

# Effects of a non-steroidal pure antioestrogen, ZM 189,154, on oestrogen target organs of the rat including bones

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## Abstract

ZM 189,154 ([1RS,2RS]-2-(4-hydroxyphenyl)-2-methyl-1-[9-(4,4,5,5,5-penta-fluoropentyl)sulphonylnonyl]-1,2,3,4-tetrahydronaphth-6-ol) is a non-steroidal pure antioestrogen. It has a high relative affinity for the oestrogen receptor, completely blocks the trophic action of oestradiol (OE<sub>2</sub>) on the uterus in immature and ovariectomized (OVX) adult rats and, in the latter, also completely blocks the trophic action of OE<sub>2</sub> on vagina, bone and growth rate. ZM 189,154 displays no intrinsic oestrogen-agonist activity on uterus, vagina, bone, LH secretion or growth rate in OVX rats. Differential sensitivity of OE<sub>2</sub>-regulated processes was more apparent in intact rats. Daily doses of 0.6 mg/kg per day of ZM 189,154 blocked ovulation;

2 mg/kg per day achieved maximal uterine atrophy but did not affect bone density or growth rate; 10 mg/kg per day produced a broader spectrum of effects (reduced bone density, increased basal LH, slightly increased growth rate), but the magnitude of these was smaller than after ovariectomy; the 10 mg/kg dose also produced multiple ovarian follicular cysts. The failure of ZM 189,154 to achieve complete ovariectomy-like effects in intact rats may be due to the action of ovarian factors other than OE<sub>2</sub>, or to the circulating OE<sub>2</sub> levels resulting from the disturbance to ovarian function posing too strong a challenge to the antagonist.

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## Introduction

The properties of 7 $\alpha$ -alkylamide and 7 $\alpha$ -alkynylsulphonyl analogues of oestradiol-17 $\beta$  (OE<sub>2</sub>) which characterize them as pure antioestrogens have been described (Wakeling & Bowler 1987, Wakeling *et al* 1991). These agents are pharmacologically distinct from the partial agonist antioestrogens such as tamoxifen, notably in their capacity to completely block the trophic actions of either OE<sub>2</sub> or tamoxifen on oestrogen target organs such as the uterus and mammary gland in rodents and primates. (Wakeling & Bowler 1987, Nicholson *et al* 1988, Wakeling *et al* 1991, Dukes *et al* 1992). However these studies also demonstrated that there are significant differences in organ sensitivity to the action of pure antioestrogens; for example in rats, complete inhibition of oestrogen action on the uterus can be achieved without affecting LH secretion or bone density (Wakeling & Bowler 1988, Wakeling 1993).

In addition to steroidal pure antioestrogens, non-steroidal pure antioestrogens have also been described (von Angerer *et al* 1990, Sharma *et al* 1990, Nishino *et al* 1991, Day *et al* 1991). The activity of a new agent of this type, ZM 189,154, (European Patent, EP0124369 B1), a 2-methyltetrahydronaphthalene substituted with a side-chain like that of ICI 182,780 (Fig 1), is reported here to illustrate further the range of effects these agents elicit in

oestrogen-dependent tissues. Attention is focussed on the differences in dosage needed to affect different oestrogen-dependent processes with particular reference to effects on bone because of concerns that long term clinical use of pure antioestrogens might adversely affect bone density to cause an ovariectomy-like onset of osteoporosis (Jordan 1992).

## Materials and Methods

The antioestrogens tamoxifen (ICI 46,474 (trans-1-(4-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene), ICI 164,384 (N-n-butyl-N-methyl-11-[3,17-dihydroxy-oestra-1,3,5(10)-triene-7-yl]undecanamide) and ZM 189,154 ([1RS,2RS]-2-(4-hydroxyphenyl)-2-methyl-1-[9-(4,4,5,5,5-pentafluoropentyl)sulphonylnonyl]-1,2,3,4-tetrahydronaphth-6-ol) were synthesized in the laboratories of Zeneca Pharmaceuticals.

Competitive binding assays to measure the relative binding affinity of antioestrogens for rat uterine oestrogen receptors were as described elsewhere (Wakeling & Slater 1980) except that the competitor dilutions were prepared in Tris:dimethylformamide (1:1) and mixed together with [<sup>3</sup>H]OE<sub>2</sub> (Amersham International, Amersham, UK) with cytosol at a ratio of 1:20.

