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D-RING ALKYLAMIDE DERIVATIVES OF ESTRADIOL: EFFECT ON ER-BINDING AFFINITY AND ANTIESTROGENIC ACTIVITY

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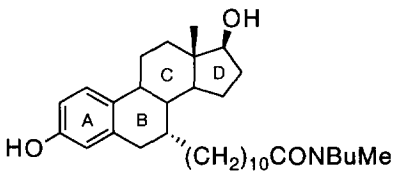
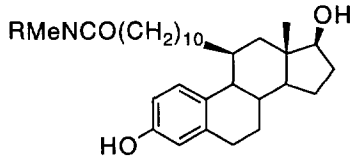
Abstract: A series of 17α-, 15α-, and 15β-(butyl methyl alkylamide)-estradiol derivatives (**4-10**) were synthesized from estrone and tested for the estrogen receptor binding affinity, uterotrophic activity, and antiuterotrophic activity. By moving the alkylamide side chain from B-ring (7α-position) or C-ring (11β-position) to D-ring (17α, 15α or 15β-positions) a dramatical decrease of the RBA and no antiestrogenic activity in uterine weight assay was observed. These results extend to D-ring the SAR study of this family of steroid antiestrogens. In addition, we also correct the C15-stereochemistry of compounds **5-10**, which was incorrectly attributed in a previous paper. Copyright © 1996 Elsevier Science Ltd

The search for pure antiestrogens or compounds devoid of agonistic activity is of major interest for the improvement of estrogen-sensitive cancer therapy. The report by Wakeling et al.[1] that a 7α-undecanamide derivative of estradiol (**1**; ICI 164384) has the characteristics of a pure antiestrogen stimulated the interest for steroidal antiestrogens. Thus, modifications of side-chain functionnalization, side-chain position, and steroidal skeleton were tested to improve the antiestrogenic activity of such compounds.[2] Focusing on the side-chain position, Roussel-Uclaf introduced an undecanamide side chain at position 11β of estradiol (**2**; RU 50667, and **3**; RU 51625) and obtained compounds without estrogenic activity, when given subcutaneously in mice, but exhibiting a slight agonistic effect when administered orally.[3] This result, and others from Roussel-Uclaf, showed that the 11β-position of estradiol, as the 7α-position, can accept a bulky side chain while maintaining high binding affinity to the estrogen receptor. It was also well established by Wakeling et al.[4] that a 7α-orientation of the alkylamide side chain is crucial for pure antiestrogenic activity. We then decided to introduce the same butyl methyl undecanamide chain at three other positions located in the D-ring, namely 17α, 15α, and 15β.

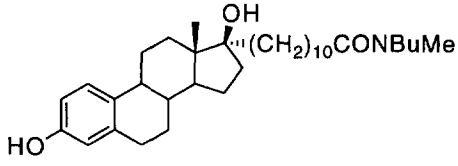
Herein, we report the estrogen receptor binding affinity, estrogenic activity (as uterotrophic activity), and antiestrogenic activity (as antiuterotrophic activity) of compounds **4-10**. In addition, we have corrected the C15-stereochemistry of compounds **5-10**, which was incorrectly attributed in a previous paper.[5]



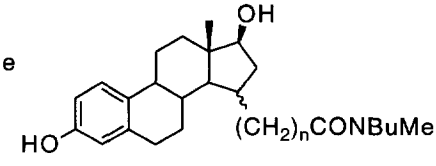
1 (ICI 164384)



2 (R = Bu; RU 50667)
3 (R = *i*-Pr; RU 51625)



