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Structural Derivatives of Tamoxifen and Oestradiol 3-Methyl Ether as Potential Alkylating Antioestrogens*

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Abstract—*The oestrogenic and antioestrogenic activity of potential alkylating derivatives of tamoxifen and oestradiol 3-methyl ether have been compared with tamoxifen and oestradiol benzoate in the immature rat. Although all the tamoxifen derivatives demonstrated an ability to inhibit the binding of* [^{3}H]*oestradiol to rabbit or rat uterine oestrogen receptors* in vitro, *none of the compounds was as potent as tamoxifen in tests for antioestrogenic activity* in vivo. *The potential alkylating derivatives of oestradiol 3-methyl ether were not antioestrogenic. The properties of all the compounds* in vivo *did not suggest irreversible effects upon the uterus. Since the assays* in vitro *did not predict activity* in vivo *the results indicate that only agents with very high affinity for the oestrogen receptor that do not potentially require metabolic activation may be useful* in vivo.

INTRODUCTION

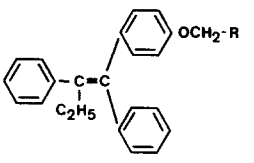
TAMOXIFEN [1-(*p*-*β*-dimethylaminoethoxyphenyl)-*trans*-1,2 diphenyl-but-1-ene] has been reported to have antioestrogenic [1] and antitumour [2–5] properties in the rat. The compound is used clinically in the treatment of breast cancer [6].

Hormone responsiveness in human breast cancer has been associated with the presence of the oestrogen receptor [7]; however, a proportion of tumours that contain oestrogen receptors do not respond to therapy. With this in mind, we have synthesised a number of derivatives of tamoxifen and oestradiol 3-methyl ether which contain alkylating moieties. The aim of the study was to exploit the oestrogen receptor as a cellular transport system so that alkylation of nuclear sites in any oestrogen receptor-containing cells would occur and ultimately lead to target cell death.

In the present study we describe the interaction of potential alkylating derivatives of tamoxifen (Fig. 1) with rat and rabbit uterine oestrogen receptors *in vitro*, the effect of these compounds upon the rat uterine oestrogen receptor system *in vivo* and the effect of potential alkylating oestrogens (Fig. 2) on oestrogen-stimulated uterine weight.

MATERIAL AND METHODS

All test compounds (Figs. 1 and 2) were synthesised at ICI Ltd. (Pharmaceuticals



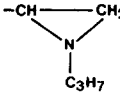
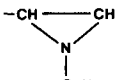
		R	
TAMOXIFEN	ICI 46,474	$-CH_2N(CH_3)_2$	(trans)
	ICI 111,610	$-CH_2NHCH_2CH_2OH$	(trans)
	ICI 111,611	$-CH_2NHCH_2CH_2Cl$	(trans)
	ICI 115,387	$-CH_2N(CH_3)CH_2CH_2OH$	(trans)
	ICI 115,513	$-CH_2N(CH_3)CH_2CH_2Cl$	(trans)
	ICI 119,647		(trans)
	ICI 121,919		(cis)

Fig. 1. Derivatives of tamoxifen used in the study.

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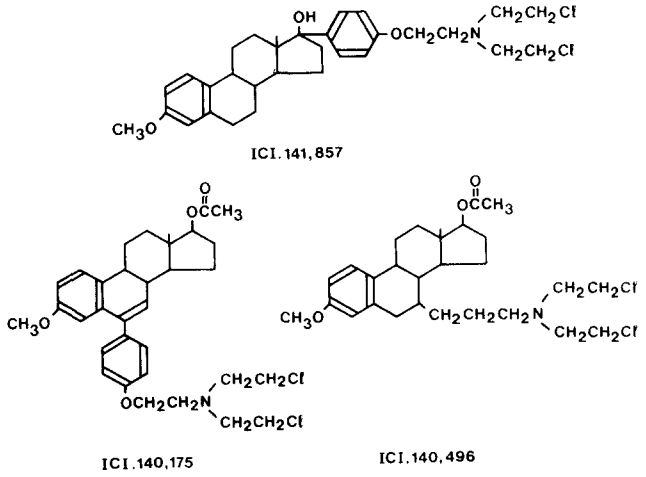

Fig. 2. Steroidal derivatives used in the study.

