Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 99–102
Bioorganic & Medicinal Chemistry Letters

Amide derivatives of 9,11-seco-estra-1,3,5(10)-trien-11-oic acid as modified orally active estrogen agonists with moderate antagonistic activity☆

Arvind Singh Negi,[a] Devdutt Chaturvedi,[b] Atul Gupta,[b] S. Ray,[b,*] Anila Dwivedy[c] and M. M. Singh[c]

[a]*Phytochemistry Division, Central Institute of Medicinal and Aromatic Plants, PO CIMAP, Kukrail Road, Lucknow 226015, India*
[b]*Medicinal Chemistry Division, Central Drug Research Institute, Lucknow 226015, India*
[c]*Endocrinology Division, Central Drug Research Institute, Lucknow 226001, India*

Received 25 July 2004; accepted 9 October 2004
Available online 28 October 2004

Abstract—Synthesis of amide derivatives of 9,11-seco-estra-1,3,5(10)-trien-11-oic acid containing alkyl and aromatic amine residues has been carried out with an aim to prepare orally active estrogen antagonists. Modification of the estradiol molecule in the form of *C*-seco-amide derivatives has led to their high oral absorption. Compounds **7** an *n*-propyl amide, **8** an *n*-butyl amide, and **16** a *p*-anisidyl amide of *C*-seco-estrane showed significant estrogen antagonistic activity (>20%), while, the majority of compounds possessed high estrogen agonistic activity on oral administration at 10 mg/kg dose in rats.

1. Introduction

The endogenous estrogens namely 17β-estradiol (**1**), estrone (**2**), and estriol (**3**) (Fig. 1) mainly promote proliferation and growth of specific cells in the body and are responsible for the development of most secondary sexual characteristics in females. Besides regulation of female reproductive system, estrogens play an important role in the maintenance of bone, CNS, and CVS functioning. However, estrogens have also been indicated with certain major diseases including hypertension and cancer. This dual nature of estrogens has led to the development of the several nonsteroidal and steroidal molecules, which meet the requirement of estrogens in the body in fertility regulation, osteoporosis, CNS disorders such as Alzheimer's, and also antagonize the effect of estrogens in case of estrogen dependent cancers. Such tissue selective estrogens have been termed as selective estrogen receptor modulators (SERM).[1–4]

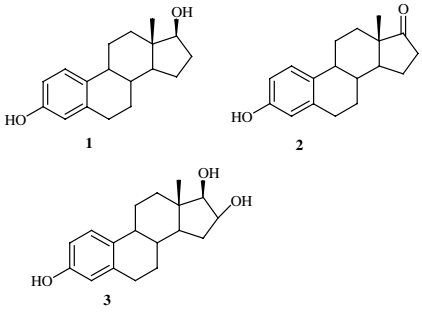


Figure 1. Endogenous estrogens.

In the field of steroidal estrogens it has been found that presence of a long chain amide at 7α-position of estradiol leads to pure estrogen antagonists.[5] Two of the potent estrogen antagonists are ICI 163,964 and ICI 164,384, shown in Figure 2. Estrogen antagonizing effect has also been observed in molecules having a nitrogen containing residue at 11β-position as in compound RU-3941, RU-51625, RU-53637 (Fig. 2).

A primary requirement for a molecule to act as estrogen agonist or an antagonist is its affinity for estrogen receptors. It has been observed that the receptor binding

Keywords: Antiestrogens; Estrogen antagonists; seco Estranes; RBA.
☆CDRI Communication number 6437.
*Corresponding author. Tel.: +91 522 2629558; e-mail: suprabhatray@yahoo.co.in

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.031

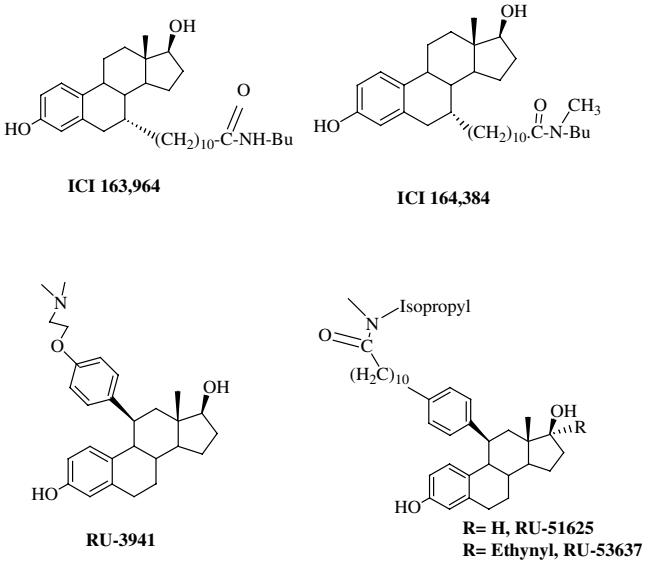
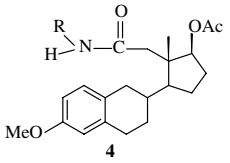


Figure 2. Steroidal estrogen antagonists.

