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Contraceptive progestins. Various 11-substituents combined with four 17-substituents: 17α-ethynyl, five- and six-membered spiromethylene ethers or six-membered spiromethylene lactones

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Abstract

Norethisterone (NET) is a 19-nortestosterone derivative with progestagenic and some androgenic activity, which was used in the first generation of contraceptives. NET was succeeded by levonorgestrel (LNG) and later on by desogestrel (DSG) and gestodene (GSD). Although these latter two progestins had increased potency, there was still androgenicity with gestodene and to a lesser extent with desogestrel. New progestins were synthesized in order to further enhance progestagenic and to reduce androgenic activity. Four different chemical moieties were introduced in position 17 of 19-nortestosterone, viz. 17α-ethynyl, five- and six-membered spiromethylene ethers, and a six-membered-spiromethylene lactone. In combination with these structures seven different substituents were added at position 11, i.e. methylene, methyl, ethyl, ethenyl, ethynyl, 2-propenyl and 1-propynyl. All substituents except for methylene occupied the 11β-position. All these 32 compounds were synthesized and analysed in vitro and in vivo against etonogestrel (ETG, 3-keto-desogestrel), the biologically active metabolite of desogestrel. Their relative binding potency to progesterone (PR), androgen (AR) and estrogen (ER) receptors were determined in cell lysates of human breast tumor MCF-7 cells and to glucocorticoid (GR) receptors in that of human leukemic IM-9 cells. Moreover, their relative agonistic activities were assessed in Chinese hamster ovary cell-based transactivation assays. All in vivo activities were determined in McPhail (progestagenic), ovulation inhibition (progestagenic and estrogenic), Hershberger (androgenic), hormone screening (glucocorticoid and estrogen) and Allen-Doisy (estrogenic) tests after oral and for the McPhail test also after subcutaneous administration. The progestagenic binding and transactivation potencies of all compounds in the three 17-spiro series were higher than those of the corresponding analogues in the 17α-ethynyl series. None of the compounds showed estrogenic or clear androgenic binding and transactivation potential except for a six-membered-spiromethylene lactone with a propynyl group. This compound showed strong androgenic binding. The glucocorticoid binding and transactivation were very low for the compounds with the 17α-ethynyl and the five-membered-spiromethylene ether groups, whereas both six-membered-spiro series showed, clearly with methyl and ethynyl substituents, and less pronounced with methylene and ethenyl, higher binding and transactivation values. For the 17α-ethynyl series, the McPhail test showed high potencies with methylene, methyl and ethenyl substituents after oral treatment or with propenyl after subcutaneous administration. The introduction of the spiro substituents in position 17 led to high potencies for other 11-substituents as well. Besides methyl, also ethyl, ethynyl and propynyl were potent substituents. With ovulation inhibition tests, the ethyl, ethenyl and ethynyl substituents were the more potent compounds in all four series. However, compounds with methyl or ethynyl additions appeared to be glucocorticoidal in the hormone screening test irrespective of the 17-substituent, while with the three spiro series even methylene and ethenyl groups became active. Androgenicity was only observed at dose levels at or above 5 mg/kg, which is 2.5-fold weaker than ETG. Moreover, estrogenicity appeared negligible with the three spiro series, while with the 17α-ethynyl series methyl, ethyl, ethenyl and ethynyl substituents, a very high estrogenic potential was assessed. Based on the high efficacy and low side-effects, the following compounds show a high selectivity: 17α-ethynyl with ethyl, ethenyl and 2-propenyl substituents, six-membered spiromethylene ether with ethyl and six-membered-spiromethylene lactone with ethyl, 2-propenyl or 1-propynyl substituents. These compounds have relatively high binding and transactivation values for PR, and have high biopotencies in the McPhail and ovulation inhibition tests, while showing very weak

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androgenic and glucocorticoid activities. These compounds may be very useful for contraception for either oral and/or subcutaneous administration.

Keywords: Steroid receptors; Competition studies; Progestational activity; Androgenic activity; Glucocorticoid activity; 3-Keto-desogestrel; Etonogestrel; New progestagens

1. Introduction

Norethisterone (NET) is a synthetic 19-nortestosterone derivative, that has been and still is widely used for birth control and for climacterial complaints in a number of pharmaceutical formulations with or without (ethynyl)estradiol [1–4]. With respect to birth control it belongs to the first generation of contraceptives. Its successor, levonorgestrel (LNG) belongs to the second generation [5–7] and contains an extra 18-methyl addition with respect to NET (Fig. 1). LNG is five times more progestagenic, but also five times more androgenic than NET [7–9]. This androgenicity is associated with the induction of atherosclerosis, and it may also increase the likelihood of hypertension and other arterial diseases [10]. Therefore a huge effort was put into a further reduction of this androgenicity. Desogestrel (DSG) and gestodene (GSD) both belong to the third generation of contraceptives [11,12]. DSG is metabolized in the liver into the active derivative etonogestrel (ETG, 3-keto-desogestrel). ETG contains an extra 11-methylene substituent with respect to LNG, and GSD has an extra double bond between carbon atoms 15 and 16 (Fig. 1). Both ETG and GSD are twice as potent as LNG in progestagenic activity. With respect to androgenicity, GSD is slightly less active than LNG, while ETG is significantly better than GSD and LNG [7–9,13,14]. Furthermore NET, LNG, ETG and GSD are well known for their progestagenic effects on hypothalamus and/or pituitaries by the suppression of the mid-cycle surge of gonadotropins, which impairs ovulation [9].

The aim of the present investigation is to search for even more potent and selective progestins. Moreover, at the relevant physiological progestagenic dose levels, these compounds should induce only minor or no side-effects at all towards androgen receptor regulated processes. In order to reach these goals, seven different modifications were introduced to NET on the 11-position, i.e. methylene, methyl, ethyl, ethenyl, ethynyl, 2-propenyl, and 1-propynyl. Except of course in the case of methylene all substituents occupied the 11β-position. These modifications in the 11-position were combined with four structural modifications in position 17: besides the 17β-hydroxy and 17α-ethynyl groups, five- and six-membered spiromethylene ethers, and a six-membered-spiromethylene lactone were introduced (Fig. 2). To establish the differences between these 32 compounds and the standard ETG, in vitro binding assays on progesterone (PR), androgen (AR), glucocorticoid (GR), and estrogen (ER) receptors were combined with functional in vitro transactivation bioassays in CHO cells [15–17]. Finally, five in vivo tests were carried out, i.e. McPhail (progestagen), ovulation inhibition (combined progestagen and estrogen), Hershberger (androgen), hormone screening (glucocorticoid and estrogen) and Allen-Doisy (estrogen). All these in vivo assays were performed after oral administration and for the McPhail test also after subcutaneous treatment.

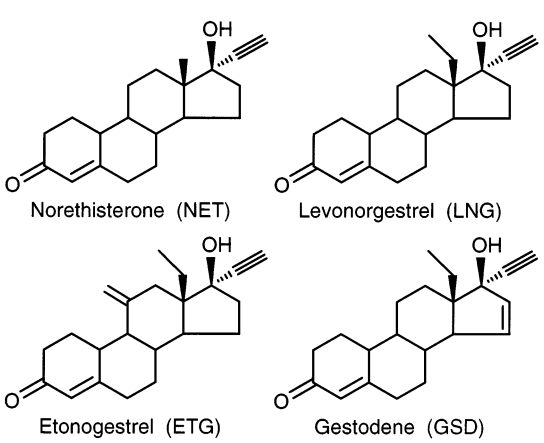

Fig. 1. Structures of norethisterone (NET), levonorgestrel (LNG), and the reference compounds etonogestrel (ETG) and gestodene (GSD).