The rat uterine weight assay for uterotrophic and antiuterotrophic activity has been described (Wakeling *et*

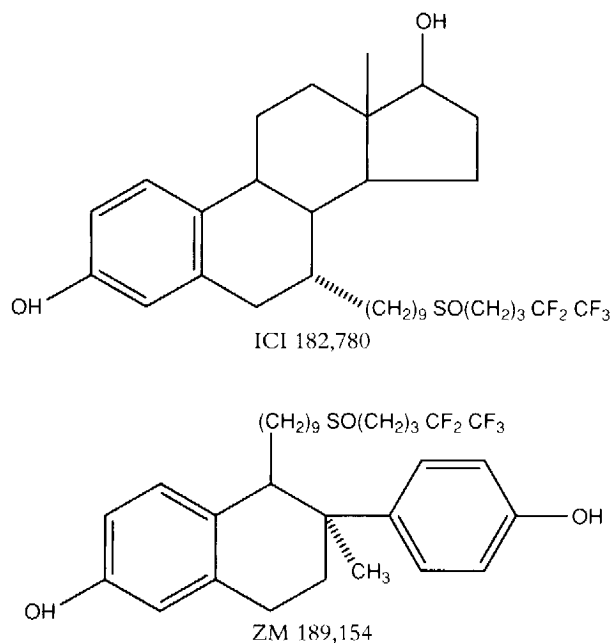


FIGURE 1. Structures of the pure antioestrogens ICI 182,780 and ZM 189,154.

*al* 1983). Details of doses, routes of administration and duration of treatments are reported in the present Figures and Tables.

ZM 189,154 and oestradiol benzoate (Sigma Chemical Company, Poole, Dorset, UK) were prepared for administration by diluting an ethanol stock solution into the required volume of arachis oil with gentle warming (60 °C). Tamoxifen was prepared for oral administration as a dispersion in aqueous 0.5% Tween 80. Dose volumes were 0.5 and 0.1 ml/100 g body weight for immature and mature rats respectively.

In uterotrophic/antiuterotrophic studies in ovariectomized (OVX) rats, ovariectomy was performed at least 2 weeks before treatment began. For ovulation inhibition studies, rats having vaginal smear patterns consistent with 4-day-oestrous cycles were given either a single dose of ZM 189,154 on day 2 or 3 of the cycle, or daily doses on days 1 to 4 of the cycle. The rats were then killed by CO<sub>2</sub> exposure on the morning of the next scheduled day 1, their Fallopian tubes excised and the contents gently expressed onto a microscope slide and the number of eggs present counted.

Effects on uterine, ovarian and body weights and plasma gonadotrophin concentrations in intact rats were assessed after 14 days of dosing, effects on bone parameters were assessed after 28 days of dosing, this being the shortest convenient interval following ovariectomy at which significant reductions in bone density were readily measurable; body, uterine and ovarian weights were also monitored in these longer experiments. All the rats used in

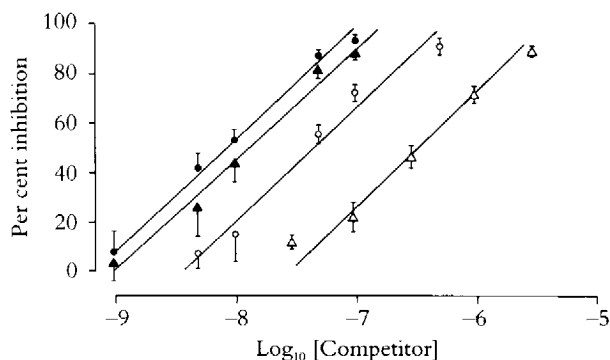


FIGURE 2. Competition for binding of  $5 \times 10^{-9}$  M [<sup>3</sup>H]oestradiol-17 $\beta$  (OE<sub>2</sub>) to rat uterine oestrogen receptor by unlabelled OE<sub>2</sub> (●) ZM 189,154 (▲), ICI 164,384 (○) and tamoxifen (△). Percent inhibition refers to specific binding corrected by subtraction from total [<sup>3</sup>H]OE<sub>2</sub> bound, the non-specific component recorded in the presence of  $5 \times 10^{-7}$  unlabelled OE<sub>2</sub>. Each point and bar represents the mean  $\pm$  S.E.M. of nine observations in three different experiments. Estimates of competitor concentration which reduced [<sup>3</sup>H]OE<sub>2</sub> by 50% (IC<sub>50</sub> values) were calculated by linear regression analysis of per cent inhibition versus log<sub>10</sub>[competitor].

