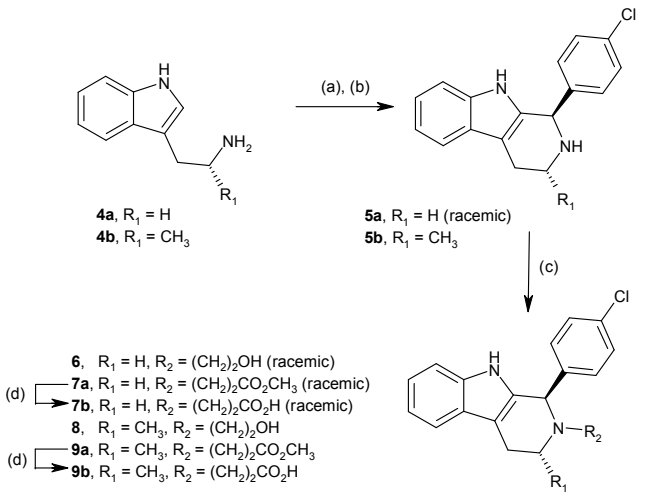
RESULTS

Biological data for illustrative compounds selected from several hundred diverse examples prepared during the course of this work are listed in Table 1, along with reference data for **1a-b**, **2** and **3a-b**. As outlined in Schemes 1-4, central to synthesis of the novel compounds listed in Table 1 was use of the Pictet-Spengler cyclisation[25-27] to construct the 1-aryl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole skeleton from appropriate tryptamine and aryl aldehyde precursors.

Racemic screening hit **6** was obtained *via* alkylation of the readily accessible racemic unsubstituted amine **5a**[28] with 2-bromoethanol, and screening hit **7b** was similarly obtained by alkylation of **5a** with methyl 3-bromopropionate and subsequent hydrolysis of ester **7a** (Scheme 1). Synthesis of *trans*-1*R,* 3*R* compounds containing a methyl group of defined stereochemistry adjacent to the tetrahydro piperidine nitrogen atom commenced with the appropriate chiral α-methyltryptamine (Schemes 1-4).

In accord with published work,[25-27] Pictet-Spengler cyclisation of *N*-unsubstituted (*R*)-α-methyltryptamine **4b** with 4-chlorobenzaldehyde gave predominantly the *cis* isomer as the kinetic product that could be enriched in *trans* isomer **5b** under acidic conditions. Alkylation of **5b** gave **8** and **9a**, and the latter hydrolysed to **9b** (Scheme 1).

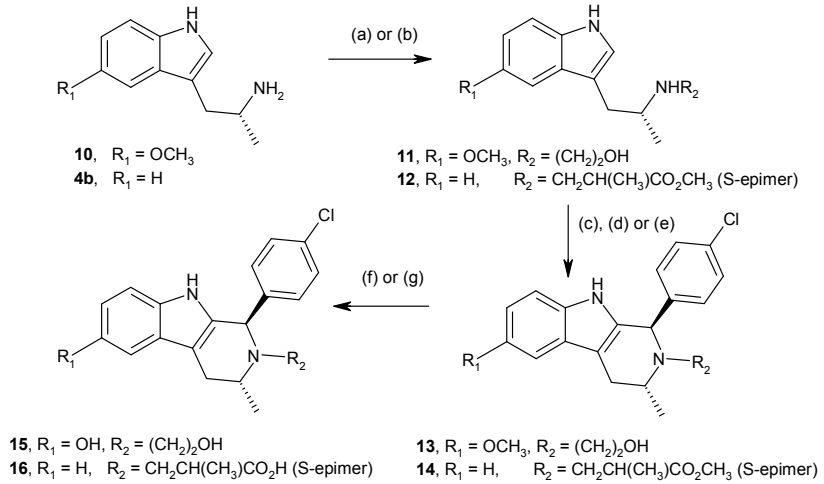
Scheme 1. Synthesis of compounds **6, 7b, 8, 9b**[a,b]



[a]Compounds **6** and **7b** prepared as racemates; [b]Reagents and conditions: (a) 4-chlorobenzaldehyde, PhMe, 105 °C; (b) AcOH, PhMe, 105 °C, 50-75%; (c) 2-bromoethanol or methyl 3-bromopropionate, Et_3N, MeCN, 140 °C, 72-76%; (d) $NaOH/H_2O$/THF, 65 °C, 94-96%.

It is also well established[25-27] that substitution of the α-methyltryptamine nitrogen atom leads predominantly to the desired *trans* stereochemistry during the Pictet-Spengler cyclisation. Compounds **15** and **16** were thus accessed as shown in Scheme 2. Alkylation of the appropriate α-methyltryptamines **10** and **4b** gave **11** and **12,** respectively. Pictet-Spengler cyclisation with 4-chlorobenzaldehyde and subsequent deprotection afforded the desired products **13** and **14**.

Scheme 2. Synthesis of compounds **15-16**[a,b]

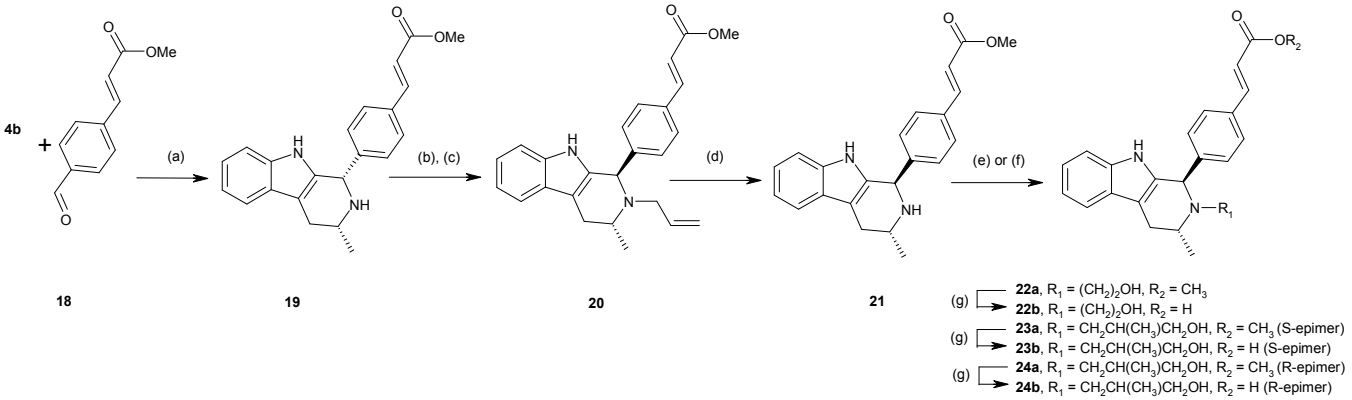


[a]Synthesis of **15** carried out using racemic precursor and enantiomerically pure material obtained by chiral chromatography at the final step; [b]Reagents and conditions: (a) 2-bromoethanol, K_2CO_3, MeCN, 80 °C, 53%; (b) (*S*)-methyl 2-methyl-3-(trifluoromethylsulfonyloxy)propanoate (**17**), *i*-Pr_2NEt, dioxane, 80 °C, 79%; (c) 4-chlorobenzaldehyde, AcOH, 80 °C; (d) NaOH, H_2O, MeOH, 60 °C, 47%; (e) 4-chlorobenzaldehyde, AcOH, PhMe, 90 °C, 72%; (f) BBr_3, DCM, -78 °C, then RT, 35%; (g) NaOH, H_2O,THF, 65 °C, 93%.

For synthesis of initial acrylic acid derivatives **22b-24b**, a route was developed (Scheme 3) that enabled variation of the basic nitrogen substituent at a late stage of the synthesis. Reaction of **4b** with aldehyde **18** gave predominantly the expected *cis* product **19**. Alkylation with allyl bromide and treatment with acid provided a 65:35 mixture of the *trans* isomer **20** and the corresponding *cis* isomer, from which the desired *trans* intermediate **21** could be isolated pure after removal of the allyl group. Alkylation and ester hydrolysis then gave the acrylic acid derivatives **22b-24b**.

Finally, compounds **27b-30b** were obtained by the optimised route shown in Scheme 4, in which **4b** was first alkylated to give **25a-b**. Pictet-Spengler cyclisation under acidic conditions with the appropriate aldehyde **18** or **26** provided the pure *trans* products **27a-30a** in good yield after chromatography, and ester hydrolysis then gave **27b-30b**.

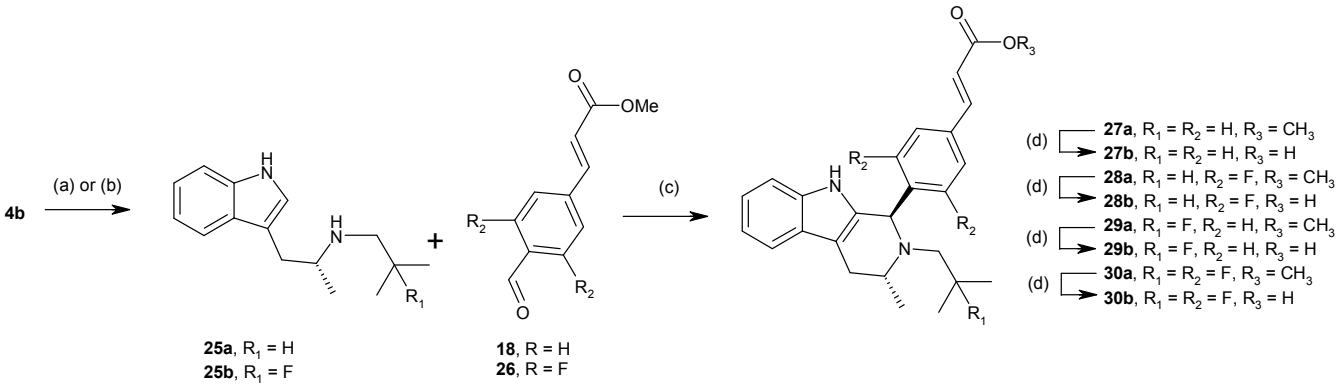
Scheme 3. Synthesis of compounds **22b-24b**[a]



[a]Reagents and conditions: (a) AcOH, 80 °C, 74%; (b) allyl bromide, *i*-Pr_2NEt, MeCN, 145 °C; (c) TFA, DCM, 20 °C, 82%; (d) $(Ph_3P)_3$RhCl, MeCN, 100 °C, 56% ; (e) 2-bromoethanol, *i*-Pr_2Net, MeCN, 140

^{o}C, 76%; (f) (*R*)- or (*S*)-3-bromo-2-methyl-1-propanol, *i*-Pr_2Net, MeCN, 135 ^{o}C, 64%; (g) NaOH, H_2O, THF, MeOH, 20 ^{o}C, 75-93%.

Scheme 4. Synthesis of compounds **27b-30b**[a]



[a]Reagents and conditions: (a) isobutyraldehyde, $Na(OAc)_3BH$, THF, 0 ^{o}C, 44%; (b) 2-fluoro-2-methylpropyl trifluoromethanesulfonate (**31**), *i*-Pr_2NEt, dioxane, 90 ^{o}C, 91%; (c) AcOH, PhMe, 80 ^{o}C, 68-74%; (d) NaOH, H_2O, THF, MeOH, 20^{o}C, 89-92%.

Table 1. ER binding, ER downregulation, PR agonism, PR antagonism[29] and MCF7 antiproliferation[42,43] 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	5.0	<4	6.89	7.24
1b	9.51	5.31	<4	9.1	9.27
2	9.09	10.2	<4	9.68	10
3a	8.49	5.89	<4	5.4	6.53
3b	9.84	8.17	<4.5	7.92[b]	ND[c]
6	5.69	5.93	<4	5.57	ND[c]
7b	4.86	5.43	<4.5	ND[d]	ND[c]
8	7.4	7.07	<4	6.14	4.86
9b	5.75	6.11	<4	5.82	<4.6
15	7.65	7.36	7.48	6.04	<4.5
16	7.15	7.52	7.89	5.68	<4.5
22b	6.16	5.88	<4	5.64	6.09
23b	7.16	7.33	<4	7.31	7.65
24b	7.01	7.34	<4	7.3	ND[c]
27b	8.19	8.68	<4	8.44	9.11
28b	9.15	9.38	<5.5	9.08	9.64[e]
29b	9.0	9.07	<5.5	8.46	9.49

30b	9.17	9.86	<5.5	9.55	10.4

[a]n ≥ 3, SEM values are available in the supplementary material; [b]n = 2; [c]Not determined; [d]Unable to determine PR antagonist pIC_{50} due to inconsistent response across concentration range; [e]n = 1.