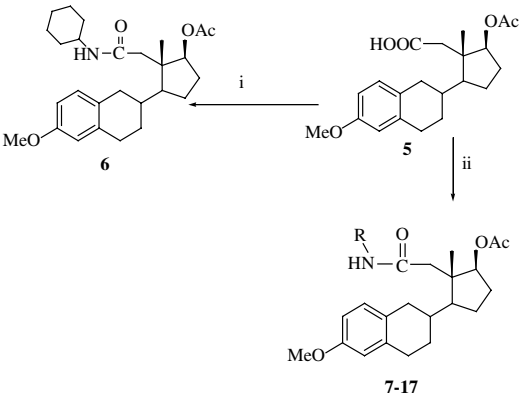
affinity of a seco-estrane depends upon the position of the bond cleaved.[6] We have observed that a properly designed *C*-seco-estradiol retains its binding affinity (80% of estradiol) with significantly reduced estrogenic activity.[6] Reduced estrogenic activity has also been reported in *C*-seco-estranes having B-ring aromatic.[7] Further, the endogenous estrogen, estradiol shows poor oral absorption. The antiestrogen ICI 163,964 has also been reported to exhibit poor bioavailability. Thus, keeping in mind the above findings, it was considered of interest to develop *C*-seco-estradiol molecules having a nitrogen containing chain at a position comparable to 11β-substituted antiestrogens shown in Figure 2. Thus, synthesis of 9,11-seco-estranes having a nitrogen containing residue in the form of an amide chain around C-11 (**4**) was undertaken.



4

2. Chemistry

In order to have the right stereochemistry of estradiol for effective receptor binding, synthesis of seco-estrane derivatives was achieved (Scheme 1) through removal of specific bonds of estradiol in a manner that the stereochemistry of the estradiol framework was retained. Thus, synthesis of 3,17β-dihydroxy-9,11-seco-estra-1,3,5(10)-trien-11-oic acid 17-acetate, 3-methyl ether, (**5**) was carried out following known procedure.[8] Treatment of **5** with aliphatic amines such as *n*-propyl amine, *n*-butyl amine, 2-phenyl ethyl amine etc., in presence of DCC (dicyclohexyl carbodiimide) in THF/dichloromethane at room temperature and also under refluxing conditions led to the formation of the same product. It



Scheme 1. Reagents and conditions: (i) DCC, dichloromethane, TEA, amine, reflux, 2–3 h, 40%; (ii) HOBT, dichloromethane, TEA, amine, reflux, 3 h, 42–68%.

showed the presence of an amide peak in IR (1666 cm^{-1}), FAB mass analysis of the compound obtained from these reactions showed molecular ion peak at 442 (M^+). From ^1H NMR, ^{13}C NMR and elemental analysis the product of this DCC reaction has been characterized as cyclohexyl amide derivative of the 11-oic acid (**6**).[11] Preparation of the desired amides (**11**–**21**) was finally achieved through condensation of respective amines with HOBT (hydroxy benzotriazole) ester of 11-oic acid prepared in situ.

2.1. General procedure for the synthesis of amide derivatives (Compounds 7–17)

3,17β-Dihydroxy-9,11-seco-estra-1,3,5(10)-trien-11-oic acid 17-acetate, 3-methyl ether, *n*-butyl amide (**8**).