these studies weighed between 240 and 260 g at the start of the experiments. At the end of the dosing period, left and right femurs were dissected, freed of adherent soft tissues, weighed and their volumes determined by Archimedes' principle (by subtraction of the weight of a 25 ml specific gravity bottle filled with water containing each femur, from the sum of the weights of the femur and the specific gravity bottle filled only with water) to estimate gross density. The femurs were then reduced to ash and the ash weighed. Gross bone density was calculated by dividing femur weight by volume; mineral density was calculated by dividing femur ash weight by volume. One group of rats in each of these studies was subjected to ovariectomy on day 1 to provide an estimate of the maximum antioestrogenic effect potentially attainable.

Plasma luteinizing hormone (LH) concentrations were assayed by a modification of the double-antibody technique described by Niswender *et al* (1969).

Treatment effects were analysed by comparison of group means using Student's *t*-test.

## Results

### *Interaction with oestrogen receptor*

Competition of ZM 189,154 with [<sup>3</sup>H]-OE<sub>2</sub> for binding to the rat uterus oestrogen receptor was measured (Fig 2) and compared with that of tamoxifen and the steroidal pure antioestrogen ICI 164,384. Competitive

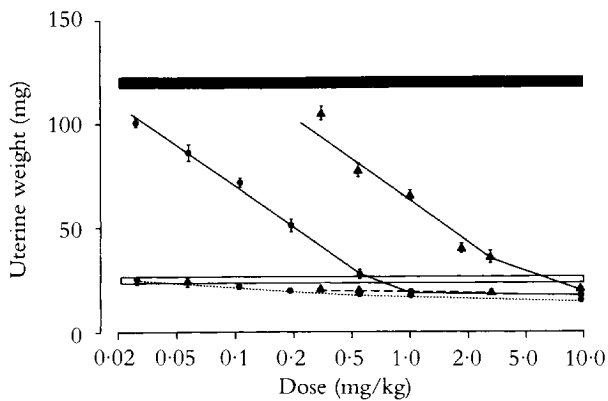


FIGURE 3. Effects of ZM 189,154 on uterine weight of immature rats. Animals received daily a single dose of arachis oil vehicle alone (open bar), 0.5 g oestradiol benzoate s.c. alone (solid bar), or the indicated doses of ZM 189,154 alone s.c. (●—●) or orally (▲—▲), or oestradiol benzoate together with ZM 189,154 s.c. (●—●) or orally (▲—▲) for 3 days. Points and bars represent means  $\pm$  s.e.m. for a minimum of 10 observations in at least two different experiments. Where no bar is present errors are smaller than the symbols.

displacement of [ $^3\text{H}$ ]- $\text{OE}_2$  by ZM 189,154 reflected by the parallel displacement curves, allowed calculation of a relative binding affinity of 0.66 for ZM 189,154 ( $\text{OE}_2=1$ ), compared with 0.19 and 0.025 for ICI 164,384 and tamoxifen respectively.

#### Antiuterotrophic activity in immature rats

When administered orally or parenterally at doses in the range 0.025–10 mg ZM 189,154/kg, the weight of the uterus in treated rats was always similar to or less than that in vehicle treated immature rats (Fig 3). Co-administration of ZM 189,154 together with a maximally effective dose of  $\text{OE}_2$  inhibited the trophic action of  $\text{OE}_2$  on the immature rat uterus in a dose-dependent manner (Fig 3). Complete blockade of  $\text{OE}_2$ -induced uterine growth was achieved with daily subcutaneous (s.c.) doses of 0.5 mg/kg or oral (p.o.) doses of 3–5 mg/kg. Estimates of the dose required to reduce uterine weight by 50% ( $\text{ED}_{50}=0.09$  and 0.7 mg/kg, s.c. and p.o. respectively) indicated that ZM 189,154 is seven- to eightfold less potent via the oral route compared with parenteral administration. Similar assays in adult OVX rats and mice confirmed that ZM 189,154 alone did not stimulate the uterus and did not induce vaginal cornification;  $\text{OE}_2$ -stimulated growth was also blocked by ZM 189,154 (data not shown,  $\text{ED}_{50}$  values of 1.3 and 6.2 mg/kg, p.o. in rats and mice respectively).