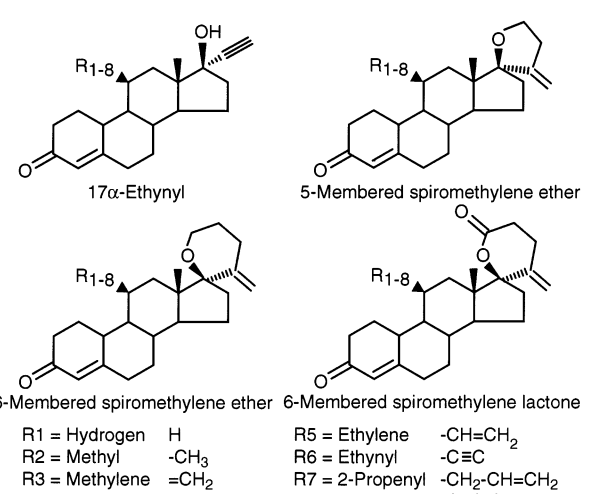

Fig. 2. Structures of several progestagenic structures with modifications on position 17 or 11, indicated with R1–R8.

2. Materials

2.1. Materials

The 32 steroids used as well as the references Org 2058, 5α-dihydrotestosterone (DHT), dexamethasone (DEX), and 17β-estradiol (E_2) were obtained from N.V. Organon (Oss, The Netherlands). The standards ethynyl estradiol, 17β-estradiol benzoate (E_2-B) and methyltestosterone (MT) were obtained from Diosynth (Oss, The Netherlands). Trypsin was obtained from Flow Laboratories (Irvine, Scotland), Dulbecco's Modified Eagles Medium/Nutrient Mixture F-12 (DMEM/HAM F12 medium in a ratio of 1:1) was from Gibco (Paisley, UK), characterized fetal calf serum (FCS) and defined bovine calf serum supplemented (dBCS) from Hyclone (UT, USA), 96-well plates from Greiner (Nürtingen, Germany) and 96-well white culture plates and LucLite from Packard (Meriden, USA). Tritiated Org 2058 (s.a. 1.7 Tbq/mmol), and DEX (s.a. 3.29 Tbq/mmol) were obtained from Amersham ('s-Hertogenbosch, The Netherlands) and E_2 (s.a. (specific activity) 4.66 Tbq/mmol) and 5α-DHT (s.a. 4.07 Tbq/mmol) from NEN (du Pont, 's-Hertogenbosch, The Netherlands). All other chemicals were of analytical grade.

2.2. Cell lines

MCF-7 cells for binding assays were obtained from Dr McGrath (Michigan Cancer Foundation, USA) and IM-9 cells from Dr M. Lesniak (National Institute of Health, USA). These cells were cultured in medium with FCS. The CHO cells, derived from CHO K1 cells obtained from the American Type Culture Collection (Rockville, MD, USA), contained hPR-B-MMTV-LUC (clone 1E2-A2), hAR-MMTV-LUC (clone 1G12-A5-CA), hGR-MMTV-LUC (clone B4-8), or hER-RO-LUC (clone 2B1-1E9), and these cells were cultured in medium with charcoal-treated dBCS. All cell lines were cultured at 37°C in Roux flasks (175 cm^3) flushed with 5% CO_2 in air until pH 7.2–7.4 was reached. Complete medium was refreshed every 2 or 3 days. Then 1 day before harvesting MCF-7 cells, these cells were cultured on charcoal-treated FCS.

2.3. Animals

The Harlan Sprague Dawley/Central Institute for the Breeding of Laboratory Animals of the Dutch Organisation for Applied Scientific Research (HSD-CPB), Zeist, The Netherlands supplied:

1. SPF-bred immature female HSD/Cpb:ORGA rats,
2. SPF-bred young female HSD/Cpb:ORGA rats,
3. SPF-bred female HSD/Cpb: ORGA rats with known fertility, and
4. immature female HSD/Cpb:CH rabbits.

The rats were housed in light- and temperature-controlled rooms (14 h light/10 h dark; 21–23°C). Tap water and pelleted food (RMH-B, Hope Farms, Linschoten, The Netherlands) were given ad libitum. The rabbits were housed in light- and temperature-controlled rooms (14 h light/10 h dark; 19–21°C). They were fed daily with 50 g pelleted food (LKK-20, Hope Farms) and had free access to tap water.

2.4. Pharmaceutical formulations

A suspension of the compounds or the reference compounds were given in an aqueous solution of gelatine (5 g/l) and mannitol (50 g/l) (gel.mann.) for oral treatment of rats. Rabbits were treated using a tablet formulation of the following constituents: potato starch 10%, magnesium stearate 0.5% and tabletting powder with 2% amylopectin to 100%. For subcutaneous administration of rats and rabbits the compounds were dissolved in arachis oil ± benzyl alcohol (100 g/l).

3. In vitro studies

3.1. Displacement studies

For displacement analysis MCF-7 and IM-9 cells were used. The cells were cultured, harvested and cytosolic preparations prepared as described by Schoonen et al. [15]. Prior to use 1 g of cells was prepared into the cytosol. The cytosol was diluted with buffer solution to a final receptor concentration of 1:20 (w:v) for hPR, 1:5 for hAR, and 1:10 for hER in MCF-7 cells. For the intact hGR assay 6×10^4 IM-9 cells were used per well. Samples were counted in a Topcount microplate scintillation counter (Packard). Specific binding was determined by subtracting non-specific from total binding. The relative binding affinities of the compounds were obtained by analysis by using a three-point parallel line assay [16]. The mean RBA values with standard deviations (S.E.M.) of different independent tests were calculated.

3.2. Transactivation studies

For transactivation studies the stably transfected CHO cells described above were used [16,18]. Steroids for treatment were diluted in ethanol and diluted with medium to such a concentration that in the wells of the 96-well white culture plate only 1% ethanol was present. Thereafter cells were seeded at 5×10^4 cells/well and incubated in an incubator during 16 h in medium with charcoal-treated dBCS at 37°C in 5% CO_2 in air. Then part of the medium was removed and

LucLite added for cell lysis and luciferase measurement. Luciferase activity was measured in a Topcount luminescence counter. Relative agonistic activity (RAA) studies were carried out with various concentrations of the standards (1:2:4 dilutions) and compounds of interest. The relative agonistic activities of the compounds were obtained by analysis by a three-point parallel line assay [19]. The mean RAA values with S.E.M. of different independent tests were calculated.

4. Pharmaceutical in vivo tests

4.1. Progestagenic activity in immature rabbits

The progestational activity of the compounds was assessed as described by McPhail [20] and modified by van der Vies and de Visser [8]. The minimal active dose (MAD) was considered to be the total dose at which the mean McPhail index attained a value of 2.0.

4.2. Ovulation inhibition in rats

Ovulation inhibition in rats was determined according to van der Vies and de Visser [8]. An ovulation inhibition of 60% was considered as MAD.

4.3. Androgenic-anabolic activity in orchidectomized rats

The test for androgenic activity was performed according to the Hershberger test [21], which was slightly modified by van der Vies and de Visser [8]. The results are presented as MAD: the daily dose at which the ventral prostate weight was 1.8 times higher than the placebo value.

4.4. Hormone screening test

The glucocorticoid and estrogenic activity of the compounds in immature female rats was determined according to van der Vies and de Visser [8]. The reduction on the effect of the weight is indicated as zero if identical to controls and as 100% if the organ weight would have been reduced completely. An effect of more than 30% on adrenal and thymus weights is indicative of an individual glucocorticoidal effect. An effect of more than 30% on thymus weight alone indicates an estrogenic effect.

4.5. Estrogenic activity in ovariectomised rats

The estrogenic activity of the compounds in rats was determined according to Allen and Doisy [22] with a slight modification by van der Vies and de Visser [8]. The total dose at which 50% of the animals showed one or more positive smears is given as MAD.