A mixture of compound **5** (0.25 g, 0.7 mmol), hydroxy benzotriazole (HOBT) (0.09 g, 0.7 mmol), and dicyclohexyl carbodiimide (DCC) (0.2 g, 0.9 mmol) in dry dichloromethane (15 ml) was stirred at room temperature. On completion of esterification when the dicyclohexyl urea (DCU) started precipitating, *n*-butyl amine (0.2 ml, 3.8 mmol) was added to it. The reaction mixture was refluxed for 4 h. On the completion of reaction, mixture was poured into water (50 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with water, dried over anhydrous sodium sulphate, and distilled off. The crude oil thus obtained was purified through silica gel column taking hexane–ethyl acetate as eluent. The pure compound **8** was crystallized from dichloromethane.[11]

3. Biological evaluation and discussions

3.1. Estrogen receptor binding affinity

The relative binding affinity (RBA) of the compounds for estrogen receptors was determined[9] by competition assay, employing radio labeled estradiol (^3H-E_2) as the reference compound. The test ligands and (^3H-E_2) were incubated (4 °C) with cytosol estrogen receptors obtained from immature 20–21 days old rat uteri. Aliquots

of the uterine cytosol (200 L concd 1 uterus per mL) prepared in TEA buffer (10 mM Tris, 1.5 mM EDTA, 0.02% sodium azide, pH 7.4) were incubated in triplicate with a fixed concentration of radio labeled estradiol with or without various concentrations of the competitor substance dissolved in 60 μL of the TEA buffer containing DMF as co-solvent (final concentration of DMF in the incubation medium never exceeded 5%) for 18 h at 4 °C. At the end of this period, dextran coated charcoal (DCC) (5% Norit, 0.5% dextran) suspension in 100 μL of TEA buffer was added into each tube, which were briefly vortexed and allowed to stand for 15 min. DCC was precipitated by centrifugation (800 g × 10 min) and the supernatants counted for radioactivity in 10 mL of a dioxane-based scintillation fluid. RBA of the text compound was computed from a graph plotted between percent bound radioactivity versus log concentration of the test substance. At 50% inhibition, log of the competitor concentration relative to that of estradiol, gave the affinity of the test compound to estrogen receptor relative to estradiol. This when multiplied with 100 gave the percentage value designated as RBA.

3.2. Estrogen agonistic activity[10]

Twenty one day old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for seven days were randomized into different treatment groups. Each rat received the compound of the invention once daily for three consecutive days on days 28–30 of age by oral route. A separate group of animals received only the vehicle for similar duration served as control. At autopsy 24 h after the last treatment on day 31 of age, vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed. Premature opening of vagina, cornification of vaginal epithelium, and increase in uterine fresh weight were taken as parameters for evaluation of estrogen agonistic activity in comparison to rats of vehicle control group. The objective was to evaluate estrogen agonistic effect of the compounds on the uterus and vagina.

3.3. Estrogen antagonistic activity[10]

Twenty one day old immature female Sprague–Dawley rats were bilaterally ovariectomized under light ether anesthesia and after post-operative rest for seven days were randomized into different treatment groups. Each rat received the compound of the invention and 0.02 mg/kg dose of 17α-ethynylestradiol in 10% ethanol–distilled water once daily for three consecutive days on days 28–30 of age by oral route. A separate group of animals receiving only 17α-ethynylestradiol (0.02 mg/kg) in 10% ethanol–distilled water for similar duration were used for comparison. At autopsy on day 31 of age, vaginal smear of each rat was taken and uterus was carefully excised, gently blotted, weighed, and fixed for

Table 1. Yield%, mp, oral dose, estrogen antagonistic and estrogen agonistic activities, and RBA of different amide derivatives of *C*-seco estrane