When the immature rat uterus was stimulated by treatment with tamoxifen instead of  $\text{OE}_2$ , co-administration of ZM 189,154 antagonized the action of tamoxifen in a dose-dependent manner and complete blockade of tamoxifen-induced growth was achieved with a dose of 10 mg ZM 189,154/kg (Fig 4).

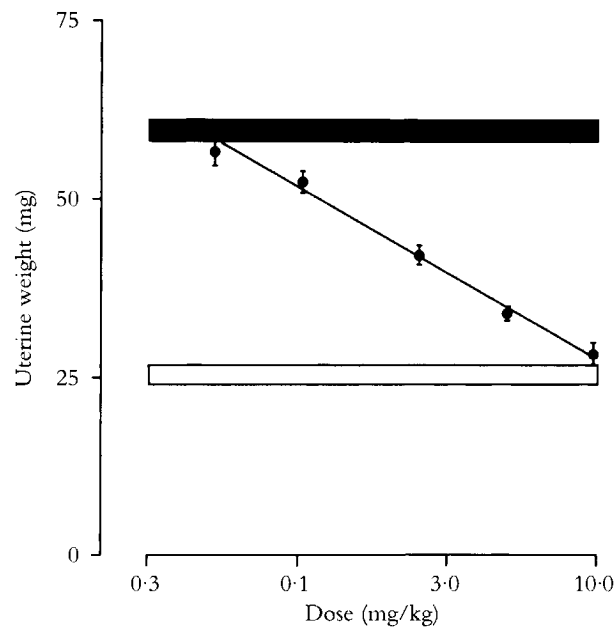


FIGURE 4. Antagonism of the uterotrophic effect of tamoxifen by ZM 189,154. Immature rats received daily a single dose of arachis oil vehicle alone (open bar), 1.0 mg tamoxifen/kg orally (solid bar), or the indicated doses of ZM 189,154 s.c. together with tamoxifen for 3 days. Points and bars represent means  $\pm$  s.e.m. for a minimum of ten observations in at least two different experiments. Where no bar is present errors are smaller than the symbols.

#### Effects in OVX mature rats

The trophic and inhibitory effects of ZM 189,154 on the uterus, vagina and growth rate of adult OVX rats were measured to determine whether this agent showed the differential effects on different oestrogen target organs described previously for the steroidal pure antioestrogens (Wakeling & Bowler 1988, Wakeling *et al* 1991). In animals treated for 14 days with  $\text{OE}_2$  alone uterine weight increased fourfold compared with OVX controls, growth rate was reduced and full cornification of the vagina was recorded after 4 days. In contrast, at a daily oral dose of 10 mg/kg administered alone to OVX rats, ZM 189,154 had no effect on the uterus, growth rate or vagina (Table 1) but, given together with  $\text{OE}_2$ , ZM 189,154 achieved 72, 96 and 100% blockade of the uterotrophic action of  $\text{OE}_2$  with daily oral doses of 1.5, 4 and 10 mg/kg (Table 1). However, the lowest dose of ZM 189,154 had little effect on  $\text{OE}_2$ -induced vaginal cornification and none of the doses reversed the  $\text{OE}_2$ -induced suppression of body weight gain in OVX rats (Table 1).

Since ZM 189,154 was more potent parenterally than orally, the effects of 10 mg/kg s.c. were studied alone and in combination with  $\text{OE}_2$  or tamoxifen. Again, there was no evidence for an oestrogenic action of ZM 189,154 on the uterus or on growth rate or plasma LH concentration,

TABLE 1. Agonist and antagonist activity of ZM 189,154 (1.5–10 mg/kg, orally) and oestradiol (OE<sub>2</sub> benzoate; 0.5 µg/day s.c.) in ovariectomized rats. Values are means ± s.e.m. for groups of five rats treated for 14 days