Division). For experiments *in vitro*, 2, 4, 6, 7 [^{3}H] oestradiol-17β (90 Ci/mmol) was obtained from New England Nuclear Corporation, dissolved in benzene:ethanol (9:1 v/v) 97% pure, and was used without further purification. Storage was at 4°C. Before use, required aliquots were evaporated and dissolved in a few drops of ethanol before the addition of the required volume of buffer. TED buffer (Tris 0.01 mol/l; EDTA 0.0015 mol/l; dithiothreitol 0.0005 mol/l, pH 7.4) was used throughout. Oestradiol-17β was obtained from Sigma Chemicals and diethylstilboestrol was obtained from British Drug Houses.

Experiments in vitro

Uteri from mature virgin rabbits were frozen immediately, after excision, in liquid N_2 and then powdered with a Thermovac tissue pulverizer with liquid N_2 cooling. Powdered tissue was homogenised in TED buffer using an Ultraturax tissue homogeniser (2 × 5 sec bursts with ice water cooling). Homogenate was centrifuged at 100,000 ***g*** for 1 hr (4°C) to prepare cytosol. The ability of all compounds to inhibit the oestrogen-specific binding of [^{3}H]oestradiol was determined using a dextran-coated charcoal method previously described [8].

The ability of ICI 111,610 and ICI 111,611 to inhibit the binding of [^{3}H]oestradiol to rat uterine oestrogen receptors was determined by sucrose density gradient analysis (5–20%) using methods previously described [9].

Experiments with tamoxifen derivatives in vivo

Immature female rats (35–50 g) of the Sprague–Dawley strain were used throughout. All compounds were injected s.c. in 0.1 ml peanut oil. Injection solutions were made by taking aliquots of freshly made ethanolic solutions in the required volume of peanut oil and evaporating the ethanol under N_2 by gently warming (60°C) on a water bath.
Three experiments were undertaken:

(a) Rats were divided into 6 groups each containing 8 animals. Groups were injected on 3 consecutive days with either oestradiol (0.08 μg), ICI 115,513 (1 or 10 μg) or oestradiol (0.08 μg) and ICI 115,513 (1 or 10 μg). Controls received peanut oil alone (0.1 ml). On the fourth day animals were killed and the uteri weighed wet on a torsion balance.

(b) Rats were divided into 8 groups each containing 8 animals. Pairs of groups were injected with 50 μg of tamoxifen, ICI 115,513 or ICI 111,611. Controls received peanut oil alone (0.1 ml). One group of each pair was killed 1 day and the other 5 days after injection. Uteri were weighed and nuclear and cytoplasmic oestrogen receptor levels were determined (see below).

(c) Rats were divided into 5 groups each containing 8 animals. Three groups were injected with 50 μg of tamoxifen, ICI 115,513 or ICI 111,611. Two control groups were injected with peanut oil. Six days later treated groups and one control group were injected with oestradiol (0.08 μg) on three consecutive days. Nine days after the first injection animals were killed, uteri were weighed and cytoplasmic oestrogen receptor levels were determined (see below).

Nuclear and cytoplasmic oestrogen binding assays

Paired uteri were homogenised together in 2 ml TED buffer by 2 × 5 sec bursts with an Ultraturax tissue homogeniser (with ice water cooling) and then immediately centrifuged for 10 min at 800 ***g*** (4°C). Supernatants were then centrifuged at 100,000 ***g*** (4°C) for 1 hr to prepare cytosol. The 800 ***g*** pellet was resuspended in 1.5 ml TED buffer containing 0.25 mol/l sucrose (TEDS) and centrifuged at 100 ***g*** for 5 min; the supernatant was decanted and stored. The pellet was resuspended in TED buffer and the procedure was repeated. The pellet (P_1) was stored for DNA determinations. Pooled supernatants were centrifuged at 800 ***g*** for 10 min and the resulting supernatants were discarded. The nuclear pellet was resuspended and centrifuged through TEDS buffer three times to wash. The nuclear pellet (P_2) was resuspended in 1.6 ml TEDS buffer and 800 μl (4 × 200 μl) were used for

nuclear exchange assays and 800 μl after pelleting (1/2 P_2) for DNA extraction and determination.

