**Supplementary Table 1: Crystallographic data collection and refinement statistics**

|  |  |
| --- | --- |
|  | **AZD9496** |
| **Data processing** |  |
| Space group | P6522 |
| Cell parameters  |  |
|  Lengths a, b, c (Å) | 57.86, 57.86, 274.04 |
|  Angles α, β, γ (º) | 90.00, 90.00, 120.00 |
| Unique reflections | 26,271 |
| Resolution range (Å)**a** | 54.81-1.80 (1.90-1.80) |
| Multiplicity **a** | 6.9 (7.2) |
| Completeness (%)**a** | 99.1 (99.4) |
| Mean I/σI **a** | 13.4 (2.8) |
| Rmerge (%) **a,b** | 6.1 (52.0) |
|  |  |
| **Refinement** |  |
| Reflections used in refinement | 21,907 |
| Reflections used for Rfree | 1,588 |
| Rvalue (%)**c** | 22.7 |
| Rfree (%)**c** | 27.2 |
| Rmsd  |  |
|  Bonds (Å) | 0.006 |
|  Angles (º) | 1.025 |
|  |  |
| **Final model** |  |
| Number of atoms |  |
|  Non-hydrogen protein | 1,800 |
|  Non-hydrogen ligand | 32 |
|  Solvent | 79 |
| Average B (Å2) |  |
|  Protein main chain atoms | 28.76 |
|  Protein all atoms | 29.87 |
|  Ligand | 29.47 |
|  Solvent | 41.52 |
| Ramachandran statistics (%)**d** |  |
|  In most favoured regions | 96.5 |
|  In additional allowed regions | 3.5 |
|  In generously allowed regions | 0.0 |
|  In disallowed regions | 0.0 |
| PDB accession code | 5ACC |

**a** Values in parentheses refer to the outer resolution shell

**b** *R*merge = S*hkl*[( Σ*i*|*Ii* - ‹*I*›| )/ Σ*i Ii*]

**c** *R*value = S*hkl*||*F*obs| - |*F*calc|| / S*hkl*|*F*obs|

*R*free is the cross-validation *R* factor computed for the test set of 5% of unique reflections

**d** Ramachandran statistics as defined by PROCHECK(Laskowski et al, 1993)

Supplementary Table 2

Summary of AZD9496 binding kinetics to human ERα LBD protein

|  | k ass (M-1s-1) | S.E. (M-1s-1)  | k diss (s-1)  | S.E. (s-1)  | KD (nM) | S.E. (nM) | t ½ (min) |
| --- | --- | --- | --- | --- | --- | --- | --- |
| AZD9496 | 1.29 x 106 | 3.34 x 104 | 0.00043 | 0.00003 | 0.33 | 0.03 | 27 |

**Supplementary Table 2. Summary of AZD9496 binding kinetics to human ERα LBD protein.** Tetra-His antibody was covalently immobilised on to the biacore sensor surface using amine coupling chemistry and injection of ERα LBD performed to obtain a target protein immobilisation level of ~1500 RU of ERα LBD. Biacore sensorgrams for AZD9496: ERα LBD interaction was double referenced using Scrubber2 software (v2.0c, Biologic, Australia). The kinetic interaction analysis was performed using BIAevaluation software, fitted separately to a 1:1 binding model binding interaction and the ratio of the rate constants (kdiss/kass) used to determine an equilibrium dissociation constant (kD).

Supplementary Table 3

Summary of AZD9496 binding to NHR ligand binding domains

| Assay Details: | Estrogen Receptor α | Estrogen Receptor β | Androgen Receptor | Glucocorticoid Receptor | Progesterone Receptor |
| --- | --- | --- | --- | --- | --- |
| AZD9496 | 0.82 nM  | 0.7 nM  | 30 M  | 9.25 M  | 0.54 M  |

Supplementary Table 3. Summary of AZD9496 binding to NHR ligand binding domains

Binding to NHR LBDs was assessed using LanthaScreen® assays apart from the AR screen which used a FP end-point. GST labelled NHRs were added to the relevant fluorescently labelled ligand and a terbium-labeled anti-GST antibody used to indirectly label the NHR LBD. Competitive binding was detected by a test compounds ability to displace the fluorescent ligand resulting in a loss of FRET signal. A 12 point half-log concentration response curve (100 μM top concentration) was used to generate IC50 values and the TR-FRET emission ratios were acquired on a BMG Pherastar plate reader. The results are the mean of at least three independent experiments.

**Supplementary Table 4**

| Cell Line | Cell Type | ER status | Mean EC50 (no. of expts) |
| --- | --- | --- | --- |
| MCF-7 | breast | + | 0.0028 μM (n=6) |
| Cama-1 | breast | + | 0.0034 μM (n=6) |
| BT474 | breast | + | 0.002 μM (n=6) |
| MDA-MB-134 | breast | + | 0.007 μM (n=2) |
| T47D | breast | + | 0.0026 μM (n=3) |
| LnCAP | prostate | - | >10 μM (n=3) |
| PC9 | prostate | - | >10 μM (n=4) |
| HCT116 | colon | - | >10 μM (n=3) |
| MDA-MB-468 | breast | - | >10 μM (n=3) |
| HCC70 | breast | - | >10 μM (n=4) |

Supplementary Table 4. Cell growth inhibition in a panel of ER+ve and ER-ve cell lines

Cells were seeded into 384-well plates at various seeding densities (500-2000 cells/well) in RPMI 1640 media containing 1% L-glutamine and 10% FBS. The cell plates were incubated for 24 hours at 37 °C before a 10 point concentration range (10 μM to 0.3 nM) of AZD9496 was added. % confluency was measured over several days using an Incucyte platform and pEC50 values were determined once vehicle control confluency was between 60-85% after at least 96 hours. Data shown is the mean of the number of experiments indicated.