

# **Sex Hormones and Antihormones in Endocrine Dependent Pathology: Basic and Clinical Aspects**

Proceedings of an International Symposium,  
Milano, 10–14 April 1994

*Editors:*

**Marcella Motta**

Department of Endocrinology  
University of Milan  
Milan, Italy

**Mario Serio**

Endocrinology Unit  
Department of Clinical Physiopathology  
University of Florence  
Florence, Italy



1994

**Elsevier**

Amsterdam – Lausanne – New York – Oxford – Shannon – Tokyo

©1994 Elsevier Science B.V. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher, Elsevier Science B.V., Permissions Department, P.O. Box 521, 1000 AM Amsterdam, The Netherlands.

No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, the Publisher recommends that independent verification of diagnoses and drug dosages should be made.

Special regulations for readers in the USA — This publication has been registered with the Copyright Clearance Center Inc. (CCC), 27 Congress Street, Salem, MA 01970, USA. Information can be obtained from the CCC about conditions under which photocopies of parts of this publication may be made in the USA. All other copyright questions, including photocopying outside the USA should be referred to the copyright owner, Elsevier Science B.V., unless otherwise specified.

International Congress Series No. 1064  
ISBN 0-444-81879-0

*This book is printed on acid-free paper.*

*Published by:*

Elsevier Science B.V.  
P.O. Box 211  
1000 AE Amsterdam  
The Netherlands

*Library of Congress Cataloging in Publication Data:*

Sex hormones and antihormones in endocrine dependent pathology :  
proceedings of an international symposium, Milano, 10-14 April 1994  
/ editors, Marcella Motta, Mario Serio.

p. cm. -- (International congress series ; no. 1064)  
Includes bibliographical references and indexes.  
ISBN 0-444-81879-0 (alk. paper)

1. Breast--Cancer--Endocrine aspects--Congresses. 2. Prostate--  
Cancer--Endocrine aspects--Congresses. 3. Hormones, Sex--  
Antagonists--Therapeutic use--Congresses. 4. Hormones, Sex--  
Therapeutic use--Congresses. 5. Uterus--Diseases--Endocrine  
aspects--Congresses. I. Motta, Marcella. II. Serio, Mario.  
III. Series.

[DNLM: 1. Neoplasms, Hormone-Dependent--congresses. 2. Sex  
Hormones--congresses. 3. Prostatic Neoplasms--congresses.  
4. Breast Neoplasms--congresses. 5. Genital Diseases, Female--  
congresses. W3 EX89 no. 1064 1994 / QZ 200 S518 1994]

RC280.B8S48 1994

616.99'449--dc20

DNLM/DLC

for Library of Congress

94-32151

CIP

In order to ensure rapid publication this volume was prepared using a method of electronic text processing known as Optical Character Recognition (OCR). Scientific accuracy and consistency of style were handled by the author. Time did not allow for the usual extensive editing process of the Publisher.

Printed in the Netherlands

## Pure antioestrogens in breast cancer: experimental and clinical observations

R.I. Nicholson<sup>1</sup>, J.M.W. Gee<sup>1</sup>, C.L. Eaton<sup>1</sup>, D.L. Manning<sup>1</sup>, R.E. Mansel<sup>2</sup>, A.K. Sharma<sup>2</sup>, A. Douglas-Jones<sup>2</sup>, M. Price-Thomas<sup>3</sup>, A. Howell<sup>4</sup>, D.J. DeFriend<sup>4</sup>, N.J. Bundred<sup>4</sup>, E. Anderson<sup>4</sup>, J.F.R. Robertson<sup>5</sup>, R.W. Blamey<sup>5</sup>, M. Dowsett<sup>6</sup>, M. Baum<sup>6</sup>, P. Walton<sup>7</sup> and A.E. Wakeling<sup>7</sup>

<sup>1</sup>Tenovus Cancer Research Centre, Cardiff, UK; <sup>2</sup>Departments of Surgery (REM, AS) and Pathology (AD-J), UWCM, Cardiff, UK; <sup>3</sup>Royal Gwent Hospital, Newport, UK; <sup>4</sup>Withington and Christie Hospitals, Manchester, UK; <sup>5</sup>Department of Surgery, City Hospital, Nottingham, UK; <sup>6</sup>Royal Marsden Hospital, London, UK; and <sup>7</sup>Zeneca Pharmaceuticals, Macclesfield, UK