DISCUSSION

Compounds listed in Table 1 were evaluated in a previously-described cascade of assays[29] designed to identify compounds with the requisite ER downregulator-antagonist profile in an MCF7 cell line, in which measurement of progesterone receptor (PR) levels was used as a downstream biomarker of ER agonism *via* an ER-mediated or indirect mechanism, such as inhibition of histone deacetylase. These assays were designed specifically to measure ERα down-regulation, agonism and antagonism through simultaneous measurement of ER and PR levels in cells after compound treatment. To confirm the mechanism of ERα down-regulation, assays were performed in the presence of a fixed concentration of tamoxifen (a representative output in Figure 6b) or cyclohexamide (assay not shown) at 5 hours. If ER downregulation seen with cyclohexamide treatment is unaltered from the 24 hour value, this indicates down-regulation is not as a result of an alternative pathway that requires further protein synthesis. Adding a fixed concentration of tamoxifen lowers the IC_{50} value should a compound and tamoxifen be competing for binding to the LBD.[29]

In order to identify novel, drug-like ER binding cores, a directed high-throughput screening set was generated. A computational approach was used that focused on covering a productive drug-like subspace with lower complexity compounds. The approach had two main tenets, in firstly choosing a set of lower complexity compounds as measured by number of heavy atoms, and number of rotatable bonds, and secondly in using chemical clustering to limit the number of compounds representing each point in chemical space.

Application of this approach allowed the AstraZeneca collection of 1.5 million compounds to be covered by a subset of 100K exemplars. This subset of lower-complexity compounds was chosen to represent four different categories of molecules: 7.5% phenol containing compounds, 7.5% compounds with phenol isosteres, 46% with generalized ER pharmacophores and 39% covering chemotypes not represented by the preceding categories.

A hit rate of 4.2 % was obtained[30] when the resulting set of 90K non-phenol compounds was evaluated at a concentration of 10 μM in a directed high-throughput screen that measured affinity for the human ERα ligand binding domain by fluorescence polarization.[29] It is notable that the hit rate of 19.2% was significantly higher with the 10K phenol compounds tested than that of the non-phenol compounds at 4.2%. As discussed earlier, despite the high hit-rate it was not the phenol hits but the non-phenolic compounds that proved to be the more promising start for identifying drug-like compounds which possessed low clearance and high oral bioavailability.

Determination of IC_{50} values identified the 1-aryl-2-(2-hydroxyethyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole derivative **6** as a novel and moderately potent ER binder (pIC_{50} 5.7) that appeared to be a particularly attractive chemical starting point (MW 317, measured LogD = 3.7). Good selectivity was seen versus a core panel of nuclear hormone receptor binding assays ($pIC_{50} < 4.5$ vs AR, PR and GR).

Compound **6** showed moderate activity (pIC_{50} 5.9) in the ER downregulation assay, along with minimal activity in the PR agonism assay.[29] The structurally related, more hydrophilic carboxylic acid **7b** (measured LogD 1.6) was also detected in the ER binding screen and showed a similar pharmacological profile to **6**. Performing the downregulation assay in the presence of tamoxifen shifted the IC_{50} curves of **6** and **7b** in a manner consistent with a competitive interaction at the same site in the ER ligand binding domain. As a measure of ER antagonism, compound **6** also decreased the downstream estradiol-driven PR response in the MCF-7 cell line.

Summarised in Table 2 are data for selected compounds from a number of routine physical property and metabolic stability assays. Although the more lipophilic screen hit **6** was highly turned over in rat and human hepatocytes, the more hydrophilic variant **7b** was significantly more stable, and dosing as a racemate in a rat PK study showed encouragingly low clearance and high oral bioavailability (Table 3). Further incentive to pursue this novel ER binding motif as a medicinal chemistry start point was provided by the presence of the 1-aryl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole core as a substructure in the approved PDE5 inhibitor tadalafil[33] and in clinical candidates NITD-609[34] (antimalarial) and PTC299[35-37] (VEGFR expression inhibitor), implying that this motif can be regarded as a "privileged substructure"[38-39] for drug discovery.

Table 2. LogD,[32] aqueous solubility, rat and human protein binding and hepatocyte stability data for **6, 7b, 16, 23b, 27b** and **30b**

Entry	LogD $pH_{7.4}$	Solubility µM $pH_{7.4}$[a]	% Free rat/human[b]	Rat/Human hepatocyte Clint (µl/min/10^6 cells)[c]
6	3.7	15 (semi-crystalline)	4.5/ND[d]	300/70
7b	1.6	>1600 (undefined)	7.2/ND[d]	29/19
16	2.8	1440 (amorphous)	3.2/1.5	82/23
23b	1.6	>1400 (semi-crystalline)	9.1/4.6	21/26
27b	2.6	>1000 (semi-crystalline)	0.69/0.51	18/40
30b	2.8	110 (crystalline)	0.37/0.26	4.2/32

[a]24 hr thermodynamic solubility of solid sample determined in 0.1M phosphate buffer (parentheses refer to visual examination of physical form of undissolved sample under a microscope at 24 hr timepoint); [b]Determined from DMSO stock solution by equilibrium dialysis in 10% plasma from Han Wistar rats or 10% human plasma supplied by Quintiles; [c]Rate of metabolism determined from DMSO stock solution in isolated rat or human cryopreserved hepatocytes diluted to $1x10^6$ cells/ml; [d]Not determined.

Varying the nature, position and multiplicity of the pendant or fused aryl ring substituents (halogens, OMe, CF_3, CN) or removal of the *N*-substituent reduced binding potency and downregulation activity (data not shown). As depicted in Figure 2, a crystal structure of compound **7b** bound to a construct of the ERα ligand binding domain[31] could be readily overlaid with the phenol metabolite of tamoxifen (**1b**).[21,22]

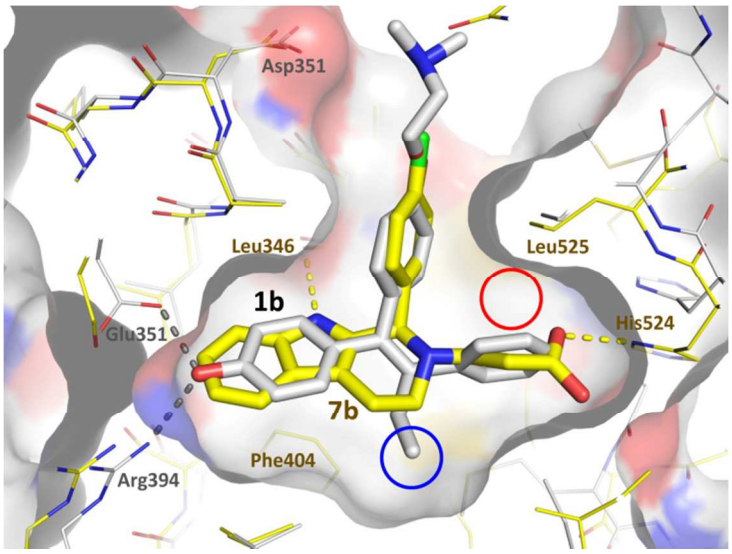


Figure 2. Crystal structure of **7b** (yellow, pdb code **5AAU**) superimposed on reference structure of **1b** (grey, pdb code **3ERT**) bound to the ERα ligand binding domain construct.[31] The blue circle indicates how the ethyl group of **1b** occupies a region of the ligand binding domain not accessed by compound **7b** termed Phe-404:Phe-425 lipophilic hole. The red circle indicates an additional liphophilic pocket unoccupied by both **1b** and **7b** termed the Leu-525:Leu384 lipophilic hole.

A number of key interactions are evident from the crystal structure of **7b**, with the benzenoid ring of the indole overlaying with the phenolic moiety of **1b**, the indole NH picking up a novel interaction by forming a hydrogen bond to the carbonyl of Leu-346, the carboxylic acid moiety sitting adjacent to His-524 and the pendant aryl substituent orientated towards the Helix-12 region of the ER, an interaction proposed to be key for ER downregulation.[12,40,41]

Of particular significance, the overlay of **7b** and **1b** suggested that introduction of a methyl substituent adjacent to the ring nitrogen in **7b** could boost potency by exploiting the "Phe-404:Phe-425 lipophilic hole" occupied by the ethyl group of **1b** (Figure 2, blue circle). Chiral synthesis of the 4 stereoisomers containing the additional methyl group showed that activity resided exclusively with the *trans-(1R,3R)* enantiomers **8** and **9b**, and that these derivatives were significantly more potent ER binders and downregulators than progenitors **6** and **7b**.

Although Figure 2 showed an imperfect overlay of the benzenoid ring of the indole with the phenolic ring of **1b**, we considered it worthwhile to probe the effect of introducing a phenol moiety into **8**. In contrast with **1a-b**, no significant increase in binding and downregulation potency was seen for the resulting phenol **15**, and the PR agonism readout revealed that introduction of the phenol moiety had switched the profile of **15** to an ER agonist.

Structural changes elsewhere also subtly influenced mechanism of action. Further examination of the crystal structure suggested that introduction of a methyl group on the N-substituent of **9b** could pick up additional binding, an observation supported by the increased potency of compound **16**, but once again with adoption of an agonist profile. No clearcut rationale for the change of profile was evident from the crystal structure of **16** bound to the ERα ligand binding domain. We were encouraged, however, by the rat oral bioavailability and overall property profile of **16** continuing to represent a viable start point for further medicinal chemistry (Tables 2 and 3).

Table 3. Rat,[a] mouse and dog[b] pharmacokinetic parameters for **7b, 16, 27b** and **30b**.

Entry	Species	Vdss l/kg	Cl ml/min/kg	Bioavailability %
7b	Rat[c]	0.6	4	85
16	Rat[c]	0.34	14	42
27b	Rat[d]	1.1	6.2	80
	Mouse[e]	3.7	21	86
	Dog[f]	0.3	0.96	111
30b	Rat[g]	0.43	1.0	63
	Mouse[h]	2.2	43	128
	Dog[f]	0.40	0.28	79

[a]Dosed to 2 male Han Wistar rats; [b] Dosed to 1 male and 1 female Alderley Park beagle; [c] blood PK parameters, dosed at 2 μmol/kg i.v. and 5 μmol/kg p.o.; [d]blood PK parameters, dosed at 5 μmol/kg i.v. and 13 μmol/kg p.o.; [e]blood PK parameters from 2 male CD-1 mice dosed at 5 μmol/kg i.v. and 13 μmol/kg p.o.; [f]plasma PK parameters, dosed at 2.5 μmol/kg i.v. and 5 μmol/kg p.o.; [g]plasma PK parameters, dosed at 2.2 μmol/kg i.v. and 4.5 μmol/kg p.o.; [h]blood PK parameters from 2 male SCID mice dosed at 2.3 μmol/kg i.v. and 5.6 μmol/kg p.o.

Lack of activity of **15** and **16** in an MCF7 breast tumor cell antiproliferation assay used historically to determine antioestrogenic activity[42,43] was consistent with an agonist profile. In contrast with prototypic downregulators **3a-b**, earlier compounds **8** and **9b** also showed no effect in the MCF7 antiproliferation assay. On closer examination of the PR agonism data, at the top concentration in the assay **8**, **9b** and related compounds showed evidence of weak agonism (typically 15-30% inhibition at 100 μM), whereas **3a-b** gave no detectable inhibition at the highest concentration. We therefore concluded that whereas **3a-b** had a pure downregulator-antagonist profile,[12] the lack of antiproliferative activity of **8** and **9b** in the MCF7 cell line could reflect partial agonism.[40,41]

An overlay of the crystal structures of **3a** and the original screen hit **7b** bound to the ERα ligand binding domain (Figure 3) showed the acrylic acid moiety of **3a** colocalizing with Asp-351 in the Helix-12 region of the ER, in an unusual acid-acid interaction that has been proposed to be crucial for achieving a downregulator-antagonist profile.[12] Hybridization of compound **8** with **3a** thus gave the moderately potent downregulator-antagonist **22b**, with no detectable agonism at the highest test concentration and ER-downregulation and antagonism activity that translated well into the MCF7 antiproliferative assay.

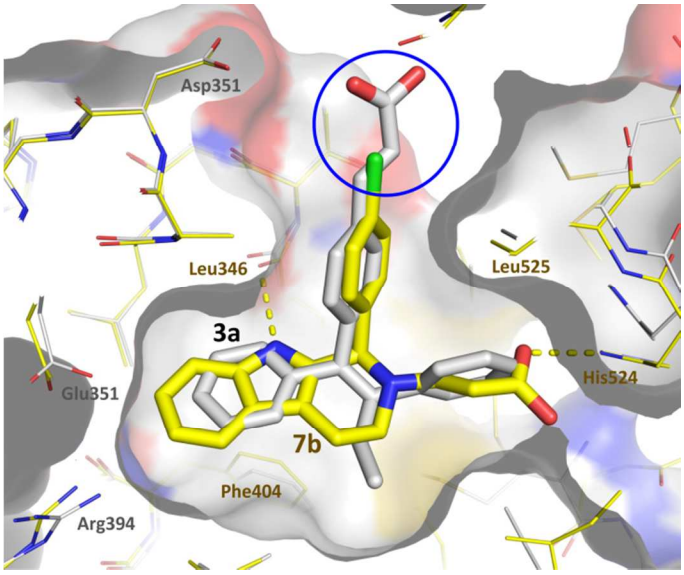


Figure 3. Crystal structure of **7b** (yellow, pdb code **5AAU**) superimposed on reference structure **3a** (grey, pdb code **5AAV**) bound to the ERα ligand binding domain construct.[31] The blue circle indicates how the acrylic acid moiety of **3a** occupies a region of the ER Helix-12 not accessed by compound **7b**.