4

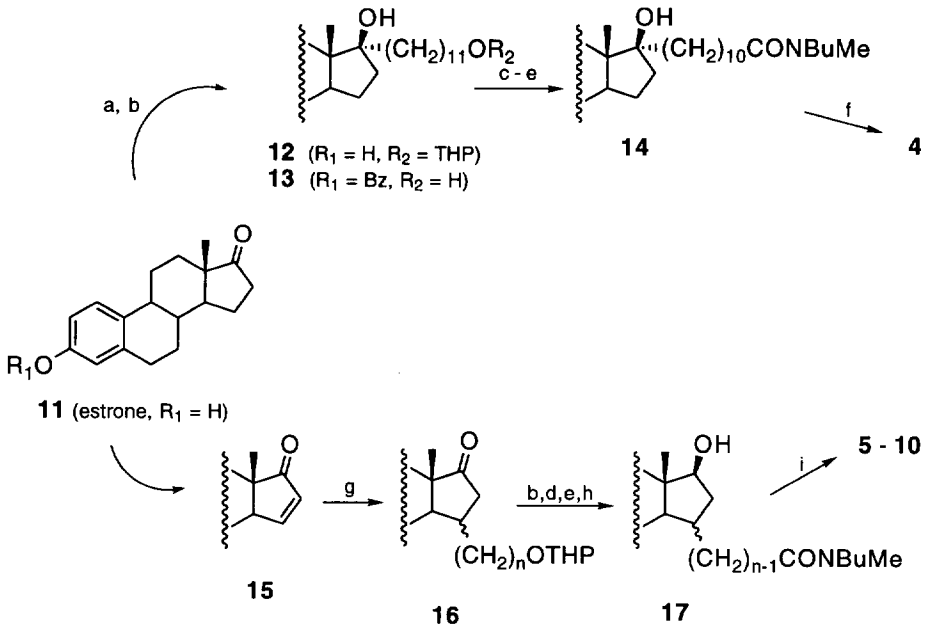


5 (n = 8, α:β/12:88)
6 (n = 10, α:β/18:82)
7 (n = 10, α)
8 (n = 10, β)
9 (n = 12, α)
10 (n = 12, β)

Chemistry:

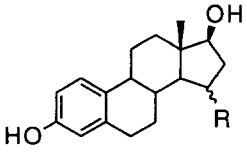
17α-Undecanamide derivative of estradiol **4** was synthesized according to the sequence of reactions shown in Scheme 1. The Grignard reagent generated from the corresponding THP-protected 11-bromoundecanol was added to ketone of estrone (**11**) leading the tertiary alcohol **12** in low yield (10%) with mainly the reduction product (estradiol). However, the yield of this Grignard addition to such sterically hindered 17-ketosteroids can be greatly enhanced (72%) by $CeCl_3$ as reported by Li et al.[6] After hydrolysis of THP and selective benzoylation (73%) of the phenolic group, the primary alcohol of **13** was oxidized to carboxylic acid before transformation to amide **14** (46% two steps). Removal of benzoyl group (96%) yielded the 17α-analog of ICI 164384, compound **4**.[7]

The synthesis of compounds **5-10** was already reported by us.[5] As shown in Scheme 1, the 1,4-addition of organocopper reagent on the 15,16-unsaturated ketone of compound **15** was used to access the steroidal C15-position. At that time, the isomeric mixture (~ 85:15) was attributed to 15α and 15β-alkylated derivatives, respectively. A recent study of Bojack and Kunzer[8] prompted us to reevaluate our conclusion. Indeed, they demonstrated the formation of a 15β-alkylated compound by organocopper addition, while, the 15α-alkylated compound was unambigously obtained by an elegant procedure using an oxy-Cope rearrangement as key step.[8] By these approaches, we recently prepared 15α-allyl and 15β-allyl estradiols (**18** and **19**) and determined NMR-probes (Table 1).[9] Since we previously observed that the 17α-allyl or 17α-alkylamide group induces a similar effect on carbons that surround the substitution site,[10] 15-allyl-compounds can be used as a representative model. Based on chemical shifts of 18-CH_3 and 17-CH, we concluded that the major isomer obtained from organocopper addition to enone **15** was the 15β-alkylated compound **10**, while the minor isomer was the 15α-alkylated compound **9**. This conclusion also apply for compounds **5-8**. Thus, in accord with Bojack and Kunzer,[8] the major 15α-stereochemistry claimed for organocopper addition in our previous paper (see ref 5) should be changed to 15β.



