Estrogen Receptor-Targeting Antiproliferative Benzoxepins

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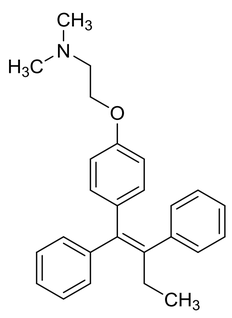
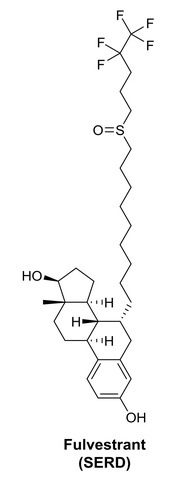
INTRODUCTION



Tamoxifen (SERM)

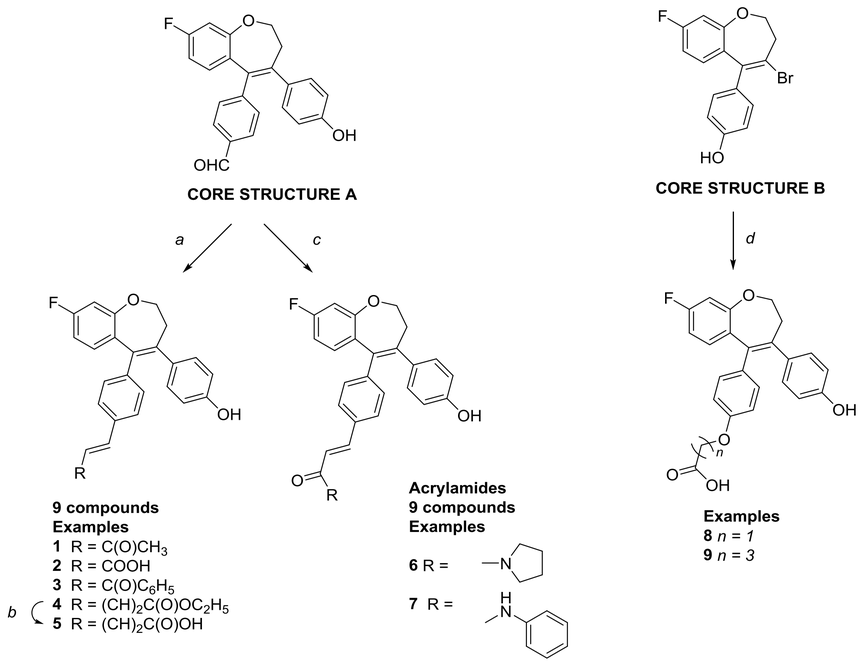
Estrogen receptor-α (ERα) is an important target for the design of drugs useful in the treatment and prevention of hormone-dependent breast cancer and osteoporosis. The selective estrogen receptor modulator (SERM) tamoxifen is an effective drug used in the treatment of estrogen-dependent breast cancer, but is associated with increased incidences of endometrial tumours. Selective estrogen receptor downregulators (SERDs, e.g. fulvestrant) are promising agents for the treatment of tamoxifen-resistant breast cancer. SERDs reduce ER expression, inducing arrest of cell proliferation and apoptosis of estrogen-dependent breast cancer cells. In this work, a series of ER ligands based on the benzoxepin scaffold with different substituents was synthesised. These compounds were shown to be high-affinity ligands for the ER with nanomolar IC_{50} binding values. Their synthesis, antiproliferative potency and further effects on ER subtypes α and β are reported.



Fulvestrant (SERD)

CHEMICAL SYNTHESIS OF BENZOXEPINS

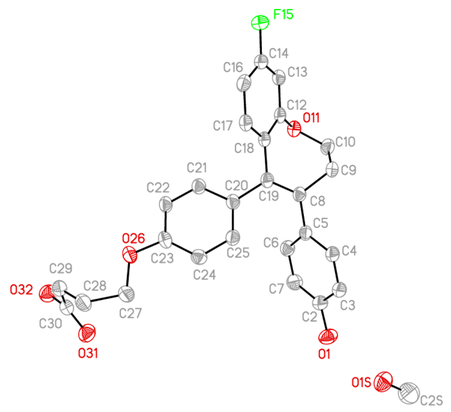
Initially, the benzoxepin scaffold and related core structures were evaluated. Core structures A and B were found to be optimal, with a fluoro-substituent and 7-membered oxygen-containing ring. Core structures A and B were obtained via a four-step synthesis. Structural diversity was added to these benzoxepins as shown in Scheme 1 (below).