Treatment	Weight gain (g)	Uterus wt (mg)	Vaginal cornification <sup>1</sup>
Ovariectomy	49.0 ± 2.4 <sup>a</sup>	85 ± 3 <sup>a</sup>	0
OE <sub>2</sub>	27.0 ± 2.4 <sup>b</sup>	342 ± 21 <sup>b</sup>	60
ZM 189,154 (10 mg/kg per day)	42.0 ± 3.8 <sup>a</sup>	80 ± 4 <sup>a</sup>	0
OE <sub>2</sub> +ZM 189,154			
1.5 mg/kg per day	19.8 ± 4.6 <sup>b</sup>	157 ± 15 <sup>c</sup>	48
4.0 mg/kg per day	20.4 ± 2.9 <sup>b</sup>	91 ± 2 <sup>a</sup>	9
10.0 mg/kg per day	28.8 ± 3.0 <sup>b</sup>	81 ± 3 <sup>a</sup>	0

<sup>a-c</sup>Indicate values which differ significantly, i.e. at least  $P < 0.01$  (Student's *t*-test).  
<sup>1</sup>Per cent total days with pro-oestrous or oestrous smears.

TABLE 2. Agonist and antagonist activity of ZM 189,154 (10 mg/kg per day s.c.), oestradiol (OE<sub>2</sub> benzoate; 0.5 µg/day s.c.) and tamoxifen (1 mg/kg per day orally) in ovariectomized rats. Values are means ± s.e.m. for groups of 6 rats treated for seven days

Treatment	Weight gain (g)	Uterus wt (g)	Plasma LH (ng/ml)
Ovariectomy	34.0 ± 2.8 <sup>a</sup>	173 ± 10 <sup>a</sup>	15.0 ± 1.3 <sup>a</sup>
OE <sub>2</sub>	13.5 ± 3.2 <sup>b</sup>	421 ± 27 <sup>b</sup>	2.2 ± 0.3 <sup>b</sup>
Tamoxifen	0.2 ± 2.6 <sup>c</sup>	242 ± 10 <sup>c</sup>	3.2 ± 0.2 <sup>b</sup>
ZM 189,154	36.0 ± 1.9 <sup>a</sup>	158 ± 5 <sup>a</sup>	10.6 ± 1.9 <sup>a</sup>
ZM 189,154+OE <sub>2</sub>	25.8 ± 2.0 <sup>a</sup>	156 ± 10 <sup>b</sup>	16.2 ± 3.2 <sup>a</sup>
ZM 189,154+Tamoxifen	15.0 ± 0.9 <sup>b</sup>	192 ± 5 <sup>a</sup>	13.1 ± 1.8 <sup>a</sup>
Tamoxifen + OE <sub>2</sub>	3.4 ± 2.8 <sup>c</sup>	235 ± 13 <sup>c</sup>	2.4 ± 0.5 <sup>b</sup>

<sup>a-c</sup>Indicate values which differ significantly, i.e. at least  $P < 0.01$  (Student's *t*-test).

whereas both tamoxifen and OE<sub>2</sub> significantly reduced growth rate and LH concentration and stimulated the uterus (Table 2). In combination, ZM 189,154 completely reversed the uterotrophic action of OE<sub>2</sub> and tamoxifen and the suppression of LH, and partially reversed the reduction of body weight gain (Table 2).

#### Effects in intact adult rats

**i. Inhibition of ovulation** Single doses of ZM 189,154 administered on day 2 or 3 of the oestrous cycle inhibited ovulation (Table 3). A dose of 2 mg ZM 189,154/kg was fully effective given on day 2 but not on day 3 of the cycle. A lower dose of 0.6 mg ZM 189,154/kg administered daily on days 1 to 4 of the cycle also completely inhibited ovulation.