Methods essentially the same as those established by Anderson *et al.* [10] were used to determine filled nuclear oestrogen binding sites. Nuclear suspensions (200 μl) were incubated with 50 μl 7.5×10^{-8} mol/l [^{3}H]oestradiol for 30 min at 37°C. Parallel incubates were undertaken with [^{3}H] oestradiol and 1000-fold excess of DES. All tubes were incubated in duplicate. Incubates were cooled to 4°C, 1.5 ml TEDS buffer was added and then samples were centrifuged at 800 g_{av} for 15 min. Nuclear pellets were resuspended and repelleted three times with 1.5 ml TEDS buffer. The washed pellet was extracted and 1 ml absolute ethanol, which was used to determine specific binding of [^{3}H]oestradiol.

The methods established by Katzenellenbogen *et al.* [11] were used to determine cytoplasmic oestrogen receptor concentrations. Initial experiments confirmed the finding of Katzenellenbogen *et al.* [11] that during exchange at 30°C for 20 hr receptor losses were between 0 and 10%. Cytosol was cooled to 4°C and 1.2–1.5 ml was incubated with 200 μl dextran-coated charcoal (DCC 1%) for 30 min at 4°C to absorb unbound ligand. After centrifugation (1000 g_{max} for 10 min) supernatants (200 μl) were incubated with 50 μl 1.5×10^{-7} mol/l [^{3}H]oestradiol at 30°C or 4°C for 16–20 hr. A parallel assay was undetaken with [^{3}H]oestradiol and 1000-fold excess DES. Exchanging cytosols (30°C) were cooled to 4°C (30 min) and unbound ligand was removed by incubation with 400 μl DCC (1%) for 30 min (4°C). After centrifugation (1000 g_{max} for 10 min) aliquots (400 μl) of the supernatants were used to determine specific binding of [^{3}H]oestradiol.

To determine tritium levels, aliquots were counted in 10 ml tritium scintillator [6 g butyl PBD 2(4′-*tert*-butylphenyl)-5(4′-biphenylyl)-1,2,5-oxadiazole, 135 ml toluene, 720 ml dioxan, 100 g naphthalene, 45 ml absolute methanol] using a Beckman LS330 liquid scintillation spectrometer. Counting efficiency was determined by an automatic external standard method and c.p.m. were converted to d.p.m.

Extraction and determination of uterine DNA content

Perchloric acid (0.5 mol/l, 2 ml) was added to the pellets P_1 and 1/2 P_2, and the mixture was incubated at 70°C for 15 min and then centrifuged at 2200 g for 10 min, the supernatants removed and the pellet extracted twice more by the same procedure. DNA was then measured on the combined acid extracts using the method of Burton [12] with calf thymus DNA standards. P_2 was calculated and total DNA was estimated (P_1 and P_2). These data were used to transpose the calculated number of femtomoles in the nuclear exchange assay to pmol/uterus.

Experiments with oestradiol 3-methyl ether derivatives in vivo

Two types of experiments were undertaken:

(a) Immature female rats were randomized into 10 groups of 10 animals. Four paired groups were each injected i.p. with either 0.1, 1, 10 or 50 μg (in 0.1 ml of 20% DMSO) of test compounds on day 1. One pair of each group was injected s.c. on days 1, 2 and 3 with 0.16 μg oestradiol benzoate in 0.1 ml peanut oil. One of the remaining groups was injected with oestradiol benzoate and the final group with vehicle alone. Animals were killed on day 4 and uteri weighed wet. Compounds ICI 140,175 and ICI 140,496 were used in this test.

(b) Twenty mature female rats (200–250 g) were bilaterially ovariectomized under ether anaesthesia and allowed 1 week for recovery. Animals were divided into 4 groups of 5 rats and treated for 5 days with:

(1) oestradiol benzoate 5 μg s.c. in 0.1 ml peanut oil;
(2) ICI 141,857 100 μg i.p. in 0.1 ml 20% DMSO, 0.9% saline;
(3) ICI 141,857 and oestradiol benzoate;
(4) injections of both vehicles alone.

Animals were killed on the fifth day 2 hr after the last injection and uteri were weighted wet.