Potency could be further improved by homologation of the N-substituent and, as seen previously, by introduction of a methyl group to occupy the additional lipophilic pocket (compounds **23b-24b**). Due to the presence of the acrylic acid and alcohol functionalities, these compounds were relatively hydrophilic (e.g. measured LogD value of 1.6 for **23b**) and consequently cellular potency could be significantly increased by removal of the alcohol moiety. By way of illustration, compounds **27b** and compound **3a** share similar profiles in terms of ER downregulation and PR agonism (Figure 4).

Compound **27b** showed an encouraging overall profile (Table 2) and excellent PK parameters across three species (Table 3). Oral exposure scaled well to higher doses in all three species (data not shown). Based on efficacy seen in an MCF7 mouse tumour xenograft model[43] at an oral once daily dose of 5 mg/kg, a preliminary predicted efficacious human dose was derived from an in-house physiologically-based pharmacokinetic (PBPK) model.[44] A predicted once daily dose of 1000 mg prompted us to seek further potency in order to reduce the predicted dose.

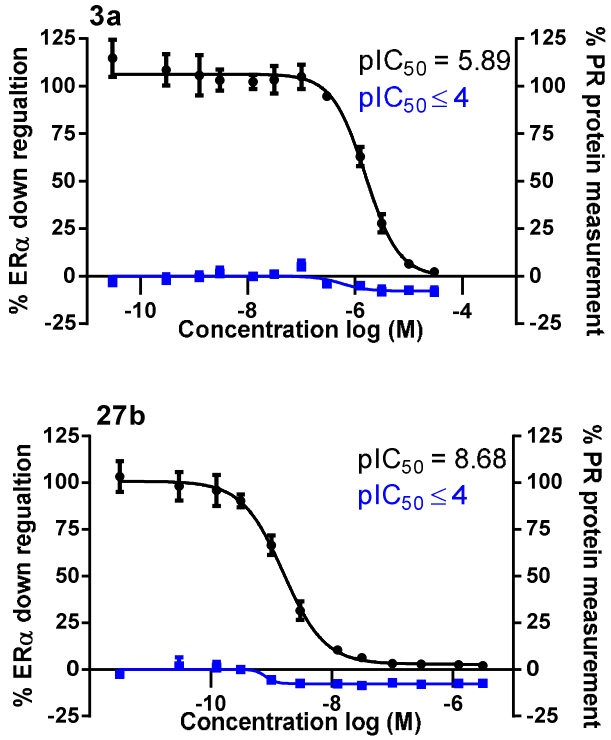


Figure 4. ER downregulation (black) and PR protein levels (blue) for **3a** and **27b** in the MCF7 cell line**.**

Previous SAR and ER co-crystallisation data suggested limited scope for improving potency through major structural changes, but from wider medicinal precedent[45] we reasoned that relatively small changes such as introduction of fluorine atom(s) could deliver the requisite increase. Of a number of variations tried, *ortho* di-substitution on the pendant aryl ring (**28b**) and monofluorination of the N-isobutyl substituent (**29b**) each provided an approximate 10-fold increase in potency across the ER binding, downregulation, antagonism and antiproliferation assays. Combining these structural changes gave optimised compound **30b**, with minimal increase in LogD relative to **27b** and potency comparable to fulvestrant (**2**). As shown in Figure 5, an overlay of **30b** and **3a** bound to the ER construct is consistent with the earlier overlays depicted in Figures 2 and 3.

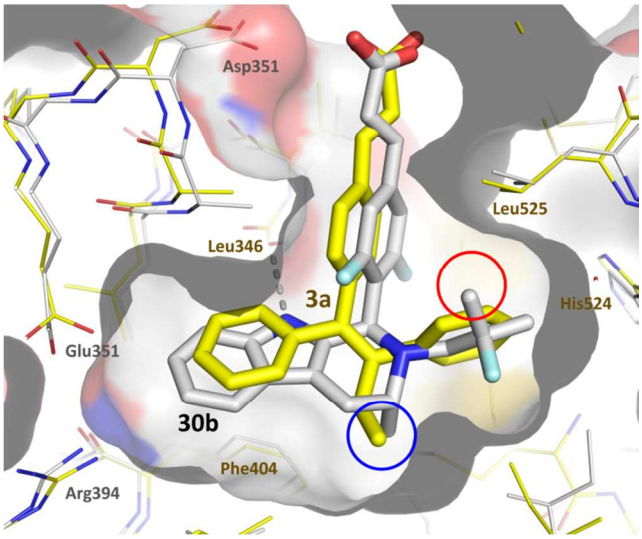


Figure 5. Depiction of **30b** (grey, pdb code **5ACC**) superimposed on reference structure **3a** (yellow, pdb code **5AAV**) bound to the ERα ligand binding domain construct.[31] Note the lipophilic holes (Phe-404:Phe-425 blue circle and Leu-525:Leu384 red circle) are filled with methyl subsitituents increasing potency for compound **30b**.

Detailed pharmacological characterisation of compound **30b**, including demonstration of ER and PR knockdown in cellular assays is described elsewehere,[46] but, for the purpose of this report, Figures 6a-c display curves from ER downregulation (Figure 6a), tamoxifen competition (Figure 6b) and antagonism (Figure 6c) assays that are wholly consistent with the desired ER downregulator-antagonist profile and comparable to fulvestrant (**2**) (downregulation curve also shown in Figure 6a).[2]

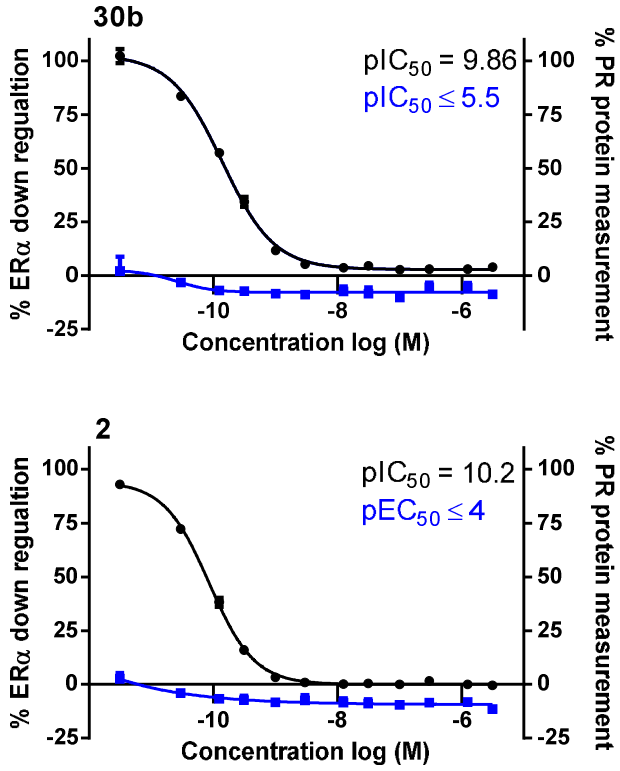


Figure 6a. ER downregulation (black) and PR protein levels (blue) for **30b** and **2** in the MCF7 cell line, showing no detectable agonism.

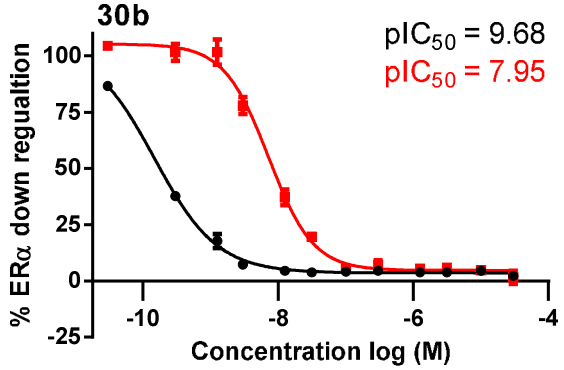


Figure 6b. ER downregulation curve (black) for **30b** and decrease in potency in the presence of 0.25 μM tamoxifen (red) due to competition at the ER ligand binding site.

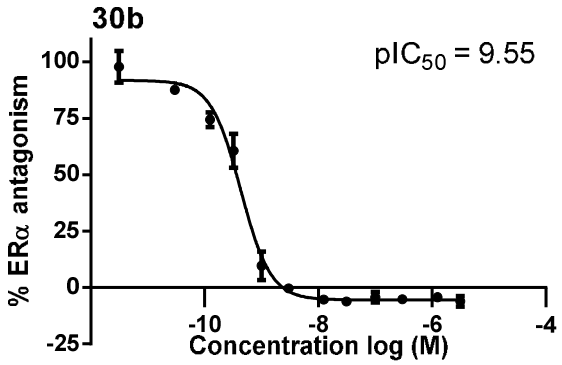


Figure 6c. Dose-dependent decrease in estradiol-driven PR response for **30b** as a measure of ER antagonism.

Compound **30b** showed an overall property profile (Table 2) broadly comparable to progenitor **27b** while maintaining excellent cross-species PK (Table 3). The significant degradation of ERα by Compound **30b** in the in cell western assay was confirmed in the Western blot assay with both Compound **2** (Fulvestrant) and estradiol (E2) as comparator compounds (Figure 7). Selectivity of compound **30b** over other tested nuclear hormone receptors is high: androgen receptor (AR); IC_{50} 30 μM, glucocorticoid receptor (GR); IC_{50} 9.2 μM and progesterone receptor (PR); IC_{50} 0.54 μM (c.f. estrogen receptor (ERα); IC_{50} 0.0008 μM). When tested in an MCF7 mouse tumour xenograft model, compound **30b** showed dose-dependent inhibition of tumour growth (Figure 8). Input of the 24 hour minimum efficacious concentration into the PBPK model[44] gave a predicted once daily human dose of below 100 mg, a value significantly lower than the maximum absorbable dose (MAD) calculated using a gastrointestinal simulated model.[47] Compound **30b** was also tested in a long term estrogen deprived model (LTED), using the HCC-1428 LTED cell line that grows in the absence of estrogen and is thought to best represent a model of aromatase inhibition.[48] Compound **30b** shows significant activity, with a dose of 5 mg/kg giving tumor regressions in this model (Figure 1 and 2, supporting information).[46]

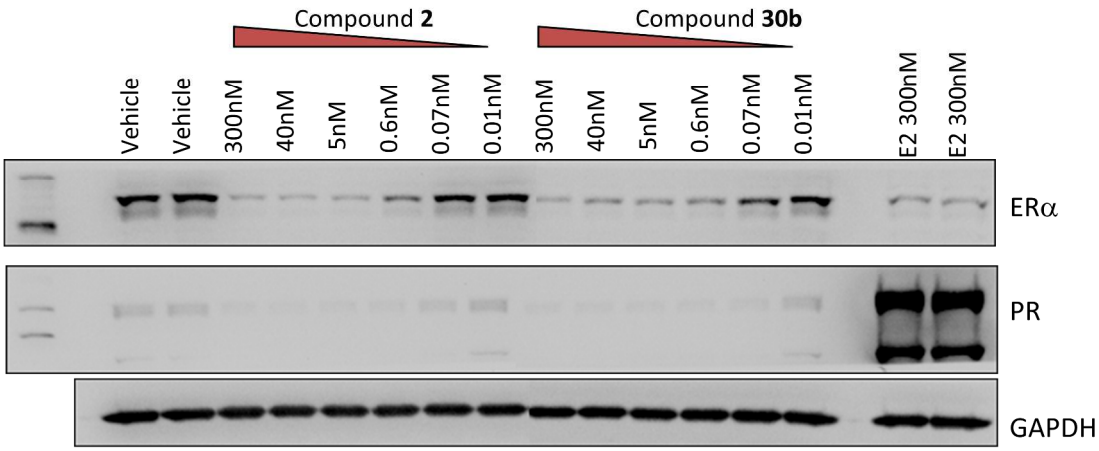


Figure 7. Degradation of ERα by Compound **30b**, Compound **2** and estradiol (E2) by Western blot analysis.[49] MCF-7 cells were incubated with Compound **2**, Compound **30b** or estradiol at the doses above. Total protein was extracted after 48 hours incubation with compound and immunoblotting performed to detect effects on ERα and PR levels.