Scheme 1: Synthesis of ER-targeting benzoxepins

Reagents and conditions: (*a*) $CH_3COCH_2P(O)(OEt)_2$, $C_6H_5COCH_2P(O)(OEt)_2$, $EtO_2CCH{=}CHCH_2P(O)OEt_2$ or $C_6H_5CH_2PPh_3$; reflux, 2 hr; NaH, THF, 0 °C → rt, 12 hr, 50-67%; (*b*) NaOH, EtOH, reflux, 1 hr, 86%; *(c)* Amine, HOBt, EDCI, Et_3N, CH_2Cl_2, 0 °C → rt, 18 hr, 18-92% (incl. compound **6**); *(c)* NaH, THF, reflux, 18 hr, 83% (compound **7**); (*d*) (3 steps) (i) K_2CO_3, KI, acetone, reflux, 8 hr, 68-77%; (ii) 4-$OHC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, 2M Na_2CO_3, THF, reflux, 6 hr, 74-86%; (iii) 1M NaOH, EtOH, reflux, 1 hr, 25-69%.

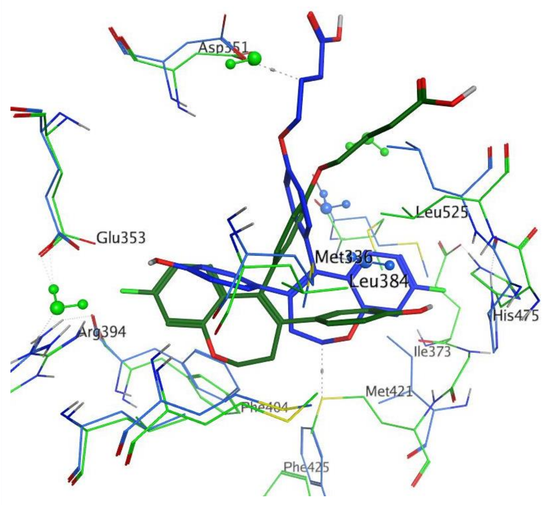
X-RAY CRYSTALLOGRAPHY



X-Ray crystallography of benzoxepin **9** (left) shows that:

1. The 7-membered ring displays a puckered conformation. This results in a molecular arrangement in which the three aromatic rings attached to the 7-membered ring are not coplanar.
2. A bond length between C8 and C19 of 1.35 Å indicates the position of the double bond in the 7-membered ring.

MOLECULAR MODELLING



Molecular modelling of benzoxepin **9** (shown as green in ERα and blue in ERβ, left) indicates:

- In ERα, **9** overlays well on the core structure of 4-hydroxytamoxifen (4-OHT) except that the fluorine maps to the hydroxyl group of 4-OHT.
- In ERβ, **9** has a 180° flipped orientation compared to other compounds in this study, in that the phenolic hydroxyl group mimics the position adopted by 4-OHT. The fluorine-containing ring is ideally positioned to accept a hydrogen bond from His475.

BIOCHEMISTRY

Antiproliferative Activity

In an initial series of acrylic acids, the presence of a fluorine at C-8 in benzoxepin compound **2**, resulted in a marked improvement in antiproliferative activity (IC_{50} = 0.26 µM) when compared with a non-fluorinated compound (IC_{50} = 21 µM; structure not shown). Compound **5** with an extended penta-2,4-dienoic acid substituent in Ring B, retains moderate antiproliferative activity (IC_{50} = 1.6 µM).