5. Results

5.1. In vitro studies

In Table 1 and Figs. 3 and 4, relative binding affinity (RBA) and relative agonistic activity (RAA) values of the reference ETG and of 32 different progestins are given. These values for PR and AR were assessed with cytosolic and for GR with intact cell binding assays as well as with transactivation assays with mouse mammary tumor virus promoter and luciferase reporter system.

5.1.1. RBA and RAA values for the human progesterone receptor

5.1.1.1. RBA. NET is 9-fold less potent than ETG. In the 17α-ethynyl group, all 11-substituents showed 2–5.5-fold higher binding potencies than NET except for 1-propynyl, which was equipotent (Fig. 3). Modification of the 17α-ethynyl group of NET into a five- and six-membered spiromethylene ether or six-membered-spiromethylene lactone improves binding by at least 10-fold. The introduction of other 11-substituents in combination with the five- and six-membered spiromethylene ethers or six-membered-spiromethylene lactone did not improve these binding potencies. For only two compounds, one with a five-membered-spiromethylene ether and 1-propynyl and another one with a six-membered spiromethylene ether and 2-propenyl, a 2–3-fold lower binding potency was found with respect to the non-substituted analogue. This implies that the introduction of five- and six-membered spiromethylene ethers and six-membered-spiromethylene lactone at position 17 has a strong impact on progesterone binding affinity.

5.1.1.2. RAA. The progestagenic activity of NET was 7-fold lower than that of ETG. In the 17α-ethynyl group, methylene, methyl, ethenyl, and ethynyl substituents showed 2–5-fold higher activities than NET, whereas 2-propenyl and 1-propynyl substituents were equipotent and an ethyl substituent 3-fold less potent (Fig. 3). Modification of the 17α-ethynyl group of NET into five- and six-membered spiromethylene ethers or into a six-membered-spiromethylene lactone improves binding by 14-fold for the ethers and 30-fold for the lactone. In the groups with five- and six-membered spiromethylene ethers, ethenyl, 2-propenyl and 1-propynyl substituents reduced the transactivation potential in comparison to the unsubstituted one, whereas the methylene addition enhanced the progestagenic activity, as did methyl in combination with the five-membered-spiromethylene ether. Moreover, ethyl in combination with the five-membered-spiromethylene ether reduced

Table 1
The relative binding affinity (RBA) and relative agonistic activity (RAA) values for various progestin derivatives to the progesterone, androgen, and glucocorticoid receptors[a]

Compounds	Progesterone receptor (Org 2058 = 100%)		Androgen receptor (DHT = 100%)		Glucocorticoid receptor (DEX = 100%)	
	RBA	RAA	RBA	RAA	RBA[b]	RAA
	MCF-7	CHO	MCF-7	CHO	IM-9	CHO
Standard						
ETG	187.0 ± 13.7 **4**	86.3 ± 16.3 **4**	6.0 ± 4.1 **29**	2.6 ± 0.4 **21**	16.0 ± 4.1 **32**	<0.1 **1**
17α-Ethynyl						
– (NET)	21.5 ± 1.0 **31**	12.4 ± 1.3 **9**	3.2 ± 0.3 **34**	1.1 ± 0.1 **32**	2.1 ± 1.0 **9**	<0.1 **3**
Methylene	105.6 ± 7.1 **24**	37.2 ± 3.6 **5**	5.0 ± 0.8 **10**	1.3 ± 0.1 **10**	17.1 ± 3.7 **4**	<0.1 **1**
Methyl	113.7 ± 9.4 **7**	79.0 ± 7.1 **2**	5.6 ± 0.8 **3**	1.5 ± 0.5 **3**	21.2 ± 3.6 **5**	1.6 **1**
Ethyl	71.0 ± 7.4 **6**	3.7 ± 0.2 **2**	2.2 ± 0.2 **13**	0.3 ± 0.1 **5**	11.2 ± 1.6 **8**	<0.1 **2**
Ethenyl	41.7 ± 6.5 **3**	22.5 ± 2.1 **2**	2.0 ± 0.2 **9**	0.3 ± 0.1 **18**	13.6 ± 4.7 **3**	0.6 ± 0.6 **2**
Ethynyl	84.0 ± 8.9 **7**	72.0 ± 17.0 **2**	4.6 ± 0.5 **12**	2.0 ± 0.1 **17**	16.3 ± 4.6 **6**	1.6 ± 2.1 **2**
2-Propenyl	90.6 ± 9.7 **8**	10.6 ± 1.4 **4**	0.6 ± 0.1 **2**	<0.1 **1**	17.5 ± 7.7 **3**	0.9 **1**
1-Propynyl	17.6 ± 0.4 **2**	10.6 ± 1.6 **3**	2.9 ± 0.8 **2**	0.4 **1**	8.9 ± 1.4 **2**	0.2 ± 0.2 **4**
5-Spiromethylene ether						
–	256.7 ± 21.5 **30**	185.7 ± 18.6 **6**	3.5 ± 0.3 **10**	0.1 **2**	3.9 ± 0.5 **3**	<0.1 **2**
Methylene	213.1 ± 21.7 **7**	304.0 ± 49.1 **4**	1.9 ± 0.4 **4**	0.1 **1**	19.9 ± 0.9 **3**	0.6 ± 0.6 **2**
Methyl	258.3 ± 23.8 **9**	277.8 ± 53.4 **5**	4.0 ± 1.1 **3**	0.3 **1**	24.0 ± 4.2 **2**	5.4 ± 0.7 **4**
Ethyl	202.5 ± 15.2 **4**	76.0 ± 32.7 **3**	2.2 ± 0.3 **2**	0.2 **1**	33.0 ± 3.1 **3**	<0.1 **2**
Ethenyl	223.7 ± 21.0 **13**	118.9 ± 10.3 **7**	3.2 ± 0.1 **2**	0.5 ± 0.7 **2**	45.5 ± 4.9 **2**	1.4 ± 0.5 **2**
Ethynyl	260.0 ± 24.5 **3**	174.0 ± 41.0 **6**	6.4 ± 1.8 **3**	3.1 **1**	39.5 ± 0.7 **2**	5.3 ± 0.1 **2**
2-Propenyl	183.0 ± 11.1 **3**	48.8 ± 13.1 **4**	1.5 ± 0.3 **2**	<0.1 **1**	21.0 ± 0.1 **2**	0.3 ± 0.2 **3**
1-Propynyl	90.3 ± 5.7 **3**	39.7 ± 17.8 **3**	5.7 ± 0.5 **2**	0.3 **1**	5.8 ± 3.5 **3**	<0.1 **2**
6-Spiromethylene ether						
–	221.3 ± 32.4 **4**	165.0 ± 21.2 **2**	3.4 ± 0.7 **3**	<0.1 **2**	30.7 ± 0.9 **2**	0.8 ± 0.5 **2**
Methylene	227.2 ± 44.2 **5**	560.0 ± 42.4 **2**	2.2 ± 0.4 **4**	0.2 ± 0.3 **2**	105.0 ± 3.7 **3**	17.1 ± 0.9 **12**
Methyl	220.8 ± 28.9 **6**	170.8 ± 73.7 **4**	3.2 ± 0.8 **2**	0.4 **1**	134.3 ± 25.0 **3**	48.5 ± 4.8 **4**
Ethyl	237.0 ± 44.0 **9**	160.2 ± 18.6 **6**	2.3 ± 0.3 **3**	0.5 ± 0.7 **2**	84.2 ± 15.0 **5**	1.6 **1**
Ethenyl	253.5 ± 91.3 **4**	65.3 ± 13.6 **4**	2.3 ± 0.3 **2**	0.5 **1**	98.5 ± 20.5 **2**	7.9 **1**
Ethynyl 2-	257.5 ± 44.1 **4**	180.0 ± 14.1 **3**	6.0 ± 1.2 **3**	0.2 **2**	269.0 ± 47.3 **3**	52.3 ± 19.3 **3**
Propenyl	123.3 ± 6.8 **4**	42.9 ± 31.3 **3**	1.0 ± 0.1 **2**	<0.1 **1**	41.0 ± 1.4 **2**	0.2 **1**
1-Propynyl	235.5 ± 29.4 **4**	46.0 ± 7.8 **4**	6.9 ± 1.1 **3**	0.3 **1**	48.5 ± 0.7 **2**	0.2 ± 0.2 **2**
6-Spiromethylene lactone						
–	232.7 ± 21.3 **7**	355.0 ± 73.1 **4**	2.2 ± 0.2 **3**	0.3 ± 0.4 **2**	14.1 ± 0.6 **2**	0.7 ± 0.4 **2**
Methylene	270.2 ± 40.5 **5**	440.0 ± 42.4 **2**	2.2 ± 0.5 **3**	0.3 ± 0.1 **2**	59.3 ± 10.2 **3**	10.1 ± 7.0 **2**
Methyl	316.0 ± 46.6 **5**	320.0 ± 36.0 **3**	4.3 ± 0.4 **2**	2.8 ± 0.5 **3**	121.0 ± 24.5 **3**	43.8 ± 3.5 **16**
Ethyl	290.0 ± 41.9 **6**	350.0 ± 40.3 **5**	6.2 ± 1.5 **3**	3.9 ± 0.8 **3**	96.5 ± 10.6 **2**	5.5 **1**
Ethenyl	282.8 ± 114.1 **4**	326.3 ± 67.2 **3**	4.7 ± 0.1 **3**	1.4 ± 0.4 **2**	82.0 ± 0.0 **2**	17.8 ± 1.4 **11**
Ethynyl	292.5 ± 35.6 **8**	410.0 ± 56.6 **2**	7.2 ± 1.6 **3**	4.0 ± 2.3 **2**	89.3 ± 16.4 **3**	44.0 ± 7.4 **4**
2-Propenyl	213.0 ± 29.9 **4**	292.0 ± 37.0 **5**	2.1 ± 0.2 **2**	0.6 ± 0.4 **4**	56.5 ± 2.1 **2**	2.8 ± 1.1 **3**
1-Propynyl	215.3 ± 47.9 **6**	342.9 ± 69.4 **7**	12.2 ± 0.9 **2**	3.0 **1**	36.5 ± 6.4 **2**	1.5 ± 0.7 **4**