Scheme 1. Synthesis of compounds **4-10**. The reagents and conditions are: (**a**) $BrMg(CH_2)_{11}OTHP$ (excess), THF, -20 °C to rt; (**b**) *p*-TSA, MeOH, rt; (**c**) NaOH (1 N), PhCOCl, acetone, 0 °C; (**d**) Jones' reagent, acetone, 0 °C; (**e**) 1. $N(Bu)_3$, ClCOO*i*-Bu, CH_2Cl_2, -10 °C; 2. HNBuMe, -10 °C to rt; (**f**) NaOH (2 N), MeOH, 0 °C to rt; (**g**) 1. $BrMg(CH_2)_nOTHP$, CuCl, HMPA, TMSCl, THF; 2. K_2CO_3, MeOH; (**h**) $NaBH_4$, MeOH; (**i**) BBr_3, CH_2Cl_2, 0 °C.

Table 1. NMR-probes for C15-stereochemistry.[a]



Signals	**18** (R = 15α-allyl)[b]	**19** (R = 15β-allyl)[b]	**9** (minor)[c]	**10** (major)[c]
18-CH_3 (^1H)	0.82	0.91	0.80	0.87
18-CH_3 (^{13}C)	12.11	14.60	---	14.55
17-CH (^1H)	3.57	3.73	3.70	3.70
17-CH (^{13}C)	80.11	81.98	---	82.10

(**a**) Chemical shifts in ppm with $CDCl_3$ as solvent. (**b**) Synthesized according the procedures described by Bojack and Kunzer.[8] (**c**) Data from ref 5.

Estrogen receptor binding affinity:

The binding affinity of compounds **4-10** for the cytosolic rat uterine estrogen receptor (ER) was determined by competition with [^{3}H] estradiol as described by Asselin and Labrie.[11] The incubations were performed at 25 °C for 3 h and nonspecific binding was determined from incubation medium containing the [^{3}H] estradiol accompanied by an excess (1 μM) of unlabeled estradiol. Table 2 shows the relative binding affinity (RBA) obtained for a series of alkylamide derivatives of estradiol. In our assay, the undecanamide side chain added to 7α-position of estradiol decreases the binding from 100 (E_2) to 1.2.[12] A lower binding affinity was however observed for all compounds bearing an alkylamide into the D-ring (RBA < 0.05%). Since the extremely hydrophobic alkylamide derivatives of estradiol generally show a high level of nonspecific binding with crude ER-preparations,[13] absolute values are of limited interest. However, when compared to ICI 164384, an estradiol B-ring undecanamide, the D-ring alkylamide analogs decrease markedly the ER-binding.

Table 2. Effect of alkylamide position on ER-binding, uterotrophic activity, and antiuterotrophic activity.

Compounds	Side chain	RBA[a] %	Uterotrophic activity[b]	Antiuterotrophic activity[b,c]
Estradiol (E_2)	---	100	Yes	No
1 (ICI 164384)	7α-undecanamide	1.2	No	Yes
4	17α-undecanamide	0.01	No	No
5	15α/β-nonamide	<0.001	No	No
6	15α/β-undecanamide	0.001	No	No
7	15α-undecanamide	<0.001	---	---
8	15β-undecanamide	0.05	No	No
9	15α-tridecanamide	<0.001	No	No
10	15β-tridecanamide	<0.001	No	No

(**a**) Relative binding affinity (RBA) in rat uterine estrogen receptor. The RBA of E_2 is taken as 100. (**b**) At a dose of 10 μg/inj (twice daily for a 10-day period) for estradiol derivatives. (**c**) The stimulation of uterus was induced by estrone (0.06 μg/inj twice daily) for a 10-day period.

Antiuterotrophic and uterotrophic activities:

The antiuterotrophic activity of compounds **4-10** and ICI 164384 (**1**) was measured by inhibition of estrone-induced stimulation of uterine weight in adult female ovariectomized BALB/c mice (Fig. 1).[14] With ICI 164384, the estrogenic effect of estrone was partially (75%) reversed at 3 μg/inj and almost completely reversed at 10 μg/inj. The antiestrogenic effect of a 7α-undecanamide was however lost by moving the side chain into the D-ring. Indeed, no antiuterotrophic activity was observed for compounds **4-10** at the two doses tested. This lack of antiestrogenic activity cannot be explained by a residual estrogenic activity since no uterotrophic activity was observed at a dose of 10 μg/inj for all tested compounds in Table 2.