**ii. Uterine weight** Daily s.c. doses of 0.3–2 mg ZM 189,154/kg for 14 days produced a dose-related reduction of uterine weight (Table 4). The maximum regression of

TABLE 3. Inhibition of ovulation by ZM 189,154 in intact rats

Dose (mg/kg s.c.)	Time of treatment	No of rats ovulating	Ova/ovulating rat (Mean ± s.d.)
1	1600 h Day 2	9/10	14.0 ± 2.1
2	1600 h Day 2	3/5	7.7 ± 4.7
1	1600 h Day 3	0/10	
2	1600 h Day 3	7/10	7.7 ± 4.5
0.3	Days 1 to 4	4/10	5.3 ± 3.0
0.6	Days 1 to 4	0/5	11.3 ± 7.3

TABLE 4. Effects of ZM 189,154 given s.c. for 14 or 28 days on uterine and ovarian weights and body weight gain in intact and ovariectomized rats. Values are means ± s.e.m. for  $n = 5$  rats (14 day treatments) or 10 rats (28 days treatments)

Dose (mg/kg per day)	Uterine wt (% of control)	Ovarian wt (% of control)	Body wt gain (% of control)
14 days			
0.3	75.2 ± 8.1 <sup>ab</sup>	77.5 ± 5.4 <sup>a</sup>	89.6 ± 17.5 <sup>b</sup>
0.6	73.7 ± 6.6 <sup>ab</sup>	82.4 ± 7.6	93.1 ± 17.5 <sup>b</sup>
1.0	65.2 ± 5.3 <sup>ab</sup>	74.5 ± 10.8	72.4 ± 17.4 <sup>b</sup>
1.5	47.9 ± 3.8 <sup>ab</sup>	70.6 ± 7.2 <sup>a</sup>	100.0 ± 10.9 <sup>b</sup>
2.0	45.2 ± 3.3 <sup>ab</sup>	83.2 ± 9.2	89.7 ± 7.6 <sup>b</sup>
Ovariectomy	36.0	—	149.5 ± 14.4
28 days			
2.0	35.0 ± 3.2 <sup>ab</sup>	83.1 ± 5.9 <sup>a</sup>	81.1 ± 12.5 <sup>ab</sup>
10.0	33.9 ± 4.5 <sup>ab</sup>	119.7 ± 4.8	125.5 ± 13.8 <sup>a</sup>
Ovariectomy	27.8 ± 3.3	—	142.9 ± 17.9

<sup>ab</sup>Indicate means (prior to conversion to %) that were significantly ( $P < 0.05$ ; Student's *t*-test) different from intact and ovariectomized controls respectively.

the uterus was 86% of that recorded in rats 14 days after ovariectomy. Extending the period of dosing to 28 days and increasing the dose fivefold to 10 mg ZM 189,154/kg, did not significantly increase the extent of uterine atrophy compared with the effect of ovariectomy (Table 4).

**iii. Ovarian weight and histology** At all doses between 0.6 and 2 mg/kg per day, ZM 189,154 caused a significant 20–30% reduction in ovarian weight, but at 10 mg/kg per day mean ovarian weight was slightly, though not significantly, greater than in controls (Table 4). Ovaries from rats given 0.3 mg ZM 189,154/kg for 14 days contained old corpora lutea showing signs of vascular congestion and degeneration, follicles in various stages of development, but no new corpora lutea; one of the old corpora lutea contained an entrapped oocyte. Ovaries from rats given 10 mg ZM 189,154 contained virtually no corpora lutea but numerous large irregular cystic follicles. In one rat, two of the latter showed extensive haemorrhage.

TABLE 5. Effects of ZM 189,154 on weight of the uterus and on bone density in rats which were ovariectomized (OVX) and given ZM 189,154 and/or oestradiol (OE<sub>2</sub> benzoate; 0.5 µg/day) for 28 days. Values are means ± S.E.M., *n*=5 animals or *n*=10 for bone data