RESULTS

Experiments in vitro

Two different techniques (a dextran-coated charcoal assay and sucrose density gradient analysis) were used to evaluate whether the derivates of tamoxifen inhibited the binding of [^{3}H]oestradiol to the oestrogen receptor. Using a rabbit uterine cytosol in a DCC assay, all compounds inhibited the binding of [^{3}H]oestradiol in a concentration related manner. Replacement of the chlorine atom in the side chain of ICI 115,513 with an alcoholic hydroxyl group (ICI 115,387) slightly increased activity (Fig. 3a). ICI 119,647 was more active than its *cis* isomer ICI 121,919 (Fig. 3b). Overall, the relative binding affi-

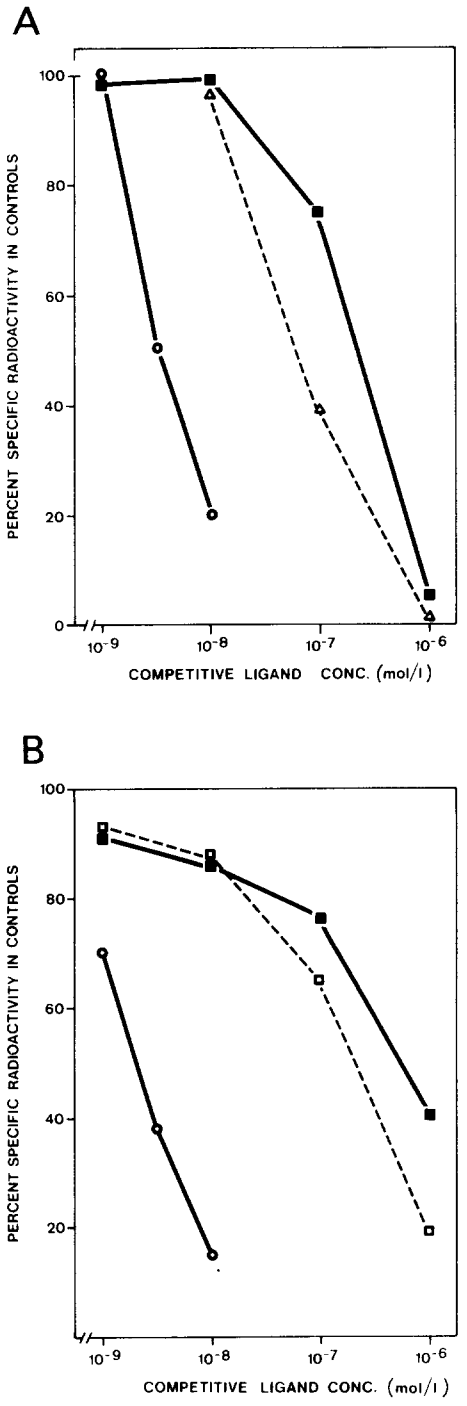

Fig. 3. Effect of (A) *increasing concentrations of oestradiol* (○), *ICI* 115,387 (△) *and ICI* 115,513 (■) *or* (B) *increasing concentrations of oestradiol* (○), *ICI* 119,647 (□) *and ICI* 121,919 (■) *on the binding of* [^{3}H]*oestradiol to uterine cytosol from mature rabbit. Cytosol* (200 µl) *was incubated with the compounds added in methanol* (10 µl) *and* 50 µl *TED buffer containing* 2.5×10^{-8} mol [^{3}H]*oestradiol*/l, *at* 4°C *for* 4 hr. *Free ligand was adsorbed by incubation with* 400 µl 1% *dextran-coated charcoal suspension at* 4°C *for* 30 min. *Tubes were centrifuged at* 2000 ***g*** *for* 5 min *and tritium counts in* 200 µl *of supernatants were determined. Specifically bound radioactivity c.p.m. is plotted as a percentage of that in the control tubes* (*cytosol incubated with methanol alone–cytosol incubated with methanol containing diethylstilboestrol to give a final incubation concentration of* 5×10^{-6} mol/l).

nities for all the compounds were: oestradiol-17β (100), ICI 115,513 (2), ICI 115,387 (7.7), ICI 119,647 (2.5), ICI 121,919 (0.8), ICI 111,611 (3), ICI 111,610 (4) and tamoxifen (1.8).