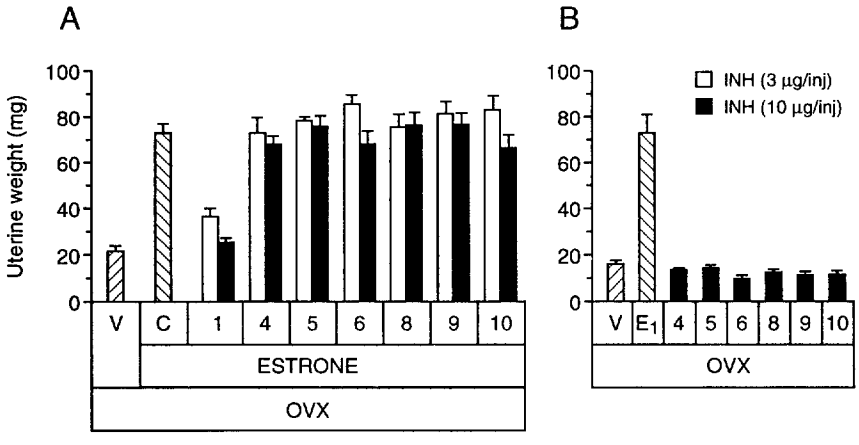


Figure 1. Uterine weight assay in female ovariectomized (OVX) BALB/c mice for compounds **1, 4-10**. Antiuterotrophic activity (**A**) and uterotrophic activity (**B**) at doses of 3/10 µg/inj twice daily for a 10-day period. The stimulation of uterus was induced by estrone (0.06 µg/inj twice daily) for a 10-day period. V, vehicle; C, control; INH, inhibitor; inj, injection.[14]

Alkylamide side chain of the steroidal D-ring:

Table 2 clearly shows that D-ring positions 15 and 17 are not suitable for the introduction of an alkylamide side chain similar to ICI 164384 or EM-139.[12] Indeed, binding affinity dramatically decreases by moving the side chain from the B- or C- ring to the D-ring and, consequently, no antiuterotrophic activity was observed. Similarly, very low binding affinity and antiuterotrophic activity were obtained for a series of seven 17α-(butyl methyl alkynylamide) derivatives of estradiol (with an unsaturated side chain).[15] The same conclusions were obtained for estradiol derivatives bearing a butyl methyl bromoalkylamide side chain at position 16α.[16] Although the mechanism of action of antiestrogens is not fully understood, their first action is thought to be binding to the estrogen receptor, thus preventing access of endogenous estradiol to its specific binding site. The low binding affinity and the lack of antiestrogenic activity observed for D-ring alkylamide estradiol derivatives suggest a limited space on the estrogen receptor to accommodate a bulky side chain. Decreasing the side chain lenght seems not a valuable strategy since binding was not sufficiently enhanced. Moreover, a long alkylamide chain was proved essential to give an antiestrogenic effect.[17] It was proposed that the 7α and 11β-positions, located in the steroidal B- or C-ring give a correct orientation of the side chain to a specific area of the estrogen receptor responsible of the antiestrogenic activity.[2,18] Indeed, energy minimization and molecular dynamic studies support the hypothesis that 7α and 11β-aminoethoxyphenyl estradiols interact with the same ER-subsite.[19] Substituted D-ring can not provide this particular orientation of a side chain or decrease the role of 17β-OH in the binding process, or simply can not enter the binding site. Study of the crystalline structure of the estrogen receptor, or at least of the binding domain, should provide an answer to these hypotheses. Before the availability of this information, the SAR study remains a crucial step in the development of antiestrogens. The results presented

above have the merit to extend the SAR study of steroidal alkylamide antiestrogens to the D-ring. Thus, contrary to the 7α- and 11β-positions, the 17α- and 15α/β-positions of estradiol are not suitable to develop potent antiestrogens.

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