Binding of Selected Compounds to ERα and ERβ

Introduction of the 8-fluoro substituent, and also the benzothiepin ring scaffold (structure not shown) increased ER binding activity for both ERα and ERβ. In our acrylamide series, compound **7** demonstrated the most effective binding activity [IC_{50} = 11.7 nM (ERα) and 0.94 nM (ERβ)], with 11-fold ERβ selectivity.

Benzoxpein **5**, containing the extended penta-2,4-dienoic acid substituent in Ring B, was found to display potent ER-binding activity with IC_{50} = 71.6 nM (ERα) and 0.55 nM (ERβ), equivalent to 129-fold ERβ selectivity.

Benzoxepin **9**, containing the 4-oxybutyric acid substituent, demonstrated extremely interesting ER-binding properties with IC50 = 147 nM (ERα) and 1.23 nM (ERβ), which is 117-fold selectivity for ERβ despite its lack of antiproliferative potency.

Table 1. Biochemical Data for Selected Benzoxepins

Compound	IC_{50} (µM)[a]	% cell death[b]	ERα IC_{50} (nM)[c]	ERβ IC_{50} (nM)[c]
1	0.89	3.7	59	175
2	0.26	5.0	14	72
3	0.97	0	104	447
5	1.6	0	72	0.55
6	14	2.3	67	2.4
7	1.3	0	11.7	0.94
8	> 20	2.6	634	34
9	> 20	5.3	147	1.23
Tamoxifen	4.1	13.3	70	170

[a]IC_{50} values are half-maximal inhibitory concentrations required to block the growth stimulation of MCF-7 cells (determined at 72 hr using the MTT assay)

[b]Cell death is the percentage cell death of MCF-7 cells at a concentration of 10 µM of compound [determined at 72 hr using the lactate dehydrogenase (LDH) assay].

[c]Values are an average of at least nine replicate experiments for ERa and six replicate experiments for ERb, obtained using recombinant ER (insect expressed, full length, untagged human ER obtained from recombinant baculovirus-infected insect cells)

Effect on the Expression Levels of ERα and ERβ

The known SERD fulvestrant reduced ERα protein levels in MCF-7 breast cancer cells, with little or no effect on ERβ. Compound **5**, which possessed good antiproliferative activity, was found to downregulate both ERα and ERβ. Compound **9** selectively downregulated ERβ in MCF-7 cells, with little effect on the expression of ERα. This result is consistent with an ER-binding assay, in which compound **9** was ERβ selective.

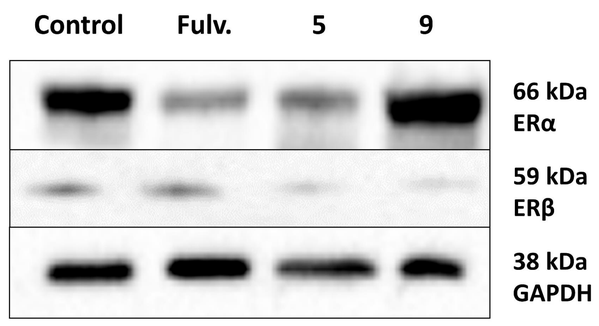


Figure (above). Effects of fulvestrant, **5** and **9** on expression levels of ERα and ERβ in MCF-7 breast cancer cells. SERD fulvestrant was used as a positive control. MCF-7 breast cancer cells were treated with compounds **5** and **9** (10 µM), and after 24 hr whole cell lysates were prepared and analysed by SDS-PAGE and Western blotting for expression levels of ERα or ERβ.

CONCLUSIONS

- The acrylic acid ligands were generally antiproliferative and ERα selective
- Compound **5** featuring the phenylpenta-2,4-dienoic acid substituent on the benzoxepin core, was shown to be antiproliferative and downregulated ERα and ERβ expression in MCF-7 breast cancer cells. This compound will be developed further as a potential clinical candidate for treatment of breast cancer.
- Compound **9** had no effect on cell viability and selectively downregulated ERβ. Compound **9** is the first reported ERβ-selective SERD. There is much research ongoing to fully elucidate the effects of ERβ in cancers. Due to its unique combination of effects, compound **9** is a useful tool for investigation of the role of ERβ in cancer cells.