To check that the compounds were active in another test system, ICI 111,611 and ICI 111,610 were selected for further study. Using a rabbit uterine cytosol, ICI 111,611 and ICI 111,610 at the higher concentrations tested (2×10^{-7} mol/l) both inhibited the binding of [^{3}H]oestradiol (2×10^{-9} mol/l) to the 8 s oestrogen receptor as demonstrated by sucrose density gradient analysis (5–20%) (Fig. 4). ICI 111,610, the compound with the hydroxyl in the side-chain, was slightly more active than ICI 111,611 with a chlorine in the side-chain.

Experiments with tamoxifen derivatives in vivo

The compounds were assessed for their ability to antagonise the effects of oestrogens in the immature and maturing rat uterus.

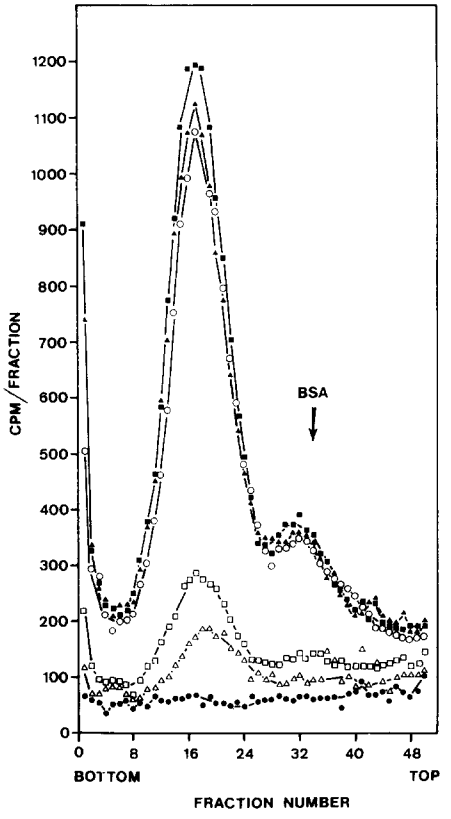

Fig. 4. The effect of increasing concentrations of ICI 111,610 (▲, 2×10^{-9} mol/l; △, 2×10^{-7} mol/l), *ICI* 111,611 (■, 2×10^{-9} mol/l; □, 2×10^{-7} mol/l) *or oestradiol* (●, 4×10^{-8} mol/l) *on the binding of* [^{3}H]*oestradiol* (○, 2×10^{-9} mol/l) *in immature rat uterine cytosol. The technique of sucrose density gradient analysis* (5–20%) *has been described previously* [9]. *The BSA standard* (4.6 s) *was determined from a separate analysis.*

ICI 115,513 at 1 or 10 μg daily demonstrated uterotrophic activity in the 3-day immature rat uterine weight test. However, neither dose was able to antagonise the uterotrophic effects of oestradiol (0.08 μg daily) (Table 1).

In order to assess the short and long term effects of the potential alkylating agents ICI 115,513 and ICI 111,611 on immature rat uterine wet weight and cytoplasmic and nuclear oestrogen receptor levels, 50 μg of each was administered and animals were killed 24 and 120 hr later. Results were compared with 50 μg tamoxifen. All three compounds were significantly uterotrophic at 24 and 120 hr (Table 2). Cytoplasmic oestrogen receptor levels were not significantly reduced at 24 hr, but nuclear oestrogen receptor levels were elevated for each compound. At 120 hr the nuclear levels were significantly elevated only in tamoxifen-treated animals. Tamoxifen decreased cytoplasmic oestrogen receptor levels by approximately 50% at 120 hr. In contrast, ICI 115,513 produced an increase in cytoplasmic oestrogen receptor levels at this time (Table 2).

Table 1. Oestrogenic and antioestrogenic effects of ICI 115,513 in the 3-day immature rat uterine weight test. Eight rats per group

Dose compounds daily (μg s.c.)		
Oestradiol	ICI 115,513	Uterine wet weight mg ± s.e.m.
—	—	35.4 ± 2.0
0.08	—	85.7 ± 3.6
—	1.0	43.7 ± 2.6*
—	10.0	55.5 ± 2.3†
0.08	1.0	80.0 ± 2.7
0.08	10.0	79.3 ± 3.3

Comparison with vehicle-treated control (Student's *t*-test).
*$P<0.05$.
†$P<0.001$.
Comparisons of ICI 115,513 and oestradiol with oestradiol alone groups; no significant difference.