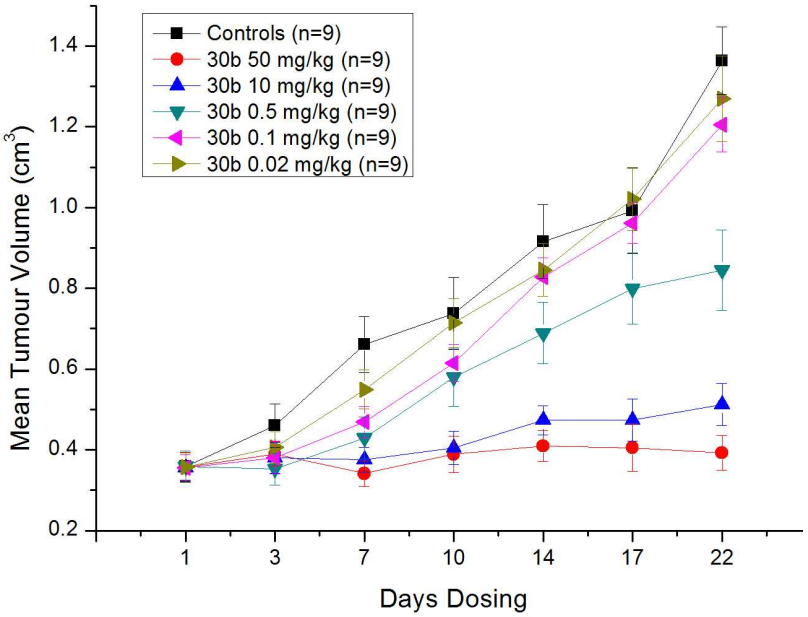


Figure 8. Dose-dependent antitumor activity of **30b** administered orally once daily in an MCF7 mouse tumor xenograft model.[41]

CONCLUSIONS

A directed screen identified the 1-aryl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole motif as a novel, druglike ER ligand. Aided by crystal structures of novel ligands bound to an ER construct, medicinal chemistry iterations led to ER downregulator **30b**, an orally bioavailable clinical candidate with comparable potency and pharmacological profile to the intramuscular SERD fulvestrant. AZD9496 is currently being evaluated in Phase I clinical trials in patients with advanced estrogen receptor (ER) positive breast cancer. More in-depth data from antitumour models, including demonstration of ER and PR knockdown *in vivo* is reported elsewhere.[46]

EXPERIMENTAL SECTION

General synthesis protocols

Operations were carried out at ambient temperature, i.e. in the range 17 to 25°C and under an atmosphere of an inert gas such as nitrogen, unless otherwise stated. Work up procedures were carried out after removal of residual solids by filtration. Microwave reactions were carried out using a Biotage® Initiator unless otherwise stated. Evaporations were carried out under reduced pressure at below 50°C by rotary evaporation or by using a Biotage v10 evaporator or Genevac equipment. Flash chromatography was performed on an automated Teledyne Isco CombiFlash® Rf or Teledyne Isco CombiFlash® Companion®, using prepacked RediSep Rf Gold™ Silica Columns (20-40 μm, spherical particles), GraceResolv™ Cartridges (Davisil® silica) or Silicycle cartridges (40 - 63 μm). Preparative high performance liquid chromatography (HPLC) was performed on a Gilson instrument using Waters Xbridge C18 reversed-phase silica gel, eluting with a decreasingly polar gradient of water containing 1% ammonium hydroxide and acetonitrile. Ion exchange purification was performed using an SCX-2 (Biotage, propylsulfonic acid functionalized silica, manufactured using a trifunctional silane, non end-capped) cartridge. ^{1}H NMR spectra were recorded on Bruker DPX400 instrument at 30 °C unless otherwise stated and are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Mass spectra (MS) were recorded on a Waters ZQ ESCi mass spectrometer scanned over an appropriate mass range. Compound purity was assessed by ^{1}H NMR and LCMS run on a Waters Alliance HT (2795) fitted with a Waters ZQ ESCi mass spectrometer and a Phenomenex Gemini-NX

(50x2.1 5μm) column. Accurate masses were determined by high resolution mass spectroscopy (HRMS) using a Waters Xevo G2 Qtof in ESI +ve ion mode.

1-(4-Chlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4b]indole-2-ethanol (6). A solution of 1-(4-chlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole[28] (200 mg, 0.71 mmol), 2-bromoethanol (0.15 mL, 2.12 mmol) and triethylamine (0.30 mL, 2.12 mmol) in MeCN (1.5 mL) was sealed in a microwave tube. The solution was heated to 140°C for 3 hours in a microwave reactor, then evaporated to dryness. The residue was purified by reverse phase chromatography, using decreasingly polar mixtures of water (containing 1% ammonium hydroxide) and MeCN as eluents to give **6** (101 mg, 44%) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO d_6) 10.33 (1H, s), 7.46 - 7.48 (1H, m), 7.42 - 7.46 (2H, m), 7.36 - 7.4 (2H, m), 7.26 (1H, dt), 7.06 (1H, ddd), 7.00 (1H, ddd), 4.83 (1H, s), 4.43 (1H, t), 3.60 (1H, ddd), 3.54 (1H, td), 3.15 - 3.22 (1H, m), 2.74 - 2.88 (3H, m), 2.65 (1H, dt), 2.57 - 2.6 (1H, m). HRMS *m/z* found 327.1282, $C_{19}H_{20}N_2OCl$ requires 327.1264.

Methyl 3-(1-(4-chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)propanoate (7a). A solution of 1-(4-chlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (**5a**)[28] (956 mg, 3.4 mmol), methyl 3-bromopropanoate (1.1 mL, 10.2 mmol) and triethylamine (1.4 mL, 10.2 mmol) in MeCN (1.5 mL) was sealed in a microwave tube. The solution was heated at 140 °C for 3 h in a microwave reactor, then evaporated to dryness and the residue was purified by reverse phase chromatography, elution gradient 55 to 100% MeCN in water containing 1% ammonium hydroxide, to give **7a** (950 mg, 76%) as a beige solid. ^{1}H NMR (400 MHz, DMSO d_6) 2.53 - 2.65 (2H, m), 2.65 - 2.89 (5H, m), 3.10 (1H, m), 3.57 (3H, s), 4.75 (1H, s), 6.99 (2H, m), 7.22 (1H, d), 7.27 - 7.33 (2H, m), 7.38 - 7.46 (3H, m), 10.30 (1H, s); *m/z* = 369, 371 $[M+H]^+$.

3-(1-(4-Chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)propanoic acid (7b). A 1.25 M solution of sodium hydroxide (5.2 mL, 6.3 mmol) was added to a stirred solution of **7a** (930 mg, 2.5 mmol) in THF (10 mL). The resulting solution was stirred at 65 °C for 2 h, then evaporated to dryness. The residue was purified by reverse phase chromatography, elution gradient 20 to 40% MeCN in water containing 1% ammonium hydroxide, to give **7b** (830 mg, 94%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.91 - 2.04 (1H, m), 2.1 - 2.25 (1H, m), 2.51 - 2.63 (1H, m), 2.63 - 2.86 (4H, m), 3.01 - 3.13 (1H, m), 4.68 (1H, s), 6.9 - 7.02 (2H, m), 7.19 (1H, d), 7.29 - 7.34 (2H, m), 7.34 - 7.38 (2H, m), 7.40 (1H, d), 10.24 (1H, s). HRMS *m/z* found 355.1201, $C_{20}H_{19}N_2O_2Cl$ requires 355.1212.

(1*R*,3*R*)-1-(4-Chlorophenyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (5b). 4-

Chlorobenzaldehyde (311 mg, 2.2 mmol) was added to a stirred slurry of (*R*)-1-(1H-indol-3-yl)propan-2-amine (**4b**) (385 mg, 2.2 mmol) in toluene (7 mL) under nitrogen. The resulting slurry was stirred at 105 °C for 30 minutes, then AcOH (0.70 mL) was added and heating was continued at 105 °C for 2 h. The reaction mixture was initially purified by ion exchange chromatography, eluting with 7M ammonia in MeOH. Product containing fractions were evaporated to dryness to give a brown gum, which was further purified flash chromatography, elution gradient 5 to 90% EtOAc in heptane, to give **5b** (330 mg, 50%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.13 (3H, d), 2.25 - 2.38 (1H, m), 2.55 - 2.65 (1H, br s), 2.78 (1H, dd), 2.93 (1H, m), 5.13 (1H, s), 6.93 - 7 (1H, m), 7 - 7.06 (1H, m), 7.23 (2H, d), 7.26 (1H, d), 7.34 - 7.39 (2H, m), 7.42 (1H, d), 10.66 (1H, s); *m/z* = 295, 297 $[M-H]^-$.

2-((1*R*,3*R*)-1-(4-Chlorophenyl)-3-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanol (8). A solution of **5b** (70 mg, 0.24 mmol), 2-bromoethanol (0.017 mL, 0.24 mmol) and triethylamine (0.033 mL, 0.24 mmol) in MeCN (1.5 mL) was sealed in a microwave tube. The solution was heated at 140 °C for 1.5 h in a microwave reactor, then further quantities of 2-bromoethanol (0.017 mL, 0.24 mmol) and triethylamine (0.033 mL, 0.24 mmol) were added and heating continued at 140°C for 15 min. The solution was evaporated to dryness, and the residue was purified by reverse phase chromatography, elution gradient 45 to 70% MeCN in water containing 1% ammonium hydroxide, to give **8** (61 mg, 76%) as a cream colored solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.07 (3H, d), 2.32 - 2.44 (1H, m), 2.63 - 2.81 (2H, m), 3.14 (1H, m), 3.27 - 3.36 (1H, m) 3.46 - 3.62 (2H, m), 4.38 (1H, br s), 4.93 (1H, s), 6.96 (1H, m), 7.02 (1H, m), 7.25 (1H, d), 7.28 (2H, d), 7.36 (2H, d), 7.42 (1H, d), 10.56 (1H, s); HRMS *m/z* found 341.1412, $C_{20}H_{22}N_2OCl$ requires 341.1421.

Methyl 3-((1*R*,3*R*)-1-(4-chlorophenyl)-3-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)propanoate (9a). Obtained from **5b** in 72% yield by an analogous method to **7a**. ^{1}H NMR (400 MHz, DMSO d_6) 1.05 (3H, d), 2.52 - 2.63 (3H, m), 2.64 - 2.73 (2H, m), 2.93 - 3.04 (1H, m), 3.09 (1H, m), 3.61 (3H, s), 4.88 (1H, s), 6.97 (1H, m), 7.03 (1H, m), 7.22 (2H, d), 7.26 (1H, d), 7.35 - 7.40 (2H, m), 7.42 (1H, d), 10.65 (1H, s); *m/z* = 381, 383 $[M-H]^-$.

3-((1*R*,3*R*)-1-(4-Chlorophenyl)-3-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)propanoic acid (9b). Obtained from **9a** in 96% yield by an analogous method to **7b**. ^{1}H NMR (400 MHz, DMSO d_6) 1.06 (3H, d), 2.12 (2H, m), 2.55 (1H, m), 2.61 - 2.73 (2H, m), 2.77 - 2.92 (1H, m), 3.11 (1H, m), 4.81 (1H, s), 6.67 (1H, br m), 6.73 (1H, br m), 7.12 (1H, m), 7.18 - 7.31 (3H, m), 7.41 (2H, m), exchangeable protons not detected; HRMS *m/z* found 369.1358, $C_{21}H_{22}N_2O_2Cl$ requires 369.1370.

2-(1-(5-Methoxy-1H-indol-3-yl)propan-2-ylamino)ethanol (11). A mixture of 1-(5-methoxy-1H-indol-3-yl)propan-2-amine (1.82 g, 8.9 mmol), 2-bromoethanol (0.63 mL, 8.9 mmol) and potassium carbonate (1.23 g, 8.9 mmol) in MeCN (6 mL) in a sealed microwave tube was heated to 80 °C for 1 h in a microwave reactor, then further quantities of 2-bromoethanol (0.63 mL, 8.9 mmol) and potassium carbonate (1.23 g, 8.9 mmol) were added. Heating was continued at 80°C for 30 min. The mixture was evaporated to dryness and the residue was purified by reverse phase chromatography, elution gradient 20 to 40% MeCN in water containing 1% ammonium hydroxide, to give **11** (2.15 g, 53%) as a pale yellow gum. ^{1}H NMR (400 MHz, DMSO d_6) 0.97 (3H, d), 2.53 - 2.71 (4H, m), 2.76 (1H, dd), 2.87 (1H, m), 3.37 - 3.53 (2H, m), 3.76 (3H, s), 4.37 (1H, t), 6.71 (1H, dd), 6.98 (1H, d), 7.08 (1H, d), 7.22 (1H, d), 10.60 (1H, s); *m/z* = 249 $[M+H]^+$.

2-(*Trans*-1-(4-chlorophenyl)-6-methoxy-3-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanol (13).

A solution of **11** (1.88 g, 7.6 mmol) and 4-chlorobenzaldehyde (1.06 g, 7.6 mmol) in AcOH (20 mL) was heated at 80 °C for 7 h under nitrogen. The solution was evaporated to dryness, and the residue was purified by ion exchange chromatography, eluting with 7M ammonia in MeOH. Product containing fractions were evaporated to dryness to give the crude acetate derivative as an orange foam, which was dissolved in MeOH (5 mL), 2M NaOH (5 mL) added and the solution heated at 60^{o}C for 30 min. The solution was evaporated to dryness and the residue purified by reverse phase chromatography, elution gradient 45 to 70% MeCN in water containing 1% ammonium hydroxide, to give **13** (1.33 g, 47%) as a cream solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.06 (3H, d), 2.37 (1H, m), 2.45 - 2.56 (1H, m), 2.63 - 2.81 (2H, m), 3.07 - 3.22 (1H, m), 3.45 - 3.65 (2H, m), 3.76 (3H, s), 4.38 (1H, s), 4.91 (1H, s), 6.68 (1H, dd), 6.93 (1H, d), 7.14 (1H, d), 7.28 (2H, d), 7.33 - 7.4 (2H, m), 10.39 (1H, s); *m/z* = 369, 371 $[M-H]^-$.