Treatment	Uterus wt (mg)	Bone gross density	Bone mineral density
Experiment 1.			
Control	386 ± 33 <sup>a</sup>	1.612 ± 0.007 <sup>a</sup>	0.742 ± 0.009 <sup>a</sup>
ZM 189,154 (2 mg/kg per day s.c.)	135 ± 8 <sup>b</sup>	1.604 ± 0.005 <sup>a</sup>	0.730 ± 0.007 <sup>a</sup>
OVX	111 ± 6 <sup>c</sup>	1.569 ± 0.008 <sup>c</sup>	0.685 ± 0.010 <sup>c</sup>
OVX+ZM 189,154	104 ± 3 <sup>c</sup>	1.582 ± 0.006 <sup>c</sup>	0.701 ± 0.008 <sup>c</sup>
Experiment 2.			
Control	411 ± 46 <sup>a</sup>	1.600 ± 0.003 <sup>a</sup>	0.730 ± 0.004 <sup>a</sup>
OVX	101 ± 3 <sup>b</sup>	1.532 ± 0.007 <sup>b</sup>	0.652 ± 0.010 <sup>b</sup>
OVX+OE <sub>2</sub>	475 ± 7 <sup>c</sup>	1.591 ± 0.007 <sup>a</sup>	0.738 ± 0.010 <sup>a</sup>
OVX+OE <sub>2</sub> +ZM 189,154 (2 mg/kg per day s.c.)	100 ± 3 <sup>b</sup>	1.532 ± 0.006 <sup>b</sup>	0.684 ± 0.006 <sup>b</sup>
Experiment 3.			
Control	369 ± 48 <sup>a</sup>	1.629 ± 0.004 <sup>a</sup>	0.766 ± 0.005 <sup>a</sup>
ZM 189,154 (10 mg/kg per day s.c.)	125 ± 4 <sup>b</sup>	1.580 ± 0.004 <sup>b</sup>	0.727 ± 0.005 <sup>b</sup>
OVX	99 ± 5 <sup>c</sup>	1.571 ± 0.007 <sup>b</sup>	0.704 ± 0.009 <sup>b</sup>

<sup>a-c</sup>Indicate values which differ significantly, i.e. at least *P*<0.01 (Student's *t*-test).

**iv. Body weight gain** Ovariectomy significantly increased growth with average daily weight gain increasing from 2.06 g in controls to 2.96 g in OVX rats. In contrast, doses of ZM 189,154 up to 2 mg/kg per day tended to reduce weight gain slightly (Table 4). However, the highest dose of 10 mg ZM 189,154/kg administered for 28 days did produce an ovariectomy-like effect, but of smaller magnitude than that caused by ovarian ablation.

**v. Plasma LH** At doses up to 1.5 mg/kg per day for 14 days, mean ± S.E.M. plasma LH concentrations (2.53 ± 0.21 ng/ml) were comparable with those in intact control rats (2.18 ± 0.12 ng/ml). In rats given 10 mg/kg for 28 days, LH was elevated (4.53 ± 0.97 ng/ml) to about half the extent seen in OVX rats (9.94 ± 1.33 ng/ml).

**vi. Bone density** Ovariectomy significantly reduced both the gross and mineral density of femur bone after 28 days; the mean ± S.E.M. reduction was 3.5 ± 0.5% in gross density and 8.8 ± 0.9% in mineral density (Table 5). Treatment with 2 mg ZM 189,154/kg did not reduce either gross or mineral bone density in intact animals, and in OVX rats did not increase bone density. Oestrogen treatment prevented ovariectomy-induced uterine regression and bone loss (Table 5, experiment 2). Administration of 2 mg ZM 189,154/kg together with OE<sub>2</sub> completely blocked this protective effect of OE<sub>2</sub> (Table 5, experiment 2) indicating a complete blockade of OE<sub>2</sub> action on the bones as well as the uterus in OVX rats.

In intact rats, 10 mg ZM 189,154/kg per day did produce significant reductions in bone density (Table 5, experiment 3): gross and mineral density were reduced 3.0% and 5.1%, respectively, compared with reductions of 3.6 and 8.1% in OVX rats.

## Discussion

The use of the steroidal pure antioestrogen ICI 182,780 in the therapy of breast cancer (Wakeling *et al* 1991) may confer advantages when compared with the well-established use of partial agonists like tamoxifen. For example, the development of resistance due to oestrogen-like activity, as has been seen with tamoxifen, is unlikely to occur (Wakeling 1993). However, a possible undesirable consequence of pure antioestrogen therapy is an adverse effect on bone mineral metabolism leading to induction or exacerbation of osteoporosis (Jordan 1992). In this respect the oestrogenic activity of tamoxifen is beneficial, particularly for long-term adjuvant therapy of breast cancer (Jordan 1992). Earlier studies with ICI 182,780 in intact adult female rats showed clear differences between the susceptibility of different oestrogen target organs to its antioestrogenic action (Wakeling *et al* 1991). For example, at doses which produced an ovariectomy-like regression of the uterus, no effect was seen on gonadotrophin secretion or on the rate of growth of the animals. Also, there was a differential between the dose of

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