[a] For the progesterone and androgen receptor binding assays cytosol of human breast MCF-7 cells was used as receptor source and for the glucocorticoid receptor that of leukemic IM-9 cells. For the transactivation assays different CHO cells were used, each expressing its individual human receptor and a luciferase reporter system. As ligands Org 2058, 5α-dihydrotestosterone (DHT), and dexamethasone (DEX) were used as reference compounds. The data are expressed as the mean with the standard error of the mean (S.E.M.). Number of experiments is in bold.
[b] Intact cell assay.

the transactivation activity as was also seen with 17α-ethynyl. In case of the six-membered-spiromethylene lactone, all transactivation values with all 11-substituents led to potencies between 292 and 440%. This level was for all the latter combinations higher than for the other five- and six-membered spiro series as well as for the 17α-ethynyl series, except for the compound with five- and six-membered spiromethylene ether and 11-methylene groups. These compounds scored with 304 and 560% the highest progestagenic activities in their series.

5.1.2. RBA and RAA values for the human androgen receptor

5.1.2.1. RBA. NET had a 2-fold lower binding potency

than ETG, being 3.2 and 6%, respectively. From the analysed progestins, the RBA values varied between 0.6 and 7.2% with the exception of a six-membered-spiromethylene lactone and 1-propynyl addition, which showed a binding potency of 12.2%.

5.1.2.2. RAA. The androgenicity of NET, being 1.1%, was 2.5-fold lower than that of ETG. The androgenic activities of all tested compounds were in between 0.1 and 4.0%. Here the six-membered-spiromethylene lactone and 1-propynyl addition scored 3.0%, which is similar to ETG.

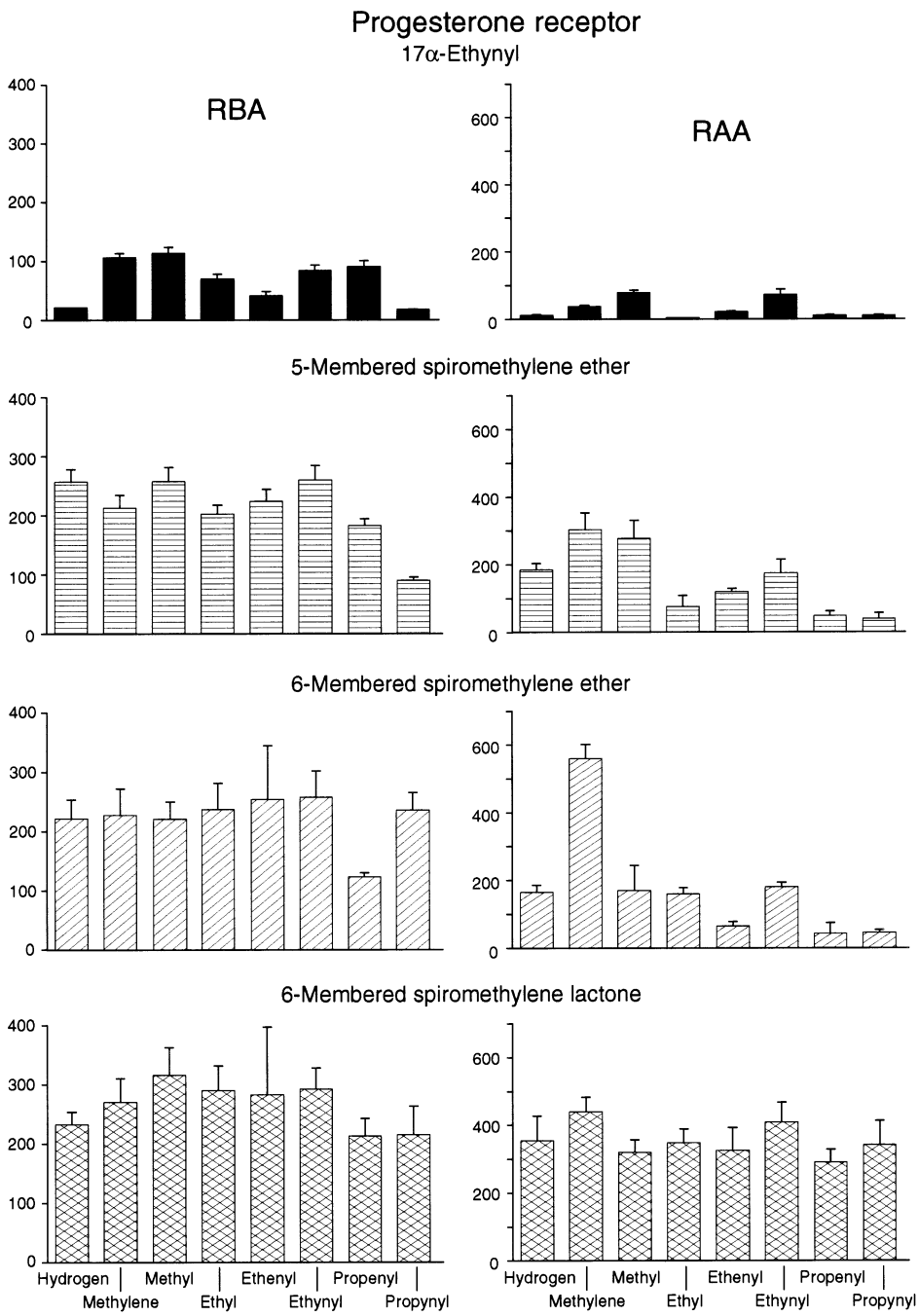

Fig. 3. Relative binding affinity (RBA) and relative agonistic activity (RAA) values with standard errors of the mean (S.E.M.) of various progestin derivatives to the progesterone receptor with binding assays in cytosol of human breast MCF-7 cells and with transactivation assays with CHO cells using Org 2058 as reference compounds.