*Key words:* ICI 182,780, ICI 164,384, oestrogen receptor, oestrogens, tamoxifen

### Introduction

The last 10 years have seen the emergence of a new class of pharmacological agents termed pure antioestrogens (reviewed in [1]). These compounds, which were originally developed by ICI Pharmaceuticals Division in the UK, have the unique property of binding to the oestrogen receptor [2] and producing a receptor complex which lacks oestrogenic activity [3,4]. If we assume that the action of oestrogens on sensitive breast cancers favours cell proliferation and survival, and that they thereby act as a driving force for the growth and development of the disease [5], pure antioestrogens have the potential to fully negate these activities by producing a state of complete oestrogen withdrawal [6].

The perceived importance of pure antioestrogens, therefore, is as alternatives to antihormonal treatments which are designed to reduce the synthesis of oestrogens, but which currently fail to nullify oestrogenic signals arising from other sources [7,8], and as potential successors to "tamoxifen-like" antioestrogens, which although widely and successfully used in the therapy of primary and advanced disease [6], possess partial oestrogenic activity [9] which may negate aspects of their effectiveness as antitumour agents.

Since pure antioestrogens are now entering clinical development, the current paper seeks to outline some of their basic cellular and antitumour properties on human

---

*Address for correspondence:* R.I. Nicholson, Tenovus Cancer Research Centre, University of Wales, College of Medicine, Heath Park, Cardiff CF4 AXX, UK.

breast cancer cells in vitro [1,10] primarily using the lead compound ICI 164,384, and to compare this information with data derived from a phase I study of ICI 182,780 in primary breast cancer patients [11]. In each instance, emphasis will be placed on immunohistochemical data as it was our original hope that such an approach would facilitate an assessment of the degree to which pure antioestrogens were fulfilling their potential as complete antagonists of oestrogen action in clinical breast cancer and thereby aid in defining the importance of oestrogens in the regulation of breast cancer growth.

Figure 1 shows the structure of ICI 164,384 and ICI 182,780 which are  $7\alpha$  long-chain analogues of oestradiol. The ER binding affinity and potency of ICI 182,780 are greater than that observed for ICI 164,384 due to the substitution of the amide function by a sulphoxide group and the fluorination of the terminal chain [12]. Such differences, however, do not alter the intrinsic biological behaviour of the drugs which are identical to other pure antioestrogens, based on substitutions in the oestradiol nucleus [13,14] or nonsteroidal forms [15].

### Properties of pure antioestrogens in vitro

One of the most important early observations arising from the functional disablement of ER signalling by pure antioestrogens in oestrogen-sensitive human breast cancer cell lines was that treated cells frequently became very efficiently growth-arrested [10,16,17]. This property is illustrated in Fig. 2a and shows the growth of MCF-7 cells in  $10^{-9}$ M oestradiol in the presence or absence of a 100-fold excess of ICI

