as osteoporosis. Nevertheless, in time, tamoxifen-resistant tumours often arise in patients and some of these could benefit from treatment with an anti-oestrogen, the mechanism of action of which is different from that of tamoxifen. It is important to establish whether other anti-oestrogens function by one or other of the mechanisms suggested for tamoxifen and ICI 164 384 or by another novel means. Clearly, a better understanding of the mechanism of hormone antagonists is likely to lead to more efficacious treatment not only of breast cancer but also other endocrine disorders.

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Development of novel oestrogen-receptor antagonists
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The discovery of non-steroidal anti-oestrogens stemmed from observations, dating back more than 50 years, that simple molecules like triphenylchloroethylene have stimulatory effects in classical bioassays of oestrogenic activity, for example the capacity to induce vaginal cornification and uterine growth in immature or ovariectomized rodents. Subsequently, it was shown that addition of a basic ether side-chain to the triphenylethylene nucleus produced compounds, like clomiphene and tamoxifen, with anti-fertility activity. Agents of this kind were of great interest because of their potential use as contraceptives, and also for the treatment of malignant and benign diseases dependent on oestrogenic stimulation. Clinical studies failed to confirm useful contraceptive activity, but opened up other therapeutic applications including the treatment of breast cancer.

Clomiphene and tamoxifen were shown to have mixed oestrogen-agonist and antagonist activity. Thus, in the immature-rat-uterus bioassay, tamoxifen stimulates growth, but to a lower maximum extent than the natural hormone 17β-oestradiol, this is a classical partial-agonist effect. Correspondingly, when tamoxifen is administered together with 17β-oestradiol the uterotrophic action of the natural hormone is reduced in a dosedependent manner, demonstrating anti-oestrogenic activity. The maximum antagonist effect in this assay is limited by the intrinsic agonist activity of...
Analysis of clinical experience and animal-oestrogens varies widely across species, target organs, cells and genes, depending on which indicator of response is measured. In respect of the application of 'Nolvadex' (tamoxifen, ICI 46 474) to breast-cancer treatment, it is legitimate to ask whether the therapeutic efficacy of the drug is in any way limited by the fact that it is a partial agonist. Analysis of clinical experience and animal-model studies suggests that this may be the case, however, a true test of this question can only be made by evaluation of the consequences of complete abrogation of all oestrogen action in patients. This cannot be achieved with any existing treatment, but could be tested if anti-oestrogens completely lacking in oestrogenic activity (i.e. pure anti-oestrogens) were available.

In instituting the search for pure anti-oestrogens, it was clear from previous experience that a novel chemical approach was necessary since all earlier attempts, modelled on existing steroidal or non-steroidal molecules, had met with limited success in reducing agonist activity. The rationale for the synthesis and testing of novel analogues of 17β-oestradiol has been described elsewhere, and led to the discovery of the first series of anti-oestrogens which satisfy the pharmacological definition of a pure antagonist. Extensive investigation, in this and other laboratories, of the exemplary compound ICI 164 384, a 7a-alkylamide analogue of 17β-oestradiol, has confirmed the following important properties of pure anti-oestrogens (see [9] for review).

In animals, ICI 164 384, unlike tamoxifen, has no oestrogen-like stimulatory activity on the uterus, vagina, mammary gland or hypothalamic-pituitary-ovarian axis. Correspondingly, ICI 164 384 blocks the trophic action of exogenous or endogenous oestrogens in a dose-dependent and complete manner. The most instructive demonstration of the difference between pure- and partial-agonist anti-oestrogens was provided by the demonstration that co-administration of tamoxifen and ICI 164 384 eliminates the trophic action of tamoxifen on the uterus or mammary gland. The latter observations suggest that pure anti-oestrogens in vivo will potentially achieve the desired complete hormone ablation.