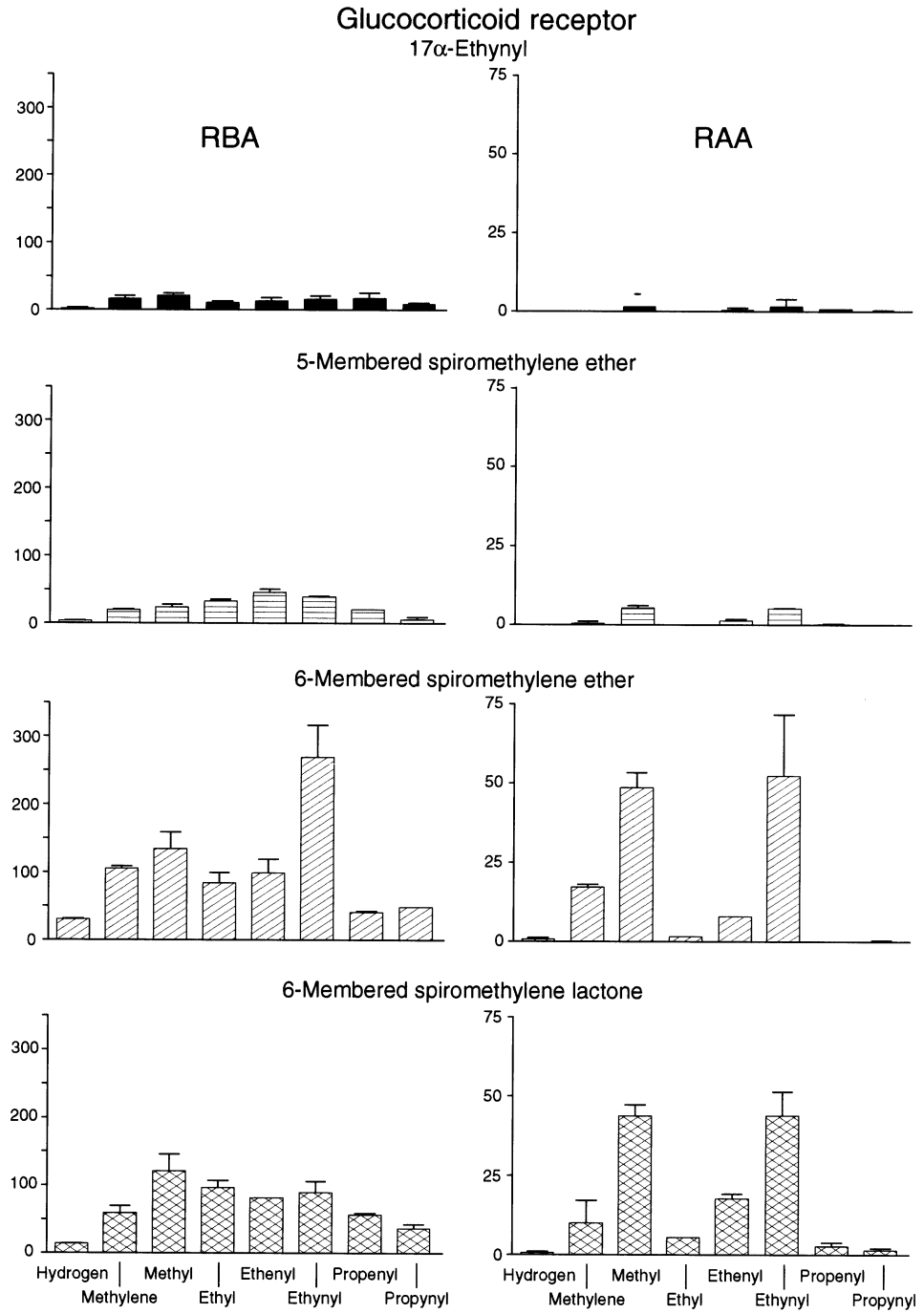

Fig. 4. Relative binding affinity (RBA) and relative agonistic activity (RAA) values with standard errors of the mean (S.E.M.) of various progestin derivatives to the glucocorticoid receptor with nuclear binding assays in IM-9 cells and with transactivation assays with CHO cells using dexamethasone (DEX) as reference compounds.

5.1.3. RBA and RAA values for the human glucocorticoid receptor

5.1.3.1. RBA. NET was 8-fold less potent than ETG, being 2.1 and 16.0%, respectively. Most 11 additions in the 17α-ethynyl group, excluding the 1-propynyl substituent, did increase binding affinity towards the level of ETG. A similar pattern was observed for the five-membered-spiromethylene ether group, although the active 11-substituents now showed activities in the range of 19.9–45.5% (Fig. 4). In the six-membered spiromethylene ether group the binding potential was in the same range of 30.7, 41.0 and 48.5% for none, 2-propenyl and 1-propynyl additions or even higher values were found of between 84 and 134% for the methylene, methyl, ethyl and ethenyl substituents. GR

binding activity reached the highest level of 269% for the ethynyl substituent in this group. For the six-membered-spiromethylene lactone group the absence of a substitution on position 11 led to RBA values of 14%. All other substitutions again increased binding activity in the range of 36–60% for methylene, 2-propenyl and 1-propynyl, and in the range of 82–97% for ethyl, ethenyl and ethynyl. The highest GR activity level was reached with a methyl substituent, being 121%.

5.1.3.2. RAA. NET and ETG did not show glucocorticoidal activity. In the 17α-ethynyl group the observed levels stayed below 1.6% (Fig. 4). In the five-membered-spiromethylene ether group a similar variation was found except for the methyl and ethynyl substituents, which showed activities of 5.4 and 5.3%, respectively. With the six-membered spiromethylene ether this pattern was also seen, but the glucocorticoid potencies of the methyl and ethynyl groups were raised towards 48.5 and 52.3%. Moreover, methylene and ethenyl additions became active at 17.1 and 7.9%, respectively. In the six-membered-spiromethylene lactone group this pattern was also present, although the ethenyl substituent became more potent than the methylene derivative.

5.1.4. RBA and RAA values for the human estrogen receptor

5.1.4.1. RBA and RAA. Neither NET nor ETG showed binding affinity towards and transactivation activity for ER, while with the other progestins none or only very weak binding affinities or transactivation activities below 0.3% were observed (data not shown).

5.2. In vivo studies

In Table 2 and Figs. 5 and 6, the in vivo MAD data are given for ETG and 32 different progestins for the following tests: the progestagenic McPhail, the combined progestagenic and estrogenic ovulation inhibition, the androgenic Hershberger, the glucocorticoid and estrogenic hormone screening (HST), and the estrogenic Allen-Doisy tests. All compounds in these assays were studied after oral administration and in the case of the McPhail test also after subcutaneous treatment.

5.2.1. Progestagenic activity in immature rabbits (McPhail test)

ETG clearly transformed the estradiol-primed endometrium in immature rabbits at total oral and subcutaneous doses of 12 and 5.4 μg/kg, respectively. NET was 20- and 12-fold, respectively, less potent than ETG. The change from 17α-ethynyl into five- and six-membered spiromethylene ethers and a six-membered-spiromethylene lactone enhanced oral activity 4-fold with the ethers, but not with the lactone. The subcutaneous activity, however, was increased by 8- to even 16-fold with the six-membered-spiromethylene (lactone) ethers. The effects of the introduction of several other 11-substituents on these different 17-derivatives is shown below and in Fig. 5.