Compd. no.	R=	Yield (%)	Mp (°C)	Dose (Oral) (mg/kg/day)	Estrogen antagonistic activity		Estrogen agonistic activity		RBA % of E_2
					Uterine weight[a] (mg)	Inhibition[b] (%)	Uterine weight[a] (mg)	Gain[c] (%)	
6	Cyclohexyl	40	145	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vehicle	—	—	—	—	17.50 ± 0.50	—	17.50 ± 0.50		—
EE	—	—	—	0.02	78.00 ± 10.00	—	78.00 ± 10.00[g]	345.70	—
12	Phenyl	48	128	10.00	69.00 ± 1.73	11.54	18.0 ± 11.52	2.91	<0.001
14	Phenethyl	57	137	10.00	73.00 ± 5.85	6.41	19.00 ± 1.00	8.57	<0.001
Vehicle	—	—	—	—	18.50 ± 0.96	—	18.50 ± 0.96		
EE	—	—	—	0.02	97.10 ± 2.30	—	97.10 ± 2.30[g]	424.86	—
16	*p*-Anisidyl	42	121	10.00	77.50 ± 2.02[e]	20.19	70.66 ± 1.20[g]	281.95	<0.001
Vehicle	—	—	—	—	16.10 ± 0.35	—	16.10 ± 0.35		—
EE	—	—	—	0.02	102.00 ± 2.31	—	102.00 ± 2.31[g]	533.54	—
11	*n*-Dodecyl	62	179	10.00	90.10 ± 1.70[e]	11.67	82.36 ± 2.90[g]	411.55	<0.001
17	*m*-Anisidyl	58	oil	10.00	86.05 ± 3.26[e]	15.64	84.93 ± 4.64[g]	427.51	<0.001
13	Benzyl	49	129	10.00	87.70 ± 4.14[d]	14.02	74.43 ± 1.78[g]	362.30	<0.001
10	*n*-Octyl	57	160	10.00	95.90 ± 1.50	5.98	87.05 ± 1.05[g]	440.68	<0.001
Vehicle	—	—	—	—	14.25 ± 0.36	—	14.25 ± 0.36		—
EE	—	—	—	0.02	92.90 ± 3.14	—	92.90 ± 3.14[g]	551.93	—
15	Phenpropyl	59	164	10.00	83.60 ± 5.90	10.01	74.33 ± 5.02[g]	421.61	<0.001
Vehicle	—	—	—	—	17.50 ± 0.90	—	17.50 ± 0.90		—
EE	—	—	—	0.02	90.96 ± 4.41	—	90.96 ± 8.41[g]	419.77	—
7	*n*-Propyl	64	oil	10.00	67.20 ± 1.40[e]	26.12	18.66 ± 0.88	6.63	<0.001
8	*n*-Butyl	51	143	10.00	70.83 ± 5.08[e]	22.13	40.73 ± 0.81[g]	132.74	<0.001
9	Butylmethyl	68	oil	10.00	75.33 ± 8.08[d]	17.18	23.66 ± 2.33[f]	35.20	<0.001

n.d. = Not determined; EE = 17α-ethynylestradiol; E_2 = estradiol.

[a] Values represents mean ± SEM of minimum of six observations in each group.

[b] Percent of 17α-ethynylestradiol per se treated group.

[c] Percent of vehicle control group.

[d] $P < 0.05$.

[e] $P < 0.01$ versus corresponding EE per se treated group.

[f] $P < 0.05$.

[g] $P < 0.01$, versus corresponding vehicle group, All other relevant comparisons were statistically not significant.

histology. Inhibition in ethynylestradiol induced cornification of vaginal epithelium and increase in uterine fresh weight were taken as parameters for evaluation of estrogen antagonistic effect of the compounds.

Estrogen receptor binding affinity, estrogenic, and antiestrogenic activity data of compounds is shown in Table 1. The low order of RBA observed with these compounds is due to the reason that the hydroxy groups at C-3 and C-17 of the steroidal molecule responsible for receptor binding were masked in all the compounds studied. Compounds **8**, **10**, **11**, **13**, **15**, **16**, and **17** administered orally, possessed high estrogenic activity, whereas moderate estrogen antagonistic activity (>20% inhibition) was shown by **7**, **8**, and **16**.

4. Conclusion

Majority of the compounds (**8**, **10**, **11**, **13**, **15**, **16**, and **17**) possessed high estrogen agonistic activity on oral administration. Unlike long chain amide (**10** and **11**) corresponding short chain derivatives (**7** and **8**) showed relatively potent estrogen antagonistic activity. No definite SAR could be drawn on incorporation of aryl or alkyl residue. This study suggests that modification of the estradiol molecule in the form of *C*-seco-amide derivatives has led to their high oral absorption. Higher estrogen antagonistic activity was elicited by compounds **7**, **8**, and **16**. The positioning of the amide group and its nature needs further modification for achieving higher anti-estrogenic activity. Inactivity of the compounds as estrogen antagonists could be due to their metabolism leading to removal of the amide chain. Compounds showing high estrogen agonistic activity may further be explored for their tissue selective action.

Acknowledgements

The authors thank Ms. Mohini Chhabra for efficient technical assistance and the Ministry of Health and Family Welfare, Government of India for financial support. The Senior Research Fellowship by Council of Scientific and Industrial Research (CSIR) to one of the author (D.D.C.) is duly acknowledged.