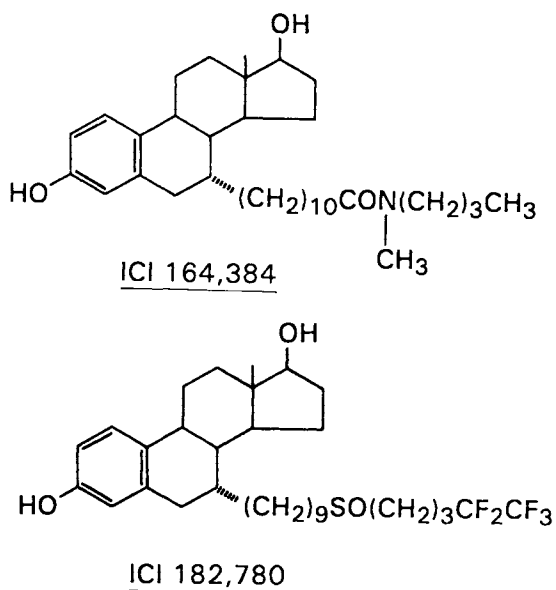
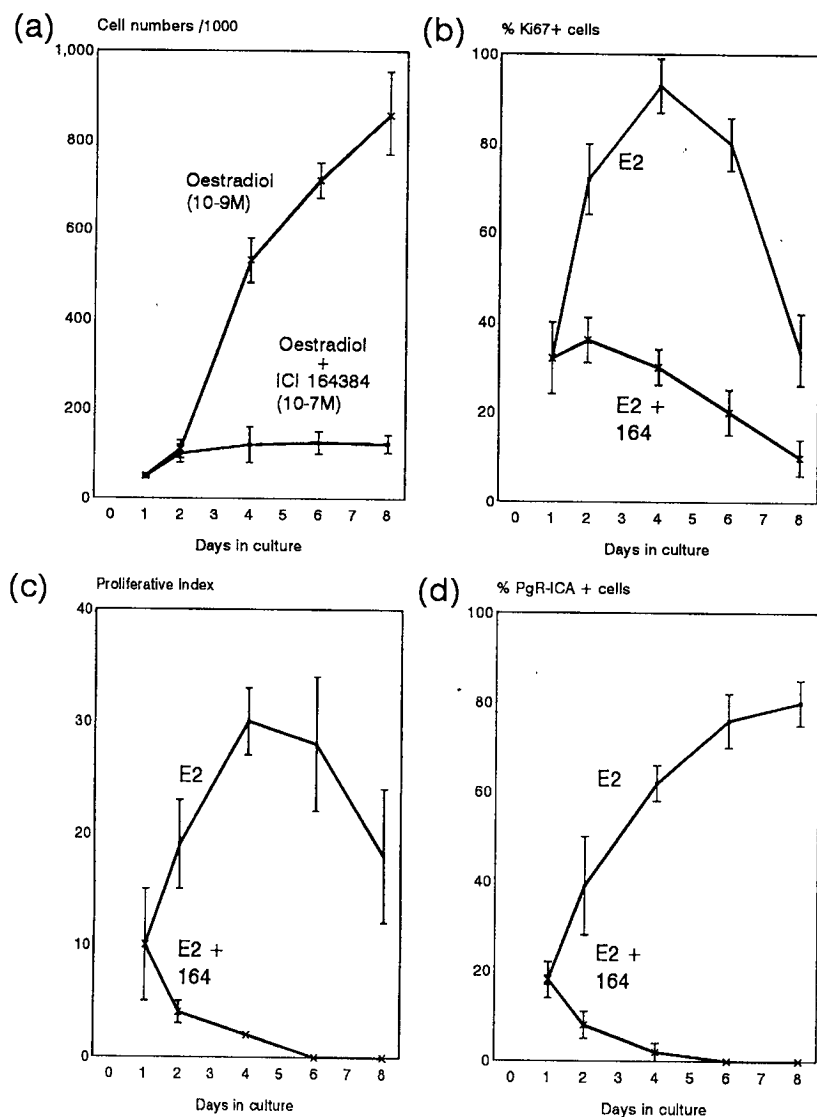


Fig. 1. Structures of ICI 164,384 and ICI 182,780.



*Fig. 2.* Growth and immunohistochemical characterisation of MCF-7 cells. The cells were grown in white RPMI tissue culture medium with 5% DCC-stripped FCS (medium A) containing oestradiol  $\pm$  ICI 164,384. (a) Cell numbers were assessed using a Coulter Counter; (b,c) Ki67; and (d) PR assays were performed according to the methods of Bouzubar et al. [19] and Walker et al. [20], respectively. The Ki67 proliferative index was calculated as the proportion of cells showing intense nucleoplasmic and nucleolar staining patterns [21]. Results are shown as the mean  $\pm$  SD of six replicates.

164,384. In contrast to the expansion of the cell population that occurs in the presence of the steroid, the pure antiestrogen virtually abolishes the growth of the MCF-7 cells, allowing at best one doubling of the initial cell number with the cells

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.