5.2.1.1. 17α-Ethynyl. Methylene, methyl, and ethenyl substituents showed 8–16-fold stronger oral activities than with NET, while ethynyl and 2-propenyl additions were only 4- and ethyl only 2-fold better. The 1-propynyl substituent was 2-fold weaker. This pattern changed drastically after subcutaneous treatment. The 2-propenyl compound was 32-fold more potent than NET and even 4-fold more than ETG. The ethynyl substituent remained 4-fold more potent than NET. Subcutaneously, methylene, ethyl, and ethenyl derivatives were less potent than orally, if compared with NET.

5.2.1.2. Five-membered-spiromethylene ether. All 11-substituents increased oral and subcutaneous activity by at least 4-fold compared with NET. Oral activity was even increased by 16-fold by the ethynyl addition, while for ethenyl 12 and for ethyl and 2-propenyl 8-fold higher potencies were found. Subcutaneous activity was again largely increased with the ethynyl and the methyl substituent, followed by methylene and ethenyl, being 16–32-fold more potent than NET.

5.2.1.3. Six-membered spiromethylene ether. All 11-substituents increased oral activity by at least 4-fold and subcutaneous activity even by 8-fold compared with NET. The ethyl addition was the most potent oral one, being 16-fold better. Ethenyl was orally again 12-fold better than NET and methyl, ethyl and 2-propenyl were 8-fold better. Not all dose finding levels were assessed for subcutaneous treatment, since the oral route is more commonly used for contraception administration. However, for 1-propynyl MAD appeared to be 4 μg/kg, being lower than for NET and ETG.

5.2.1.4. Six-membered-spiromethylene lactone. The basic structure without an 11-substitution was orally as potent as NET. All other 11-substituents increased oral activity by at least 8-fold and subcutaneous activity by at least 16-fold with respect to NET. Ethynyl and 2-propenyl additions were orally 12-fold and methylene 16-fold stronger than NET. Methyl, ethyl and 1-propynyl were orally 20-fold better than NET and as potent as ETG. After subcutaneous treatment, as far as MAD was assessed, the most potent compound was the 1-propynyl substituent, followed by ethynyl, being respectively 63- and 32-fold more potent than NET and 8–4-fold more than ETG.

Table 2
The minimal active dose (MAD) in McPhail, ovulation inhibition, Hershberger, and Allen-Doisy, tests and the adrenal or thymic activity (%) in the hormone screening test (HST) of various progestin derivatives after oral (all) and subcutaneous (McPhail) administration[a]

	McPhail		Ovulation inhibition	Hershberger	HST oral at 1 mg/kg		Allen-Doisy
	Oral (μg/kg)	s.c. (μg/kg)	oral (μg/kg)	oral (μg/kg)	A (%)	T (%)	oral (μg/kg)
Standard							
ETG	12	5.4	750	2000	6[b]	16[b]	40 000
17α-Ethynyl							
– (NET)	250	63	6000	10 000	13	54	6000
Methylene	30	15	235	20 000	20	70	250
Methyl	15	ND	70	5000	43	62	210
Ethyl	125	63	18	>20 000	19	61	96
Ethenyl	32	32	12	>40 000	19	62	16
Ethynyl	63	16	30	5000	38	62	2000
2-Propenyl	63	2	<96	ND	6	49	>2000
1-Propynyl	500	125	300	ND	15	34	ND
5-Spiromethylene ether							
–	63	8	>3000	>40 000	6	−17	ND
Methylene	≥125	4	>375	>20 000	ND	ND	ND
Methyl	48	<4	75	ND	37	8	>4000
Ethyl	32	16	<48	ND	3	47	500
Ethenyl	24	<8	<48	80 000	23	16	>4000
Ethynyl	16	2	15	80 000	61	10	>4000
2-Propenyl	32	12	32	ND	11	−15	>4000
1-Propynyl	63	16	144	ND	6	15	ND
6-Spiromethylene ether							
–	63	<8	>750	ND	9	−13	ND
Methylene	63	8	470	ND	48	6	ND
Methyl	32	<8	48	ND	54	66	ND
Ethyl	16	8	32	>40 000	24	47	>4000
Ethenyl	24	<8	9	ND	55	52	>4000
Ethynyl	32	ND	6	ND	54	50	ND
2-Propenyl	32	<8	36	ND	18	3	>4000
1-Propynyl	≥64	4	18	ND	13	−8	>500
6-Spiromethylene lactone							
–	250	<8	>1500	ND	6	−16	ND
Methylene	16	<8	>750	>80 000	44	−13	>4000
Methyl	12	<8	120	ND	61	55	>4000
Ethyl	12	4	48	20 000	56	11	>4000
Ethenyl	32	<8	400	ND	24	−10	>4000
Ethynyl	24	2	<6	ND	63	80	>4000
2-Propenyl	24	<8	<24	ND	19	−9	ND
1-Propynyl	12	1	18	>40 000	22	9	>4000

[a] In HST: A, adrenal and T, thymic effect. Moreover a value below 30% means no significant increase from controls with placebo levels. ND, not determined.
[b] For ETG a dose of 20 mg/kg was used.

5.2.2. Ovulation inhibition in rats

ETG inhibited ovulation in rats at 750 μg/kg after oral treatment. NET is 8-fold less potent than ETG. Both progestagenic and estrogenic compounds, like Org 2058 and estradiol, can induce ovulation inhibition, implying that both compounds individually or a combination of both induced this inhibitory activity. The change from 17α-ethynyl of NET into five- and six-membered spiromethylene ethers and a six-membered-spiromethylene lactone improves oral activity by at least 2-fold (Fig. 6).

5.2.2.1. 17α-Ethynyl. The activity with ethyl, ethenyl and ethynyl increased by at least 200–500-fold with respect to NET and 25–63-fold with respect to ETG. The compounds with a methylene, methyl, 2-propenyl, and 1-propynyl derivative showed potencies that were 20- to even 125-fold better than those of NET and 2–16-fold those of ETG.