(1*R*,3*R*)-1-(4-Chlorophenyl)-2-(2-hydroxyethyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-6-ol (15). A 1 M boron tribromide solution in DCM (4.8 mL, 4.8 mmol) was added dropwise to a stirred solution of **13** (1.18 g, 3.2 mmol) in DCM (30 mL) at -78 ºC. The resulting solution was stirred at -78 ºC for 30 min then at 20 ºC for 16 h. The solution was cooled to 0 ºC, quenched with water (10 mL) and diluted with DCM (10 mL). The aqueous phase was separated and passed through a SCX-2 column. Product containing fractions were combined and evaporated. The crude racemic product was purified by reverse phase chromatography, elution gradient 30 to 50% MeCN in water

containing 1% ammonium hydroxide. Product containing fractions were evaporated to dryness to give racemic **15** (960 mg, 85%) as a cream solid, of which 610 mg was purified by preparative chiral HPLC on a Lux cellulose 2 column (20 μm silica, 50 mm diameter, 250 mm length), eluting isocratically with 30% EtOH in heptane. Product containing fractions were evaporated to dryness to give the desired enantiomer as the first eluted product (228 mg). Further purification by reverse phase chromatography, elution gradient 35 to 60% MeCN in water containing 1% ammonium hydroxide, gave pure **15** (107 mg, 35% recovery) as a cream colored solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.05 (3H, d), 2.3 - 2.48 (2H, m), 2.61 (1H, dd), 2.71 (1H, m), 3.05 - 3.17 (1H, m), 3.43 - 3.61 (2H, m), 4.37 (1H, s), 4.88 (1H, s), 6.54 (1H, dd), 6.73 (1H, d), 7.03 (1H, d), 7.28 (2H, d), 7.33 - 7.39 (2H, m), 8.50 (1H, br s), 10.22 (1H, s); HRMS *m/z* found 357.1367, $C_{20}H_{22}N_2O_2Cl$ requires 357.1370.

(*S*)-Methyl 2-methyl-3-(trifluoromethylsulfonyloxy)propanoate (17). To a stirred solution of (*S*)-methyl 3-hydroxy-2-methylpropanoate (4.0 g, 34 mmol) in DCM (15 mL) at 0°C was added dropwise trifluoromethanesulfonic anhydride (6.84 mL, 41 mmol), followed by 2,6-lutidine (4.35 g, 41 mmol) dropwise over 5 min (caution large exotherm). The reaction mixture was stirred at 0 °C for 15 min, then diluted with DCM (50 mL). The solution was washed with 2M HCl (2 x 100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give **17** (8.46 g, 100%) as a pink liquid which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) 1.30 (3H, d), 2.86 - 3.07 (1H, m), 3.75 (3H, s), 4.56 (1H, dd), 4.69 (1H, dd).

(*S*)-Methyl 3-((*R*)-1-(1H-indol-3-yl)propan-2-ylamino)-2-methylpropanoate (12). A solution of **17** (607 mg, 2.4 mmol), **4b** (423 mg, 2.4 mmol) and *N,N*-diisopropylethylamine (0.93 mL, 5.3 mmol) in dioxane (10 mL) was purged with nitrogen for 5 min and then sealed in a microwave tube. The solution was heated at 80 °C for 40 min in a microwave reactor, cooled to RT and diluted with DCM (10 mL). The solution was washed sequentially with water (5 mL) and brine (5 mL), dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash silica chromatography, elution gradient 0 to 30% MeOH in DCM, to give **12** (525 mg, 79 %) as a pale yellow gum. ^{1}H NMR (400 MHz, DMSO d_6) 0.97 (3H, d), 1.03 (3H, d), 2.53 - 2.72 (3H, m), 2.78 (2H, dt), 2.83 - 2.93 (1H, m), 3.54 (3H, s), 6.94 - 7 (1H, m), 7.02 - 7.09 (1H, m), 7.13 (1H, d), 7.34 (1H, d), 7.51 (1H, d), 10.79 (1H, s), exchangeable amine N proton not detected; *m/z* = 275 $[M+H]^+$.

(*S*)-Methyl 3-((1*R*,3*R*)-1-(4-chlorophenyl)-3-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)-2-methylpropanoate (14). A solution of **12** (140 mg, 0.51 mmol) and 4-chlorobenzaldehyde (72 mg,

0.51 mmol) in toluene (4 mL) containing molecular sieves was stirred at 90 °C for 1 h. AcOH (1 mL) was added and heating continued for 5 h. The solution was evaporated to dryness and the residue was initially purified by ion exchange chromatography, eluting with 7M ammonia in MeOH. Product containing fractions were evaporated to dryness, and the residue further purified by reverse phase chromatography, elution gradient 60 to 100% MeCN in water containing 1% ammonium hydroxide, to give **14** (146 mg, 72%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.03 (6H, d), 2.3 - 2.39 (1H, m), 2.52 (1H, m), 2.63 (1H, dd), 2.81 - 2.96 (2H, m), 3.00 (1H, m), 3.67 (3H, s), 4.94 (1H, s), 6.95 - 7.02 (1H, m), 7.03 - 7.1 (1H, m), 7.25 (2H, d), 7.30 (1H, d), 7.38 (2H, d), 7.44 (1H, d), 10.71 (1H, s); *m/z* = 395, 397[M-H]$^-$.

(*S*)-3-((1*R*,3*R*)-1-(4-Chlorophenyl)-3-methyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)-2-methylpropanoic acid (16). Obtained from **14** in 93% yield by an analogous method to **7b**. ^{1}H NMR (400 MHz, DMSO d_6) 0.86 (3H, d), 1.01 (3H, d), 2.13 (1H, m), 2.23 (1H, m), 2.51 (1H, m), 2.77 (2H, m), 3.21 (1H, m), 4.79 (1H, s), 6.91 - 6.97 (1H, m), 6.97 - 7.03 (1H, m), 7.22 (1H, d), 7.31 (2H, d), 7.37 (2H, d), 7.40 (1H, d), 10.44 (1H, s), exchangeable carboxylic acid proton not detected; HRMS *m/z* found 383.1502, $C_{22}H_{24}N_2O_2Cl$ requires 383.1526.

(*E*)-Methyl 3-(4-formylphenyl)acrylate (18). 4-Bromobenzaldehyde (30 g, 162 mmol) and methyl acrylate (20.9 g, 243 mmol) were taken up in well degassed DMA (300 mL). Tri-o-tolylphosphine (4.9 g, 16.2 mmol), palladium(II) acetate (1.8 g, 8.1 mmol) and triethylamine (45.2 mL, 324 mmol) were added and the mixture was heated at 110 °C for 16 h. The mixture was poured into water (4 L) and the resulting precipitate was filtered off and dried. The solid was purified by flash chromatography, elution gradient 0 to 30% EtOAc in heptane, to give a solid which was triturated with heptane to give **18** (25.6 g, 83%) as a yellow crystalline solid. ^{1}H NMR (400 MHz, DMSO d_6) 3.75 (3H, s), 6.79 (1H, d), 7.72 (1H, d), 7.93 (4H, s), 10.03 (1H, s). No mass ion observed in LCMS.

(*E*)-Methyl 3-(4-((1*S*,3*R*)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (19). 18 (18.6 g, 97.6 mmol) was added to a stirred solution of **4b** (17 g, 97.6 mmol) in AcOH (250 mL) under nitrogen. The resulting solution was stirred at 80 °C for 2 h. The reaction mixture was evaporated to dryness, redissolved in DCM (500 mL), and the solution was washed sequentially with saturated $NaHCO_3$ (2 x 300 mL), 2 M NaOH (300 mL), water (300 mL), and saturated brine (300 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography, elution gradient 1 to 7% MeOH in DCM, to give **19** (25.1 g, 74%) as a beige foam, which contained 12% of the inseparable trans (1*R*,3*R*) epimer. ^{1}H NMR (400 MHz, DMSO d_6) 1.25

(3H, d), 2.37 - 2.48 (1H, m), 2.74 (1H, d), 3.12 (1H, s), 3.73 (3H, s), 5.18 (1H, s), 6.64 (1H, d), 6.97 (2H, dd), 7.19 (1H, d), 7.36 - 7.46 (3H, m), 7.64 - 7.75 (3H, m), 10.19 (1H, s), no NH observed; *m/z* = 347 $[M+H]^+$.

(*E*)-Methyl 3-(4-((1*R*,3*R*)-2-allyl-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (20). 19 (35 g, 101 mmol), allyl bromide (9.6 mL, 111 mmol) and *N,N*-diisopropylethylamine (19 mL, 111 mmol) were suspended in MeCN (160 mL). The mixture was purged with nitrogen for 5 min and sealed in a microwave tube. The reaction was heated at 140 °C for 3.5 h in a microwave reactor, then cooled to room temperature. The reaction mixture was evaporated to dryness, the residue was redissolved in DCM (100 mL) and the solution was washed sequentially with 1 M citric acid (100 mL), water (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography, elution gradient 0 to 20% EtOAc in heptane. Pure fractions were evaporated to dryness to afford a 50:50 mixture of **20** and the corresponding *cis* (1*S*,3*R*) epimer (10 g, 25 mmol, 26%) as a pale yellow solid. The epimeric mixture was dissolved in DCM (100 mL), TFA (5.6 mL, 76 mmol) was added, and the solution was stirred at 20 °C for 3 days under nitrogen. The reaction mixture was diluted cautiously with saturated $NaHCO_3$ solution (250 mL), and the organic layer was washed sequentially with water (250 mL) and brine (250 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash column chromatography, elution gradient 10 to 20% EtOAc in heptane, to give a 65:35 mixture of **20** and the corresponding cis (1*S*,3*R*) epimer (8.0 g, 82% recovery) as a pale yellow solid, which was used in the next stage without further separation. *m/z* = 387 $[M+H]^+$.

(*E*)-Methyl 3-(4-((1*R*,3*R*)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (21). Seven separate batches of the foregoing 65:35 mixture of **20** and the corresponding cis (1*S*,3*R*) epimer (2.0 g, 5.2 mmol) were each suspended with chlorotris(triphenylphosphine)rhodium(I) (Wilkinson's catalyst) (2.35 g, 2.5 mmol) in MeCN (12 mL) and water (2.4 mL). The mixtures were purged with nitrogen for 5 min, sealed in a microwave tube and heated to 100 °C for 60 min in the microwave reactor. The reaction mixtures were combined, evaporated to dryness and partitioned between DCM (200 mL) and saturated aqueous $NaHCO_3$ (200 mL). The organic layer was separated, washed sequentially with water (200 mL) and brine (200 mL), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by flash chromatography, elution gradient 0 to 5% methanol in DCM, to give **21** (7.0 g, 56%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.14 (3H, d), 2.27 - 2.4 (1H, m), 2.81 (1H, dd), 2.94 - 3.05 (1H, m), 3.72 (3H, s),

5.19 (1H, s), 6.60 (1H, d), 6.94 - 7 (1H, m), 7.01 - 7.09 (1H, m), 7.26 (3H, d), 7.43 (1H, d), 7.59 - 7.68 (3H, m), 10.70 (1H, s), NH not observed; *m/z* = 347 $[M+H]^+$.

(*E*)-Methyl 3-(4-((1*R*,3*R*)-2-((S)-3-hydroxy-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (23a). 21 (1.0 g, 2.9 mmol), (*R*)-3-bromo-2-methyl-1-propanol (0.45 mL, 4.3 mmol), *N,N*-diisopropylethylamine (1.51 mL, 8.7 mmol), and acetonitrile (12 mL) were degassed with nitrogen and sealed in a microwave tube. The mixture was heated at 135 ^oC overnight in a microwave reactor, cooled to room temperature, and evaporated to dryness. The residue was dissolved in DCM and filtered through a pad of silica gel, eluting with 0-50% ether in DCM to give **23a** (770 mg, 64%) as an off-white foam. ^1H NMR (400 MHz, DMSO d_6) 0.80 (3H, d), 1.07 (3H, d), 1.88 (1H, m), 2.14 (1H, dd), 2.54 - 274 (3H, m), 3.14 (1H, m), 3.32 (1H, m), 3.54 (1H, m), 3.72 (3H, s), 4.59 (1H, tr), 4.89 (1H, s), 6.60 (1H, d), 6.96 (1H, m), 7.02 (1H, m), 7.29 (3H, m), 7.42 (1H, d), 7.67 (3H, m), 10.60 (1H, s). *m/z* = 419 $[M+H]^+$.

(*E*)-3-(4-((1*R*,3*R*)-2-((*S*)-3-Hydroxy-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (23b). A solution of 2 M sodium hydroxide (1.31 mL, 9.8 mmol) was added to a solution of **23a** (412 mg, 0.98 mmol) in THF (10 mL), MeOH (2 mL) and water (2 mL). The mixture was stirred at room temperature for 4 h under nitrogen and the organics were removed by evaporation. The aqueous residue was applied to a SCX-2 column loaded with MeOH. Elution with 7 M ammonia in MeOH gave crude product, which was further purified by reverse phase chromatography, elution gradient 20 to 40% MeCN in water containing 1% ammonium hydroxide, to give **23b** (305 mg, 75%) as a solid. ^1H NMR (400 MHz, DMSO d_6) 0.80 (3H, d), 1.07 (3H, d), 1.89 (1H, m), 2.16 (1H, dd), 2.52 - 2.72 (3H, m), 3.18 (1H, m), 3.32 (1H, m), 3.54 (1H, m), 4.89 (1H, s), 6.49 (1H, d), 6.97 (1H, m), 7.03 (1H, m), 7.28 (3H, m), 7.45 (2H, m), 7.59 (2H, m), 10.60 (1H, s), carboxylic acid and hydroxyl protons seen in broad hump between 6 and 4 ppm. HRMS *m/z* found 405.2175, $C_{25}H_{29}N_2O_3$ requires 405.2178.