The pharmacological consequences of these findings were assessed in animals treated with each of the above compounds (50 μg), by challenging them on days 6, 7 and 8 with 0.08 μg oestradiol daily. Only tamoxifen demonstrated an effective antioestrogenic action and lowered the level of cytoplasmic oestrogen receptors. ICI 115,513 decreased cytoplasmic oestrogen receptor levels but was not antioestrogenic (Table 3).

Oestrogenic and antioestrogenic tests of the steroidal compounds ICI 140,175 and ICI 140,496 over the dose range 0.1–50 μg in immature rats did not detect any activity (Table 4). Compound ICI 141,857 was significantly uterotrophic ($P<0.01$) in the ovariectomized rat when administered over 5 days at 100 μg daily. The compound was not antioestrogenic at this dose (Table 5).

Table 2. Effect of a single administration of tamoxifen (50 μg s.c.), ICI 111,611 (50 μg s.c.) on immature rat uterine wet weight, cytoplasmic and nuclear oestrogen receptors determined (A) 24 hr after administration or (B) 120 hr after administration. Results represent mean ± s.e.m. with 12 rats per group. For oestrogen receptor determinations uteri were pooled into 3 groups of 4 uteri

Compound	Uterine wet weight (mg)	Oestrogen receptors (pmol/uterus) Cytoplasmic	Nuclear
A Vehicle control	33.7 ± 2.7	1.9 ± 0.4	0.05
Tamoxifen	60.2 ± 1.8§	1.35 ± 0.05	0.33 ± 0.03‡
ICI 115,513	43.4 ± 2.2	1.69 ± 0.14	0.15 ± 0.01‡
ICI 111,611	56.4 ± 1.7	1.6 ± 0.09	0.24 ± 0.03†
B Vehicle control	35.9 ± 3.6	1.68 ± 0.16	0.05
Tamoxifen	52.2 ± 1.2§	0.77 ± 0.06‡	0.16 ± 0.02*
ICI 115,513	46.7 ± 1.6†	2.17 ± 0.08	0.15 ± 0.04
ICI 111,611	45.6 ± 2.3*	1.63 ± 0.12	0.09 ± 0.04

Comparison with related vehicle-treated control (Student's *t*-test).
*$P<0.05$.
†$P<0.02$.
‡$P<0.01$.
§$P<0.001$.

Table 3. Effect of a single administration of tamoxifen (50 μg s.c.) ICI 111,611 (50 μg s.c.) on oestradiol-stimulated (0.08 μg s.c. daily on days 6, 7 and 8) rises in uterine wet weight and cytoplasmic oestrogen content measured on day 9. Twelve animals per group with uteri pooled into 4 groups of 3 uteri. Results represent means ± s.e.m.

Compound	Uterine wet weight (mg)	Cytoplasmic oestrogen receptor concentration (pmol/uterus)
—	92.6 ± 7.08	3.08 ± 0.32
Oestradiol (OE_2)	134.2 ± 8.9*	2.73 ± 0.17
OE_2 + Tamoxifen	82.92 ± 3.6‡	1.15 ± 0.07‡
OE_2 + ICI 115,513	146.9 ± 3.7	1.86 ± 0.29†
OE_2 + ICI 111,611	130.1 ± 4.4	2.56 ± 0.09

Comparison OE_2 vs vehicle-treated control.
*$P < 0.01$ (UWW).
Comparison OE_2 vs drug + OE_2.
†$P < 0.05$.
‡$P < 0.001$ by Student's *t*-test.

DISCUSSION

The primary aim of the first part of our study was to assess whether various derivatives of tamoxifen were nuclear alkylating agents and therefore irreversible oestrogen antagonists. All the compounds that were tested *in vitro* had similar activity to tamoxifen [8, 9] in inhibiting the binding of [^{3}H]oestradiol to rabbit and rat uterine oestrogen receptors. However, none of the compounds was as active as tamoxifen at reducing uterine cytoplasmic oestrogen receptor levels *in vivo* nor were they detectably antioestrogenic. It seems that these derivatives provide no advantages over tamoxifen itself as oestrogen antagonists.