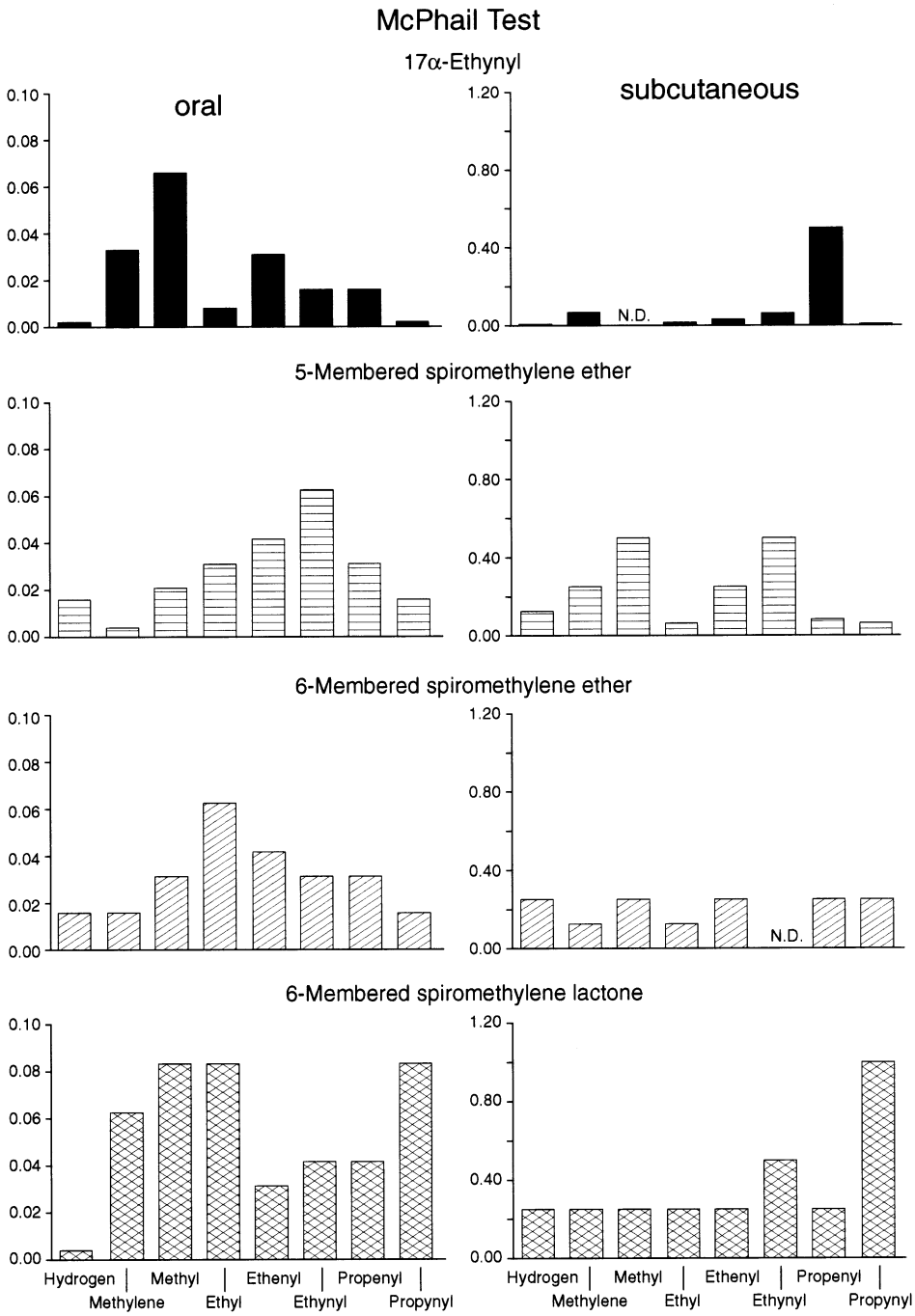

Fig. 5. Reciprocal minimal active dose (1/MAD) in the McPhail test of various progestin derivatives both after oral and subcutaneous administration. ND, not determined.

5.2.2.2. Five-membered-spiromethylene ether. Again ethyl, ethenyl and ethynyl, and also 2-propenyl, increased the activity by at least 125–400-fold with respect to NET and 20–50-fold with respect to ETG. Methylene, methyl and 1-propynyl groups also showed increased activity, being 16–80-fold higher than NET and 2–10-fold higher than ETG.

5.2.2.3. Six-membered spiromethylene ether. A tremendous increase was found with ethenyl and ethynyl, being 666–1000-fold more potent than NET and 83–125-fold more than ETG. Methyl, ethyl, 2-propenyl and 1-propynyl substituents also enhanced the activity by at least 16- and 125-fold above those of ETG and NET, respectively. Also in this six-membered spiro-

methylene ether group, the methylene addition led to the weakest activity of the substituents.

5.2.2.4. Six-membered-spiromethylene lactone. The highest activities were shown with ethynyl, 2-propenyl and 1-propynyl substituents, being 333–1000-fold more potent than NET and 42–125-fold more than ETG. The other 11-substituents are less potent in the range from 16- to 125-fold compared with NET and 1- to 15-fold with ETG.

5.2.3. Androgenic activity in orchidectomised rats (Hershberger test)

ETG and NET were orally active on the ventral prostate at dose levels of 2 and 10 mg/kg, respectively (Table 2). In general, all the other compounds tested

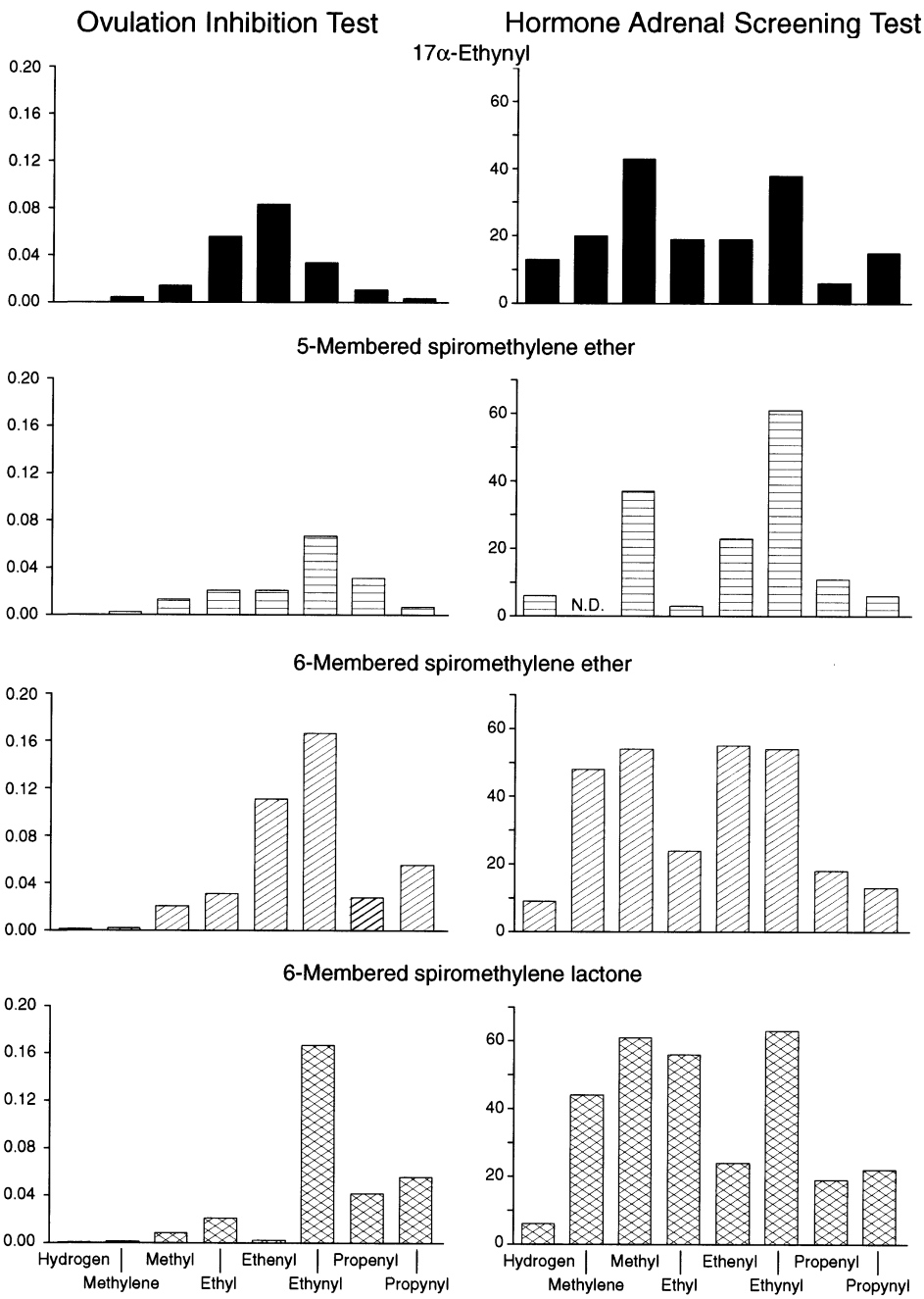

Fig. 6. Reciprocal minimal active dose (1/MAD) in the ovulation inhibition test and the percentage difference of the glucocorticoid induced effect on adrenals with respect to controls in the hormone screening test at a dose of 1 mg/kg (HST) of various progestin derivatives after oral administration. ND, not determined.

for androgenicity were selected based on a high oral potency in the McPhail test and a low glucocorticoidal effect in the hormone screening test. The selected compounds showed only activity at dose levels of 5 mg/kg or higher, which is at least 2.5-fold weaker than for the relatively weak androgenic compound ETG. This implies that the selected compounds have only a very weak or no intrinsic androgenicity.