(*E*)-3-(4-((1*R*,3*R*)-2-(2-Hydroxyethyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (22b) was obtained by an analogous method to **23b** by alkylation of **21** with 2-bromoethanol to followed by hydrolysis of ester **22a (**see Supporting Information for **22a** characterization data). ^1H NMR (400 MHz, DMSO d_6) 1.07 (3H, d), 2.36 - 2.43 (1H, m), 2.52 - 2.54 (1H, m), 2.55 (1H, dt), 2.75 (2H, ddd), 3.13 - 3.2 (1H, m), 3.48 - 3.6 (2H, m), 4.38 (1H, s), 4.96 (1H,

s), 6.48 (1H, d), 6.97 (1H, td), 7.03 (1H, ddd), 7.22 - 7.28 (1H, m), 7.31 (2H, d), 7.43 (1H, d), 7.54 (1H, d), 7.61 (2H, d), 10.58 (1H, s). HRMS *m/z* found 377.1870, $C_{23}H_{25}N_2O_3$ requires 377.1865.

(*E*)-3-(4-((1*R*,3*R*)-2-((*R*)-3-hydroxy-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (24b) was obtained by an analogous method to **23b** by alkylation of **21** with (*S*)-3-bromo-2-methylpropan-1-ol followed by hydrolysis of ester **24a (**see Supporting Information for **24a** characterization data). ^{1}H NMR (400 MHz, DMSO d_6) 0.93 (3H, d), 1.05 (3H, d), 1.84 (1H, dt), 2.34 (2H, q), 2.48 (1H, m), 2.64 (1H, dd), 3.08 - 3.23 (2H, m), 3.28 (1H, dd), 4.80 (1H, s), 6.43 (1H, d), 6.92 - 6.98 (1H, m), 6.98 - 7.05 (1H, m), 7.17 - 7.25 (3H, m), 7.27 (1H, d), 7.40 (1H, d), 7.48 (2H, d), 10.67 (1H, s), exchangeable protons not detected. HRMS *m/z* found 405.2178, $C_{25}H_{29}N_2O_3$ requires 405.2178.

(*R*)-*N*-(1-(1H-Indol-3-yl)propan-2-yl)-2-methylpropan-1-amine (**25a**). Sodium triacetoxyborohydride (42.6 g, 201 mmol) was added in one portion to **4b** (25 g, 143 mmol) and isobutyraldehyde (14.4 mL, 158 mmol) in THF (500 mL) at 0 °C under nitrogen. The resulting mixture was stirred at 0 °C for 30 min, then quenched with saturated aqueous sodium bicarbonate (50 mL) and extracted with EtOAc (2 x 500 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography, elution gradient 0 to 5% 7M ammonia in MeOH in DCM, to give **25a** (14.6 g, 44.3 %) as a waxy yellow gum. ^{1}H NMR (400 MHz, DMSO d_6) 0.82 (6H, d), 0.98 (3H, d), 1.58 (1H, dt), 2.38 (2H, qd), 2.61 (1H, dd), 2.83 (2H, ddd), 6.93 - 7.01 (1H, m), 7.02 - 7.09 (1H, m), 7.13 (1H, d), 7.34 (1H, d), 7.52 (1H, d), 10.79 (1H, s); *m/z* = 231 $[M+H]^+$.

2-Fluoro-2-methylpropan-1-ol. Lithium aluminium hydride (3.37 g, 88.6 mmol) was added portionwise over 15 min to a solution of ethyl 2-fluoro-2-methylpropanoate (9.9 g, 73.8 mmol) in diethyl ether (185 mL) at 0°C. The mixture was stirred for 1 h, then water (3.3 mL), 15% NaOH solution (3.3 mL) and water (6.7 mL) were added sequentially. The suspension was stirred for 15 min, then filtered, and the solids were washed with diethyl ether. The filtrate was evaporated to give 2-fluoro-2-methylpropan-1-ol (5.90 g, 87 %) as a colourless oil which was used without purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) 1.37 (6H, d), 3.56 (2H, d), OH not observed.

2-Fluoro-2-methylpropyl trifluoromethanesulfonate (31). Trifluoromethanesulfonic anhydride (12.1 mL, 71.2 mmol) and 2,6-lutidine (11.4 mL, 81.4 mmol) were added to a solution of 2-fluoro-2-

methylpropan-1-ol (6.25 g, 67.8 mmol) in DCM (150 mL) at -10 °C. The solution was stirred for 1 h, washed with 2M HCl (2 x 100 mL) and saturated aqueous $NaHCO_3$ (2 x 100 mL), dried over Na_2SO_4 and concentrated to give **31** (12.9 g, 85%) as a red oil which was used without purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) 1.46 (6H, d), 4.41 (2H, d).

(*R*)-*N*-(1-(1H-Indol-3-yl)propan-2-yl)-2-fluoro-2-methylpropan-1-amine (25b). 31 (8.04 g, 35.9 mmol) was added to a solution of **4b** (5.0 g, 28.7 mmol) and N,N-diisopropylethylamine (7.4 mL, 43 mmol) in dioxane (50 mL). The solution was heated to 90 °C for 3 h, cooled to room temperature, diluted with EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 100 mL). The aqueous phase was extracted with EtOAc (150 mL), and the combined organics were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with EtOAc, to give **25b** (6.49 g, 91 %) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) 1.14 (3H, d), 1.31 (3H, d), 1.37 (3H, d), 1.94 (1H, s), 2.63 - 2.87 (3H, m), 2.92 (1H, dd), 3.07 (1H, h), 7.07 (1H, d), 7.08 - 7.15 (1H, m), 7.16 - 7.24 (1H, m), 7.37 (1H, d), 7.62 (1H, d), 8.04 (1H, s); *m/z* = 249 $[M+H]^+$.

(*E*)-Methyl 3-(3,5-difluoro-4-formylphenyl)acrylate (26). 4-Bromo-2,6-difluorobenzaldehyde (10 g, 45.2 mmol) and methyl acrylate (6.1 mL, 67.8 mmol) were taken up in well degassed DMA (100 mL) and tri-o-tolylphosphine (1.38 g, 4.5 mmol), palladium(II) acetate (0.51 g, 2.26 mmol) and triethylamine (12.6 mL, 90.4 mmol) were added. The mixture was stirred and heated at 80 °C for 6 h, then filtered through a layer of celite and washed with MeOH (50 mL). The filtrate was pre-absorbed onto silica and purified by suction chromatography, eluting with 0-10% ether in DCM. Fractions containing the desired product were evaporated, and the residue was triturated successively with ether (50 mL) and water (50 mL) before being dried under high vacuum at 50 °C to give **26** (8.85 g, 87%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO d_6) 3.75 (3H, s), 6.93 (1H, d), 7.52 - 7.81 (3H, m), 10.18 (1H, s). No mass ion observed in LCMS.

(*E*)-Methyl 3-(3,5-difluoro-4-((1*R*,3*R*)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylate (30a). 26 was added to a suspension of **25b** (6.02 g, 24.2 mmol) in toluene (50 mL) and AcOH (2.8 mL, 48.5 mmol). The mixture was heated to 80 °C for 5 h and then loaded onto an SCX-2 column, eluting with 7 M ammonia in MeOH. Product containing fractions were evaporated to dryness and the residual solid purified by flash chromatography, elution gradient 0 to 30% EtOAc in heptane, to give **30a** (7.52 g, 68%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO d_6, 100°C) 1.10 (3H, d), 1.12 - 1.31 (6H, m), 2.28 - 2.72 (2H, m), 2.84 - 3.09 (2H, m), 3.52 -

3.69 (1H, m), 3.76 (3H, s), 5.30 (1H, s), 6.64 (1H, d), 6.9 - 7.11 (2H, m), 7.21 (1H, d), 7.32 (2H, d), 7.42 (1H, d), 7.58 (1H, d), 10.14 (1H, s); *m/z* = 457 $[M+H]^+$.

(*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (30b). A 7.5 M solution of sodium hydroxide (32.9 mL, 247 mmol) was added to a solution of **30a** (11.28 g, 24.7 mmol) in THF (143 mL) and MeOH (71 mL). The mixture was stirred at room temperature for 4 h. The pH was adjusted to 6.5 by addition of 2 M HCl solution, then the solution was extracted with diethyl ether (3 x 150 mL). The combined organics were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography, elution gradient 0 to 20% MeOH in DCM to give a yellow solid. Attempted trituration with acetone/heptane failed due to higher than expected solubility. The solvents were removed to give a yellow solid which was triturated in isohexane (50 mL) with a few drops of ether, and the resulting solid was filtered off and dried to give a yellow powder (11.1 g). The solid was dissolved in EtOH (100 mL) under nitrogen and in the dark. The solution was evaporated to 5 mbar using a vacuum pump at 62 ˚C in the dark. This procedure was repeated twice and the resulting yellow glass scratched with a spatula into a fine powder and subjected to 5 mbar using a vacuum pump at 62 ˚C for 60 min to give a yellow powder. The powder was then left in a vacuum oven over P_2O_5 at 62 ˚C at 300 mbar overnight to give **30b** (9.77 g, 89%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO d_6) 1.07 - 1.16 (3H, m), 1.18 - 1.29 (6H, m), 2.39 (1H, dd), 2.62 (1H, dd), 2.92 (2H, dd), 3.56 (1H, d), 5.26 (1H, s), 6.70 (1H, d), 7.02 (2H, dd), 7.22 (1H, d), 7.47 (3H, dd), 7.58 (1H, d), 10.60 (1H, s), 12.60 (1H, s); HRMS *m/z* found 443.19394, $C_{20}H_{17}ON_4$ requires 443.19409.

Compounds **27b-29b** were obtained by hydrolysis of **27a-29a,** in turn obtained by analogous routes to **30b** (see Supporting Information for characterization of intermediates **27a-29a**).

(*E*)-3-(4-((1*R*,3*R*)-2-isobutyl-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (27b): ^{1}H NMR (400 MHz, $CDCl_3$) 0.80 (3H, d), 0.93 (3H, d), 1.08 (3H, d), 1.80 (1H, dt), 2.14 (1H, dd), 2.36 (1H, dd), 2.61 (1H, dd), 2.88 (1H, dd), 3.33 (1H, q), 4.72 (1H, s), 6.41 (1H, d), 7.12 (2H, pd), 7.24 (1H, d), 7.35 (2H, d), 7.46 (2H, t), 7.5 - 7.56 (1H, m), 7.76 (1H, d), exchangeable protons not observed; HRMS *m/z* found 389.2248, $C_{25}H_{29}N_2O_2$ requires 389.2229.

(*E*)-3-(3,5-Difluoro-4-((1*R*,3*R*)-2-isobutyl-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (28b): ^{1}H NMR (400 MHz, DMSO d_6) 0.68 (3H, d), 0.82 (3H, d), 1.04 (3H, d),

1.64 - 1.76 (1H, m), 2.05 (1H, dd), 2.43 (1H, dd), 2.58 (1H, dd), 2.88 (1H, dd), 3.40 (1H, h), 5.10 (1H, s), 6.68 (1H, d), 6.95 (1H, td), 7.00 (1H, td), 7.20 (1H, d), 7.39 - 7.45 (3H, m), 7.49 (1H, d), 10.55 (1H, s). HRMS *m/z* found 425.2052, $C_{25}H_{27}N_2O_2F_2$ requires 425.2041.

(*E*)-3-(4-((1*R*,3*R*)-2-(2-Fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (29b): ^{1}H NMR (400 MHz, DMSO d_6) 1.06 (3H, d), 1.30 (3H, d), 1.47 (3H, d), 2.53 - 2.64 (2H, m), 2.79 (2H, s), 3.10 (1H, d), 5.08 (1H, s), 6.47 (1H, d), 6.98 (1H, t), 7.06 (1H, t), 7.19 - 7.37 (3H, m), 7.44 (1H, d), 7.56 (1H, d), 7.63 (2H, d), 10.81 (1H, s), 12.30 (1H, s). HRMS *m/z* found 407.2144, $C_{25}H_{28}N_2O_2F$ requires 407.2135.

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SUPPORTING INFORMATION AVAILABLE

SEM values for data in Table 1 and characterization data for precursors **22a, 24a, 27a-29a** (Table 1), selected pre-clinical comparator data between compounds **30b** and **3c** (Table 2), and *in vivo* activity of compound **30b** in a long term estrogen deprived (LTED) model are available (Figure 1 and Figure 2, supporting information) free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Jordan, V. C. Tamoxifen: a most unlikely pioneering medicine. *Nature Rev. Drug Discovery* **2003**, *2*, 205-213.