Considering the structure–activity relationships for antioestrogenicity, it seems that the correct substitution of the aminoethoxy side chain in tamoxifen is important for activity *in vivo*. In support of this observation, it has been shown that the length and substitution of the alkyl aminoethoxy side chain in nafoxidine is critical for antioestrogenic action [13]. Furthermore, the side chain needs to be at a certain position in space since dimethyl substitution *ortho* to the amino ethoxy side chain of the antioestrogen MER 25 destroys activity *in vivo* [14].

These data clearly demonstrate a disparity between the activities of the compounds *in vitro* and *in vivo* which indicates that the pharmacokinetic properties of the tamoxifen derivatives are ultimately more important than the oestrogen receptor binding activity. As one possible explanation, we have recently reported [15] that receptor binding in oestrogen target tissues is increased when tamoxifen is metabolically hydroxylated to an aro-

Table 4. Effect of a single administration i.p. of either ICI 140,175 or ICI 140,496 on either immature rat uterine wet weight or oestradiol benzoate (0.16 μg s.c. daily on the three consecutive following days)-stimulated increase in uterine wet weight. Animals were killed 24 hr after the last injection of oestradiol benzoate. Ten rats per group

Treatment and test compound	Dose test compound (μg) 0	0.1	1	10	50
Peanut oil; ICI 140,175	35.95 ± 3.06	31.0 ± 1.44	37.64 ± 2.27	40.24 ± 3.46	40.68 ± 2.26
OE_2B; ICI 140,175	117.7 ± 5.18	112.0 ± 5.08	102.4 ± 6.45	116.20 ± 7.87	114.44 ± 4.1
Peanut oil; ICI 140,496	40.84 ± 2.15	38.89 ± 1.99	35.44 ± 1.94	32.84 ± 2.3	37.18 ± 3.00
OE_2B; ICI 140,496	109.00 ± 6.25	109.00 ± 5.68	111.0 ± 4.86	113.1 ± 5.46	118.1 ± 4.99

Comparison of oestradiol benzoate treated above groups with oestradiol benzoate plus compounds by Students's *t*-test demonstrated no significant differences ($P > 0.05$) between group means.

Table 5. Effect of 5 daily s.c. injections of oestradiol benzoate (5 μg in 0.1 ml peanut oil), ICI 141,857 (100 μg i.p. in 0.1 ml 20% DMSO/0.9% saline) or a combination of the two on ovariectomized rat uterine wet weight. Five rats per group

Treatment	Uterine wet weight mg ± s.e.m.
Peanut oil and DMSO control	65.6 ± 5.15
Oestradiol benzoate (OE_2B)	214.0 ± 11.69
ICI 141,857	91.0 ± 4.73*
ICI 141,857 + OE_2B	203.6 ± 12.34

Comparison of ICI 141,857 treated with controls (Student's *t*-test).
*$P < 0.01$.

matic phenol (monohydroxytamoxifen). It is possible that the tamoxifen derivatives cannot undergo hydroxylation, or alternatively that they immediately alkylate sites within the liver. This possibility is certainly likely when considering the inactivity of the substituted derivatives of oestradiol 3-methyl ether. Oestrogen receptor binding assays were not undertaken because binding was expected to be low by analogy with the related compound, mestranol, which is known to have a low estrogen receptor binding affinity [16]. However, it is believed that mestranol is metabolically activated by demethylation to ethinyl oestradiol[17] before exerting its biological effects in oestrogen target tissues. The low biological activity of high doses of the potential alkylating steroids may reflect liver alkylation during demethylation.

The lack of activity of the compounds in the present study is similar to the reports with another potential alkylating derivative of tamoxifen, ICI 79,792 [2, 18]. This suggests that only compounds with a very high affinity for the oestrogen receptor should be considered as carriers for alkylating agents. Potentially, this approach would provide a rapid localisation in target tissue, i.e. breast or endometrial carcinomata, without a need to be metabolically activated.