These pharmacological studies are consistent with the action of all three classes of effectors, that is oestrogens, and pure- and partial-agonist anti-oestrogens, through a common receptor. This is readily demonstrable in oestrogen-receptor-binding-competition assays, where each ligand displaces [1H]17β-oestradiol in a concentration-dependent and linear fashion. ICI 164 384 has the advantage of a much higher receptor affinity than tamoxifen, which is also reflected in their relative potency as inhibitors of the growth of oestrogen-responsive-human-breast-cancer cells in vitro. The latter studies also revealed that ICI 164 384 is much more effective than tamoxifen in reducing the proportion of breast cancer cells which remain capable of DNA synthesis. This is attributed to the residual trophic activity of tamoxifen. By analogy with the animal studies, the blockade by ICI 164 384 of the trophic actions of tamoxifen in vitro can be demonstrated using both cell growth and basement-membrane invasiveness as indicators of oestrogen-like activity. Extrapolation of such observations to the clinical setting, implies that the anti-tumour action of pure anti-oestrogens might be enhanced significantly when compared with that of tamoxifen.

Since it became apparent that none of the 7a-alkylamide analogues of oestradiol was of sufficient potency to merit consideration as a drug candidate, further chemical exploration was necessary to identify more potent pure anti-oestrogens. This led us to the compound ICI 182 780 which showed a substantial (5-fold) increase of intrinsic potency compared with ICI 164 384. This was reflected by comparative measurements of receptor affinity and of cell-growth inhibition with human-breast-cancer cells. More importantly, ICI 182 780 is an order of magnitude more potent than ICI 164 384 in vivo. This results in effective and sustained anti-oestrogenic and anti-tumour activity, and provided the impetus for selection of ICI 182 780 as a candidate molecule for clinical evaluation.

Design of ligands for the glucocorticoid and progestin receptors

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Introduction
Steroid hormones act via their interaction with intracellular receptors that belong to a general class of regulatory proteins, the 'steroid-receptor superfamily'. This class includes the five 'classical' steroid receptors: oestrogen (ER), androgen (AR), progestin (PR), glucocorticoid (GR) and mineralocorticoid (MR) receptors as well as the receptors for thyroid hormone, vitamin D3, retinoic acid, and a number of 'orphan receptors' the ligands of which are not yet known.

Cloning and sequence determination of these receptors demonstrated that the common overall structure consists of essentially three domains: the variable N-terminal domain, the central DNA-binding domain and the C-terminal ligand-binding domain (LBD). Close examination of the compared sequences of the LBDs of the five 'classical' steroid-hormone receptors, confirmed what researchers in the steroid field have empirically known for many years: there is a high degree of sequence similarity between the LBDs of GR, MR, PR and AR, the receptors of the 3-oxo-A4 steroids, which are quite different from the LBD of the ER whose natural ligand, oestradiol, has an aromatic A ring (see [1] for an overview).

These similarities are even more striking if, instead of considering the straightforward amino acid to amino acid identity, one compares the sequences by hydrophobic-cluster analysis [2], a technique which has recently permitted the elaboration of a tentative three dimensional structure of the steroid receptors [3]. However, for many years and up to the present time, we have had to rely solely on structural modification of the hormonal steroids to gain some indirect information on the topography of the receptor binding site: this crude technique is called 'mapping'. It was made possible only through the availability of receptor screening, which consists of measuring the binding affinities of various compounds for the steroid receptors. In the decade following the discovery of steroid-hormone receptors [4-6 for reviews] a simple screening procedure was set up in the Roussel-Uclaf company by J. P. Raynaud and coworkers, which allowed the comparison of the relative binding affinities (RBAs) of a large number of steroids with those of reference compounds [7]. It quickly became obvious that neither the natural hormones, nor the synthetic reference compounds were perfectly specific for their cognate receptor. While oestradiol bound to a small extent to the PR (2% of progesterone) and the AR (8% of testosterone) in addition to its own ER (100%), none of the 3-oxo-A4 steroids had any affinity for the ER.

Among the latter compounds progesterone appeared as having a significant affinity already for the GR, provided a receptor preparation of thymic origin was used. Indeed, liver preparations contain enzymes which metabolize the ligand very quickly, even at 4°C, giving a false impression of selectivity. Among synthetic hormones, R 1881 especially, an androgen of the oestra-4,9,11-triene series, displayed a remarkably unspecific binding profile [7]. These first results already indicate that most receptors, especially AR, PR, GR and MR share common features which allow them to bind some ligands quite unspecifically. This suggests that the steroid receptors may present a common gross shape of