2. Ariazi, E. A.; Jordan, V. C. Estrogen receptors as therapeutic targets in breast cancer. In Nuclear Receptors as drug targets. Eckhard, O.; Weinmann, H.; Eds. Wiley-VCH Verlag GmbH: Weinheim, Germany, 2008; pp 127-199.

3. (a) Morris, C.; Wakeling, A. E. Fulvestrant ("Faslodex") – a new treatment option for patients progressing on prior endocrine therapy. *Endocrine-Related Cancer* **2002**, 267-276. (b) Nephew, K. P.; Long, X. Fulvestrant (ICI 182,780)-dependent interacting proteins mediate immobilization and degradation of estrogen receptor-α. *J. Biol. Chem.* **2006**, *14*, 9607-9615

4. Johnson, S. R. D. Fulvestrant. *Curr. Opin. Investig. Drugs* **2002**, *3*, 305-312.

5. Scott, S. M.; Brown, M.; Come, S. E. Emerging data on the efficacy and safety of fulvestrant, a unique antiestrogen therapy for advanced breast cancer. *Exp. Opin. Drug Safety* **2011**, *10*, 819-826.

6. (a) Damodaran, S.; Majumder, S.; Ramaswamy, B. Endocrine resistance: mechanisms and therapeutic targets. *Clin. Investig.* **2013**, *3*, 681-690. (b) Li, S.; Shen, D.; Shao, J.; Crowder, R.; Liu, W.; Prat, A. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep*. **2013**, *4*, 1116-30. (c) Toy, W.; Shen, Y.; Won, H.; Green, B.; Sakr, R. A.; Will, M. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet* **2013**, *45*, 1439-45.

7. Robertson, J. F. R.; Harrison, M. Fulvestrant: pharmacokinetics and pharmacology. *Br. J. Cancer* **2004**, *90* (Suppl 1), S7-S10.

8. Howell, A.; Sapunar, F. Fulvestrant revisited: efficacy and safety of the 500-mg dose. *Clin. Breast Cancer* **2011**, *11*, 204-210.

9. Wardell, S. E.; Marks, J. R.; McDonnell, D. P. The turnover of estrogen receptor α by the selective estrogen receptor degrader (SERD) fulvestrant is a saturable process that is not required for antagonist efficacy. *Biochem. Pharmacol.* **2011**, *82*, 122-130.

10. Willson, T. M.; Henke, Brad R.; Momtahen, T. M.; Charifson, Paul S.; Batchelor, Kenneth W.; Lubahn, Dennis B.; Moore, Linda B.; Oliver, Beverly B.; Sauls, Howard R.; Triantafillou, J. A.; Wolfe, S. G.; Baer, P. G. 3-[4-(1,2-Diphenylbut-1-enyl)phenyl]acrylic Acid: A Non-Steroidal Estrogen with Functional Selectivity for Bone over Uterus in Rats. *J. Med. Chem*. **1994**, *37*, 1550-1552.

11. Willson, T. M.; Norris, J. D.; Wagner, B. L.; Asplin, I.; Baer, P.; Brown H. R.; Jones S. A.; Henke, B.; Sauls, H.; Wolfe, S.; Morris, D. C.; McDonnell, D. P. Dissection of the molecular mechanism of action of GW5638, a novel estrogen receptor ligand, provides insights into the role of estrogen receptor in bone. *Endocrinology* **1997**, *138*, 3901-3911.

12. Wu Y-L.; Yang X.; Ren Z.; McDonnell D. P.; Norris J. D.; Willson T. M.; Greene G. L. Structural basis for an unexpected mode of SERM-mediated ER antagonism. *Mol. Cell* **2005**, *18*, 413-424

13. Kanbe, Y.; Kim, M-H.; Nishimoto, M.; Ohtake, Y.; Yoneya, T.; Ohizumi, I.; Tsunenari, T.; Taniguchi, K.; Kaiho, S-I.; Nabuchi, Y.; Araya, H.; Kawata, S.; Morikawa, K; Jo, J-C; Kwon, H-E.; Limb, H-S.; Kim, H-K. Newly discovered orally active pure antiestrogens. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4959-4964.

14. Hoffmann, J.; Bohlmann, R.; Heinrich, N.; Hofmeister, H.; Kroll, J.; Kuenzer, H.; Lichtner, R. B.; Nishino, Y.; Parczyk, K.; Sauer, G.; Gieschen, H.; Ulbrich, H-F.; Martin, R.; Schneider, M. R.

Characterization of new estrogen receptor destabilizing compounds: effects on estrogen-sensitive and tamoxifen-resistant breast cancer. *J. Nat. Cancer Inst.* **2004**, *96*, 210-218.

15. Tan, Q.; Blizzard, T. A.; Morgan, J. D.; Birzin, E. T.; Chan, W.; Yang, Y. T.; Pai, L-Y.; Hayes, E. C.; DaSilva, C. A.; Warrier, S.; Yudkovitz, J.; Wilkinson, H. A.; Sharma, N.; Fitzgerald, P. M. D.; Li, S.; Colwell, L.; Fisher, J. E.; Adamski, S.; Reszka, A. A.; Kimmel, D.; DiNinno, F.; Rohrer, S. P.; Freedman, L. P.; Schaeffer, J. M.; Hammond, M. L. Estrogen receptor ligands. Part 10: Chromanes: old scaffolds for new SERAMs. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1675-1681

16. Hoekstra, W. J.; Patel, H. S.; Liang, X.; Blanc, J-B. E.; Heyer, D. O.; Willson, T. M.; Iannone, M. A.; Kadwell, S. H.; Miller, L. A.; Pearce, K. H.; Simmons, C. A.; Shearin, J. Discovery of novel quinoline-based estrogen receptor ligands using peptide interaction profiling. *J. Med. Chem*. **2005**, *48*, 2243-2247.

17. Yoneya, T.; Taniguchi, K.; Nakamura, R.; Tsunenari, T.; Ohizumi, I.; Kanbe, Y.; Morikawa, K.; Kaiho, S-I.; Yamada-Okabe, H. Thiochroman derivative CH4986399, a new nonsteroidal estrogen receptor down-regulator, is effective in breast cancer models. *Anticancer Res.* **2010**, *30*, 873-878.

18. Suzuki, N.; Liu, X.; Laxmi, Y. R. S.; Okamoto, K.; Kim, H. J.; Zhang, G.; Chen, J. J.; Okamoto, Y.; Shibutani, S. Anti-breast cancer potential of SS5020, a novel benzopyran antiestrogen. *Int. J. Cancer* **2011**, *128*, 974-982.

19. Lai, A.; Kahraman, M.; Govek, S.; Nagasawa, J.; Bonnefous, C.; Julien, J.; Douglas, K.; Sensintaffar, J.; Lu, N.; Lee, K.; Aparicio, A.; Kaufman, J.; Qian, J.; Shao, G.; Prudente, R.; Moon, M. J.; Joseph, J. D.; Darimont, B.; Brigham, D.; Grillot, K.; Heyman, R.; Rix, P. J.; Hager, J. H.; Smith, N. D. Identification of GDC-0810 (ARN-810), an Orally Bioavailable Selective Estrogen Receptor Degrader (SERD) that Demonstrates Robust Activity in Tamoxifen-Resistant Breast Cancer Xenografts *J. Med. Chem.* **2015**, Article ASAP (**DOI:** 10.1021/acs.jmedchem.5b00054).

20. Rosati, R. L.; Jardine, P. D. S.; Cameron, K. O.; Thompson, D. D.; Ke, H Z.; Toler, S. M.; Brown, T. A.; Pan, L. C.; Ebbinghaus, C. F.; Reinhold, A. R.; Elliott, N. C.; Newhouse, B. N.; Tjoa, C. M.; Sweetnam, P. M.; Cole, M. J.; Arriola, M. W.; Gauthier, J. W.; Crawford, D. T.; Nickerson, D. F.; Pirie, C. M.; Qi, H.; Simmons, H. A.; Tkalcevic, G. T. Discovery and preclinical pharmacology of a novel, potent, nonsteroidal estrogen receptor agonist/antagonist, CP-336156, a diaryltetrahydronaphthalene. *J. Med. Chem.* **1998**, *41*, 2928-2931.

21. Phillips, D. H. Understanding the genotoxicity of tamoxifen. *Carcinogenicity* **2001**, *22*, 839-849.

22. Luniwal, A.; Jetson, R.; Erhardt, P. Selective estrogen receptor modulators. In Analogue-Based Drug Discovery III. Fischer, J.; Ganellin, C. R.; Rotella, D. P. Eds. Wiley-VCH Verlag GmbH: Weinheim, Germany, 2013; pp 165-185.

23. Blizzard, T. A. Selective estrogen receptor modulator chemistry at Merck. A review. *Curr. Top. Med. Chem.* **2008**, *8*, 792-812

24. Gennari, L.; Merlotti, D.; Stolakis, K.; Nuti, R. Lasofoxifene, from the preclinical drug discovery to the treatment of postmenopausal osteoporosis. *Exp. Opin. Drug Discovery* **2011**, *62*, 205-217.

25. Cox, E. D.; Cook, J. M. The Pictet-Spengler condensation: a new direction for an old reaction. *Chem. Rev.* **1995**, *95*, 1797-842.

26. Lorenz, M.; Van Linn, M. L.; Cook, J. M. The asymmetric Pictet-Spengler reaction. *Curr. Org. Synth*. **2010**, *7*, 189-223.

27. Stockigt, J.; Antonchick, A. P.; Wu, F.-R.; Waldmann, H. The Pictet-Spengler Reaction in Nature and in Organic Chemistry. *Angew. Chem. Int. Ed.* **2011**, *50*, 8538-8564.

28. Ivanov, I.; Nikolova, S.; Statkova-Abeghe, S. A simple method for the synthesis of 1-substituted β-carboline derivatives from tryptamine and carboxylic acids in polyphosphoric acid. *Heterocycles* **2005**, *65*, 2483-2492.

29. Callis, R.; Rabow, A.; Tonge, M.; Bradbury, R.; Challinor, M.; Roberts, K.; Jones, K.; Walker, G. A screening Assay Cascade to Identify and Characterize Novel Selective Estrogen Receptor Downregulators (SERDs). *J. Biomol. Screen*. **2015**, just accepted (DOI: 10.1177/1087057115580298)

30. Cut off > 36% inhibition, corresponding to 3 standard deviations from inactive compounds centred around 0% inhibition.

31. Degorce, S.; Bailey, A.; Callis, R.; De Savi, C.; Ducray, R.; Lamont, G.; MacFaul, P.; Maudet, M.; Martin, S.; Morgentin, R.; Norman, R. A.; Peru, A.; Pink, J. H.; Ple, P. A.; Roberts, B.; Scott, J. S. Investigation of (E)-3-[4-(2-Oxo-3-aryl-chromen-4-yl)oxyphenyl]acrylic Acids as Oral Selective Estrogen Receptor Down-Regulators *J. Med. Chem.* **2015**, *58*, 3522-3533.

32. van de Waterbeemd, H.; Lennernas, H.; Artursson, P.; Mannhold, R.; Kubinyi, H.; Folkers, G.; Eds. In *Drug Bioavailability: Estimation of Solubility, Permeability, Absorption and Bioavailability*. Methods and Principles in Medicinal Chemistry, Vol. 18; Wiley-VCH Verlag GmbH: Weinheim, Germany, 2003; pp 3-242.

33. Daugan, A.; Grondin, P.; Ruault, C.; Le Monnier de Gouville, A.-C.; Coste, H.; Linget, J. M.; Kirilovsky, J.; Hyafil, F.; Labaudiniere, R. The discovery of tadalafil: a novel and highly selective PDE5 inhibitor. 2: 2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione analogues. *J. Med. Chem.* **2003**, *46*, 4533-4542.

34. Yeung, B. K. S.; Zou, B.; Rottmann, M.; Lakshminarayana, S. B.; Ang, S. H.; Leong, S. Y.; Tan, J.; Wong, J.; Keller-Maerki, S.; Fischli, C.; Goh, A.; Schmitt, E. K.; Krastel, P.; Francotte, E.; Kuhen, K.; Plouffe, D.; Henson, K.; Wagner, T.; Winzeler, E. A.; Petersen, F.; Brun, R.; Dartois, V.; Diagana,

T. T.; Keller, T. H. Spirotetrahydro β-Carbolines (Spiroindolones): A New Class of Potent and Orally Efficacious Compounds for the Treatment of Malaria. *J. Med. Chem.* **2010**, *53*, 5155-5164.

35. http://www.clinicaltrials.gov/ct2/results?term=PTC299

36. Hwang, P. S.; Moon, Y.-C.; Arasu, T.; Qi, H.; Almstead, N. Process for the preparation chiral tetrahydro-β-carbolines via resolution. PCT Int. Appl. WO 2010138644.

37. Almstead, N.; Arasu, T.; Choi, S.; Cao, L.; Campbell, J.; Corson, D.; Davis, T. W.; Graci, J. D.; Gu, Z.; Hwang, P. S.; Lennox, W.; Miao, H. H.; Miller, L.; Moon, Y.-C.; Qi, H.; Trotta, C.; Weetall, M. L. Preparation of phenyltetrahydropyridoindole derivatives for use in treating cancer and non-neoplastic conditions. PCT Int. Appl. WO 2010138758.