References and notes

1. (a) Jordan, V. C. *J. Med. Chem.* **2003**, *46*(6), 883–908; (b) Jordan, V. C. *J. Med. Chem.* **2003**, *46*(7), 1081.
2. Macgregor, J. L.; Jordon, V. C. *Pharmacol. Rev.* **1998**, *50*, 151.
3. Ray, S.; Dwivedy, I. *Adv. Drug Res.* **1997**, *29*, 171.
4. Geistlinger, T. R.; McReynolds, A. C.; Guy, R. K. *Chem. Biol.* **2004**, *11*, 273.
5. Bowler, J.; Lilley, T. J.; Pitlam, J. D.; Wakeling, A. E. *Steroids* **1989**, *54*, 71.
6. Lal, K.; Sharma, I.; Agrawal, A. K.; Agnihotri, A.; Ray, S. *J. Med. Chem.* **1988**, *31*, 1261.
7. Chinn, L. J.; Dygos, J. H.; Mares, S. E.; Aspinall, R. L.; Ranney, R. E. *J. Med. Chem.* **1974**, *17*(3), 351.
8. (a) Cambie, R. C.; Manning, T. D. R. *J. Chem. Soc.* **1968**, *21*, 2603; (b) Dygos, J. H.; Chin, L. J. *J. Org. Chem.* **1975**, *40*, 685.
9. Dhar, J. D.; Dwivedi, A.; Srivastava, A.; Setty, B. S. *Contraception* **1994**, *49*, 609.
10. Ghosh, R.; Kamboj, V. P.; Singh, M. M. *Contraception* **2001**, *64*, 261.
11. *Selected physical data*: Compound **6**: yield: 40% mp = 145 °C. IR (KBr, cm^{-1}): 3332 (N–H stretching), 1732 (OAc), 1666 (C=O, amide). ^1H NMR ($CDCl_3$): 1.16 (s, 3H, 18-C*H*$_3$), 1.42–1.45 (m, 6H, 3′, 4′, and 5′C*H*$_2$ of cyclohexyl group), 1.47–1.49 (m, 3H, C*H*$_2$C*H* of steroidal nucleus), 1.63–1.66 (m, 4H, 2′ and 6′C*H*$_2$ of cyclohexyl ring), 1.56–1.75 (m, 5H, C*H*$_2$ and C*H* of steroidal nucleus), 2.01 (s, 3H, C*H*$_3$–CO), 2.10 (s, 2H, C*H*$_2$CONH), 2.81–2.85 (m, 4H, PhC*H*$_2$ of steroidal nucleus), 3.18–3.21 (m, 2H, CH$_2$NHCO), 3.73 (s, 3H, OC*H*$_3$), 3.93–3.96 (m, H, 17-C*H* of steroidal nucleus), 6.45–6.92 (m, 3H, Ar-*H*), 7.70 (bs, H, N*H*). Mass (FAB): *m/e* 442 (M^++1); Anal.: $C_{27}H_{39}NO_4$, calcd: C, 73.43; H, 8.90; N, 3.17; obsd: C, 73.74; H, 8.42; N, 3.39. Compound **8**: yield: 0.15 g (51%), mp: 143 °C; IR (KBr, cm^{-1}): 3332 (N–H stretching), 1732 (OAc), 1666 (C=O, amide); ^1H NMR ($CDCl_3$): 0.93–0.96 (t, 3H, C*H*$_3$ of *n*-butyl group), 1.16 (s, 3H, 18-C*H*$_3$), 1.30–1.34 (m, 2H, C*H*$_2$CH$_3$), 1.43–1.47 (m, 3H, C*H*$_2$C*H*), 1.52–1.55 (m, 2H, NHCH$_2$C*H*$_2$CH$_2$CH$_3$), 1.56–1.75 (m, 5H, C*H*$_2$, and C*H* of steroidal nucleus), 2.01 (s, 3H, C*H*$_3$-CO), 2.10 (s, 2H, C*H*$_2$CONH), 2.81–2.85 (m, 4H, PhC*H*$_2$ of steroidal nucleus), 3.18–3.21 (m, 2H, C*H*$_2$NH-CO), 3.73 (s, 3H, OC*H*$_3$), 3.93–3.96 (m, H, 17-C*H* of steroidal nucleus), 6.44–6.90 (m, 3H, Ar-*H*), 8.0 (bs, H, N*H*); Mass (FAB): *m/e* 415 $[M]^+$; Anal.: $C_{25}H_{37}NO_4$, calcd: C, 72.26; H, 8.97; N, 3.37; obsd: C, 72.68; H, 8.69; N, 3.19.