The oestrogen receptor binding activity *in vitro* of a large series of potential alkylating oestrogen derivatives has recently been described by LeClercq [19]. Whether these compounds will fulfil their promise as alkylating agents *in vivo* remains to be determined.

REFERENCES

1. M. J. K. Harper and A. L. Walpole, A new derivative of triphenylethylene: the effect on implantation and mode of action in rats. *J. Reprod. Fertil.* **13,** 101 (1967).
2. R. I. Nicholson and M. P. Golder, The effect of synthetic antioestrogens on the growth and biochemistry of rat mammary tumours. *Europ. J. Cancer* **11,** 571 (1975).
3. V. C. Jordan and L. J. Dowse, Tamoxifen as an antitumour agent: Effect on oestrogen binding. *J. Endocr.* **68,** 297 (1976).
4. V. C. Jordan, Effect of tamoxifen (ICI 46,474) on initiation and growth of DMBA-induced rat mammary carcinomata. *Europ. J. Cancer* **12,** 419 (1976).
5. V. C. Jordan, Antiestrogenic and antitumor properties of tamoxifen in laboratory animals. *Cancer Treat. Rep.* **60,** 1409 (1976).
6. R. C. Heel, R. N. Brogden, T. M. Speight and G. S. Avery, Tamoxifen: A review of its pharmacological properties and therapeutic use in the treatment of breast cancer. *Drugs* **16,** 1 (1978).
7. W. L. McGuire, P. P. Carbone and E. P. Vollmer, *Estrogen Receptors in Human Breast Cancer.* Raven Press, New York (1975).
8. V. C. Jordon, M. M. Collins, L. Rowsby and G. Prestwich, A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. *J. Endocr.* **75,** 305 (1977).
9. V. C. Jordan and G. Prestwich, Binding of [^{3}H]tamoxifen in rat uterine cytosols: a comparison of swinging bucket and vertical tube rotor sucrose density gradient analysis. *Mol. cell. Endocrin.* **8,** 179 (1977).
10. J. N. Anderson, J. H. Clark and E. J. Peck, Oestrogen and nuclear binding sites by [^{3}H]oestradiol exchange. *Biochem. J.* **126,** 561 (1972).
11. J. A. Katzenellenbogen, H. J. Johnson and K. E. Carlson, Studies on the uterine cytoplasmic estrogen binding protein. Thermal stability and ligand dissociation rate. An assay of empty and filled sites by exchange. *Biochemistry* **12,** 4092 (1973).
12. K. Burton, A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62,** 315 (1956).
13. D. Lednicer, S. C. Lyster and G. W. Duncan, Mammalian antifertility agents. IV. Basic 3,4 dihydro-naphthalenes and 1234 tetrahydro-1-naphthols. *J. med. Chem.* **10,** 78 (1976).
14. E. R. Clark and V. C. Jordan, Oestrogenic antioestrogenic and fertility effects of some triphenylethanes and triphenylethylenes related to ethamoxytriphetol (MER 25). *Brit. J. Pharmac.* **57,** 487 (1976).

15. K. E. Allen, E. R. Clark and V. C. Jordan, Evidence of the metabolic activation of non-steroidal antioestrogens: a study of structure activity relationship. *Brit. J. Pharmac.*, in press.
16. A. Eisenfeld, Oral contraceptives: ethinyl estradiol binds with higher affinity than mestranol to macromolecules from the sites of antifertility action. *Endocrinology* **94,** 803 (1974).
17. D. W. Hahn, J. L. McGuire, F. C. Greenslade and E. D. Turner, Molecular parameters involved in the estrogenicity of mestranol and ethinyl estradiol. *Proc. Soc. exp. Biol. Med.* **137,** 1180 (1971).
18. W. Powell-Jones, D. A. Jenner, R. W. Blamey, P. Davis and K. Griffiths, Influence of antioestrogens on the specific binding *in vitro* of [^{3}H]oestradiol by cytosol of rat mammary tumours and human breast carcinomata. *Biochem. J.* **150,** 71 (1975).
19. G. LeClercq, N. Devleeschouwer, N. Legros and J. C. Heuson, Estrogen-like cytotoxic agents of potential value for the treatment of breast cancer. *Europ. J. Cancer* in press.