38. Duarte, C. D.; Barreiro, E. J.; Fraga, C. A. M. Privileged structures: a useful concept for the rational design of new lead drug candidates. *Mini-Rev. Med. Chem.* **2007**, *7*, 1108-1119.

39. Costantino, L.; Barlocco, D. Privileged structures as leads in medicinal chemistry. *Frontiers in Med. Chem.* **2010**, *5*, 381-422.

40. Wittmann, B. M.; Sherk, A.; McDonnell, D. P. Definition of functionally important mechanistic differences among selective estrogen receptor down-regulators. *Cancer Res.* **2007**, *67*, 9549-9560.

41. McDonnell, D. P.; Wardell, S. E. The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer. *Curr. Opin. Pharmacol.* **2010**, *10*, 620-628.

42. Wakeling, A. E.; Bowler, J. Novel antioestrogens without partial agonist activity. *J. Steroid. Biochem.* **1988**, 31, 645–653.

43. Wakeling, A. E.; Dukes, M.; Bowler, J. A Potent Specific Pure Antiestrogen with Clinical Potential. *Cancer Res.* **1991**, *51*, 3867-3873.

44. Poulin, P.; Jones, R. D. O.; Jones, H. M.; Gibson, C. R.; Rowland, M.; Chien, J. Y.; Ring, B. J.; Adkison, K. K.; Ku, M. S.; He, H.; Vuppugalla, R.; Marathe, P.; Fischer, V.; Dutta, S.; Sinha, V. K.; Bjoernsson, T.; Lave, T.; Yates, J. W. T. PHRMA CPCDC initiative on predictive models of human pharmacokinetics, Part 5: Prediction of plasma concentration-time profiles in human by using the physiologically-based pharmacokinetic modeling approach. *J. Pharm. Sci.* **2011**, *100*, 4127-4157.

45. Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. Fluorine in medicinal chemistry. *Chem. Soc. Rev* **2008**, *37*, 320-330.

46. Weir, H. M.; Bradbury, R. H.; Lawson, M.; de Almeida, C.; Andrews, D. M.; Buttar, D.; Callis, R. J.; Curwen, J. O.; Hulse, M.; Donald, C. S.; Gingell, H.; Hussain, S.; Karoutchi, G.; Lamont, S. G.; MacFaul, P.; Moss, T.; Norman, R. A.; Pearson, S. E.; Rabow, A. A.; Tonge, M.; Walker, G. E.; Wilson, Z.; Powell, S.; Campbell, H.; Ghazoui, Z.; Davies, G.; Rowlinson, R.; Sadler, C.; Richmond,

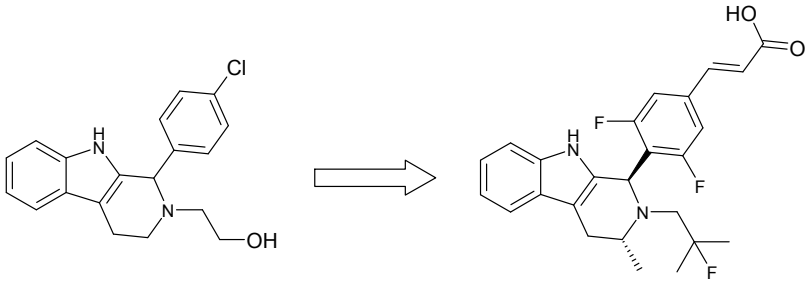
G.; Mazzola, A. M.; D'Cruz, C.; De Savi, C. AZD9496, a Novel Selective Estrogen Receptor Down-regulator drives Tumour Growth Inhibition in both ER+ve and ER Mutant Models of Resistance *Cancer Res.* **2015**, manuscript submitted.

47. Fagerholm, U. Prediction of human pharmacokinetics - gastrointestinal absorption. *J. Pharm. Pharmacol.* **2007**, *59*, 905-916.

48. Miller, T. W.; Balko, J. M.; Ghazoui, Z.; Dunbier, A.; Anderson, H.; Dowsett, M. A A gene expression signature from human breast cancer cells with acquired hormone independence identifies MYC as a mediator of antiestrogen resistance. *Clin. Cancer Res*. **2011**, *17*, 2024-2034.

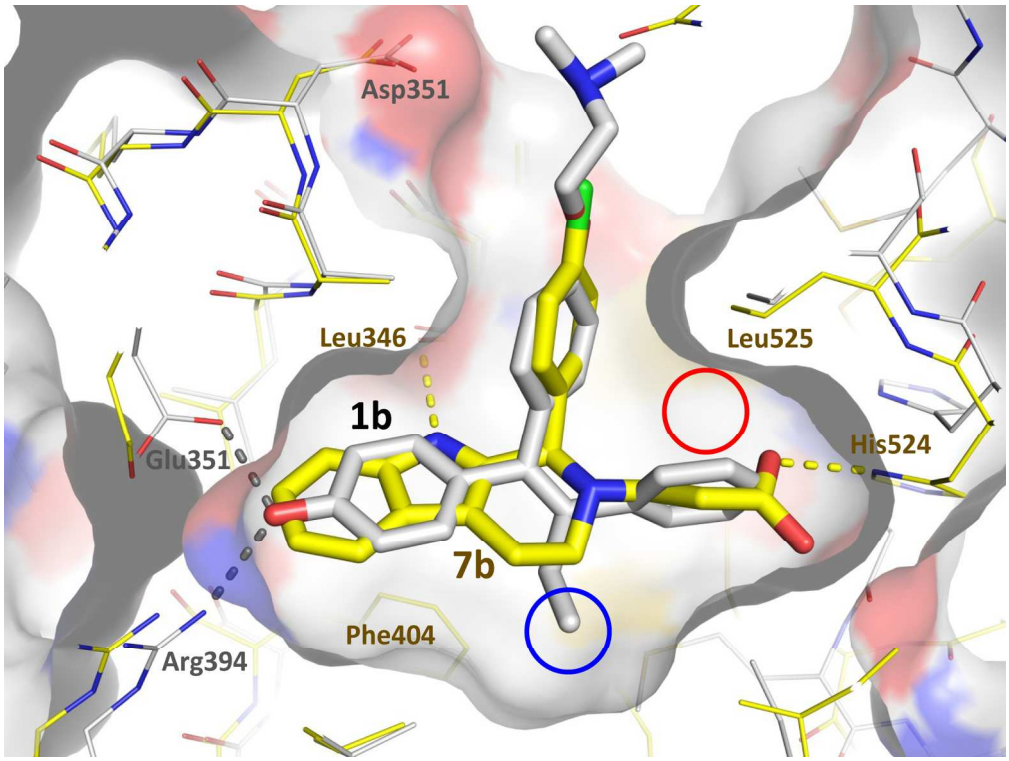
49. Immunblotting method: Cells were lysed in 25 mmol/L Tris/HCL pH6.8, 3 mmol/L EDTA, 3 mmol/L EGTA, 50 mmol/L NaF, 2 mmol/L sodium orthovanadate, 270 mmol/L sucrose, 10 mmol/L β-glycerophosphate, 5 mmol/L sodium pyrophosphate and 0.5% Triton X-100 supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Pierce) and proteins run on 4% to 12% Tris-HCl precast gels (Bio-Rad). Membranes were probed overnight with primary antibodies (ERα, Thermo Fisher Scientific SP1; PR, Dako PgR636; GAPDH, CST 2118) followed by incubation with HRP-tagged secondary antibodies (CST 7074 or 7076) and visualized on a Syngene ChemiGenius with Super-Signal West Dura Chemiluminescence Substrate (Pierce).

Table of Contents Graphic



ER binding screen hit, pIC_{50} = 5.9

AZD9496, orally bioavailable SERD, pIC_{50} = 9.9



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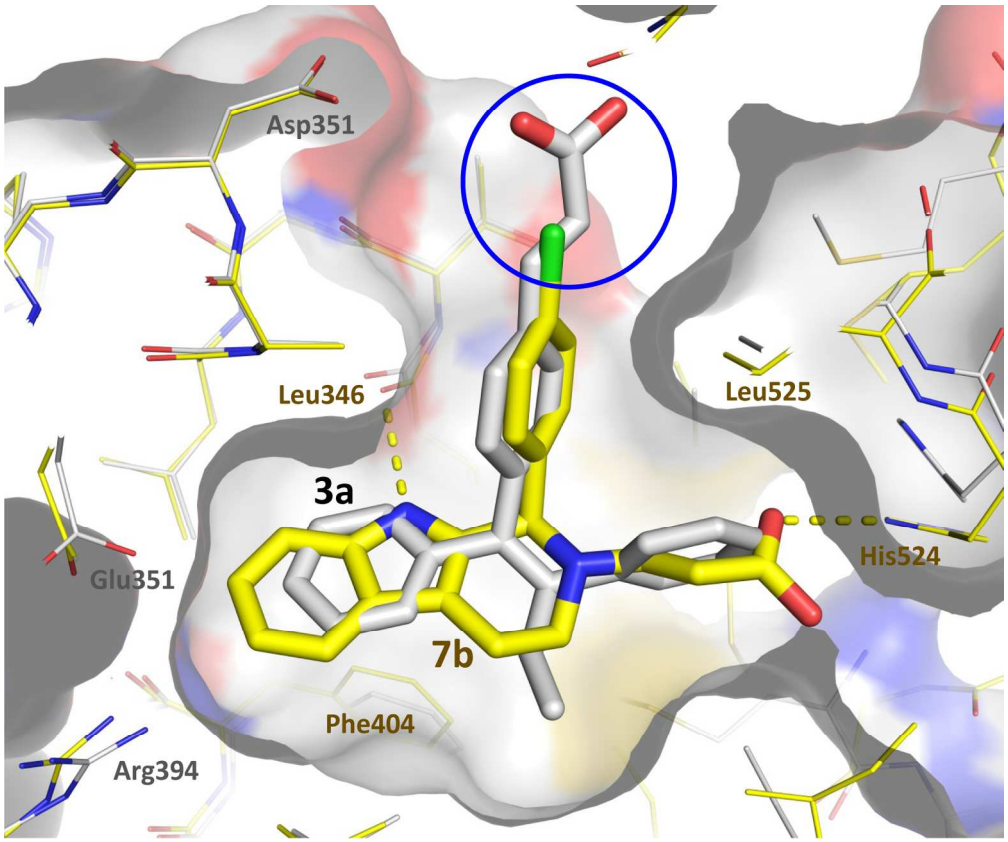

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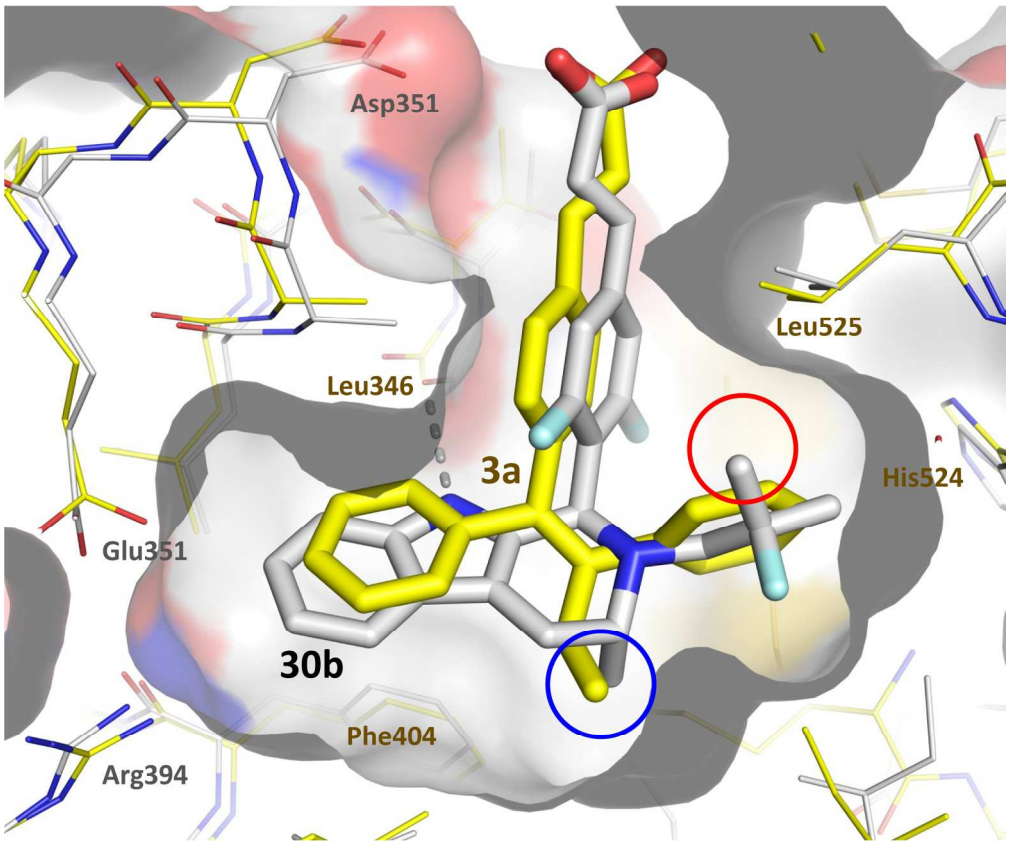

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