5.2.4. Glucocorticoid and estrogenic activity in rats (hormone screening test)

The effects of the progestagenic compounds are indicated for adrenal and thymic growth reduction at a relatively high oral dose level of 1 mg/kg against control animals. If the effect exceeds that of the control group by 30% or more, the effect is considered as biologically significant. Adrenal and thymic growth reduction is a marker for glucocorticoidal efficacy, whereas thymic growth reduction alone is a marker for estrogenicity. With ETG and NET no effects were found on the adrenals, while only a small thymus reduction of up to 46% was observed with NET, indicating an estrogenic potential of NET. Introduction of the three other 17-substituents, such as five- and six-membered spiromethylene ether and six-membered-spiromethylene lactone groups in place of the 17α-ethynyl, neither showed glucocorticoidal nor estrogenic efficacy (Fig. 6).

5.2.4.1. 17α-Ethynyl. A glucocorticoid effect was observed with methyl and ethynyl groups. Moreover, a clear estrogenic effect was shown for methylene, ethyl, ethynyl and to a lesser extent with 2-propenyl, and 1-propynyl substituents.

5.2.4.2. Five-membered-spiromethylene ether. Only methyl and ethynyl derivatives showed glucocorticoid activity, while estrogenicity was only present with the ethyl addition. The ethenyl, 2-propenyl and 1-propynyl substituents induced neither estrogenic nor glucocorticoid activity.

5.2.4.3. Six-membered spiromethylene ether. Methylene, methyl, ethenyl and ethynyl groups induced glucocorticoid activity. Again the compound with the ethyl group showed a more estrogenic profile, while the 2-propenyl and 1-propynyl groups were inactive again as estrogenic and glucocorticoidal compounds.

5.2.4.4. Six-membered-spiromethylene lactone. Again methylene, methyl, and ethynyl substituents, as well as the ethyl, showed glucocorticoid activity. However, none of these compounds showed an estrogenic potential.

5.2.5. Estrogenic activity in ovariectomised rats (Allen-Doisy test)

The estrogenic activity of ETG was compared with that of the other progestagenic compounds. In order to obtain an estrogenic response in 50% of the rats a dose of 40 mg/kg of ETG was needed, which is 20-fold less potent than the standard estradiol. NET was almost 7-fold more potent than ETG.

5.2.5.1. 17α-Ethynyl. The ethenyl substituent was remarkably effective at 16 μg/kg, being 375-fold more active than NET. Also the methylene, methyl, and ethyl groups were relatively active, being 24–62-fold more potent than NET. On the other hand, the ethynyl and 2-propenyl compounds were only 3-fold stronger or equipotent to NET, respectively.

5.2.5.2. Five- and six-membered-spiromethylene (lactone) ethers. None of the tested compounds in these groups showed activity at a level of 4 mg/kg except for an ethyl group combined with a five-membered-spiromethylene ether, which was active at 500 μg/kg. These data imply that the progestagenic compounds with the five- and six-membered-spiromethylene (lactone) ethers and/or metabolites thereof do not possess or have hardly any intrinsic estrogenicity.

6. Discussion

NET, LNG, GSD and ETG are known as compounds with progestagenic activity [2–4,14,23,24]. Within this group of compounds, GSD and ETG were 10–20-fold and LNG 5-fold more potent than NET. With respect to androgenicity, LNG was slightly more potent than GSD and almost twice as potent as ETG. Although NET was 5-fold less androgenic than ETG, its androgenicity at equivalent progestagenic effects was twice to four times as high. In this study an effort was made to further increase the progestagenic activity and to decrease the androgenic activity with respect to ETG. Therefore 32 compounds with four classes of different 17-substituted side chains and various modifications at position 11 (Fig. 2) were studied. These compounds were tested in vitro on binding affinities and transactivation activities for PR, AR, GR and ER. In vivo they were studied in the progestagenic McPhail, the combined progestagenic and estrogenic ovulation inhibition, the androgenic Hershberger, the glucocorticoidal and estrogen dependent hormone profiling and the estrogenic Allen-Doisy tests.

In the binding and transactivation tests with PR, the advantage of the introduction of five- and six-membered spiromethylene ethers and six-membered-spiromethylene lactone with respect to 17α-ethynyl is

evident. This was not only shown for the non-substituted reference compound NET, but also for the compounds with other 11-substituents. In the binding assays for PR, all compounds with the spiromethylene (lactone) ethers showed a tendency towards higher binding potential than ETG except for three compounds. These higher potential compounds have a 11β-2-propenyl substituent in combination with the five- and six-membered spiromethylene ethers or a 11β-1-propynyl substituent in combination with a five-membered-spiromethylene ether. In the transactivation assays, this improvement in progestagenic activity was clearly visible for the six-membered-spiromethylene lactone with all the 11-substituent variations, but less pronounced with the five- and six-membered spiromethylene ethers. In these ether groups, again the 2-propenyl and 1-propynyl additions were less potent, but also the ethynyl and ethyl substituents were less active than ETG. Thus it can be concluded that spiromethylene ethers have a higher potency than NET and that the following 11-additions: methylene, methyl and ethenyl all enhance the bioactivity of these spiromethylene ethers.

In the AR binding and transactivation assays, all affinities and activities of these 32 compounds were lower than or equal to those of ETG with exception of the compound with an 11β-1-propynyl and six-membered-spiromethylene lactone. This compound had an activity that was 2-fold higher in binding than that of ETG, while this effect was absent in the transactivation assay.

None of these tested compounds showed estrogenic activity with either the binding or transactivation assays.

In the binding and transactivation assays with GR, all three spiromethylene (lactone) ethers increased the GR binding affinity. However, the transactivation activity only increased with the six-membered spiromethylene ether and six-membered-spiromethylene lactone provided that the following 11-substituents were present, i.e. methylene, methyl, ethenyl and ethynyl. This implies that although the compounds do bind strongly, they are not as potent in expressing their biological activity in these transactivation assays.

In the progestagenic McPhail test in rabbits, the advantage of the introduction of the three spiromethylene (lactone) ethers is again very evident with subcutaneous administration. This pattern was also seen with binding and transactivation assays. On the other hand, in the 17α-ethynyl group an 11β-2-propenyl addition resulted in a very potent compound, while in combination with the five-membered-spiromethylene ether, methyl and ethynyl showed great activity. Moreover, in combination with the six-membered-spiromethylene lactone, ethynyl and in particular 1-propynyl were the most potent substituents. In the McPhail test with oral administration a different pattern was identified for these compounds. This was most likely due to persistence of metabolism in the stomach, intestine and/or liver of some but not of all compounds. With the 17α-ethynyl group, it appeared that the methylene, methyl and ethenyl substituents were the most potent ones, whereas with the five-membered-spiromethylene ether the ethynyl prevailed. In the six-membered spiromethylene ether, the ethyl substituent was the most active one and in case of the six-membered-spiromethylene lactone the methylene, methyl, ethyl and 1-propynyl additions were the more active ones. Thus the subcutaneous data are more in line with the binding and transactivation data. The oral data, on the other hand, show that protection against metabolic conversion by some of the 11-substituents is better in combination with for instance a 17α-ethynyl group, while other 11-additions are better with one of the other spiromethylene (lactone) ethers.

In the ovulation inhibition test in rats both estrogens and progestins can evoke ovulation inhibition. As progestins can be metabolized into compounds with a 3β-hydroxy group in combination with a Δ^4 double bond or a 5α-hydrogen atom both the parent compound as well as its metabolite might be active in this test after oral administration [25]. In this test the 17α-ethynyl group in combination with ethyl and ethenyl additions were the most potent compounds. This activity was different from binding and transactivation in which these compounds were relatively weak. The estrogenic profile of metabolites of these compounds might lead to the improved profile in ovulation inhibition. With the introduction of the spiromethylene (lactone) ethers, the estrogenicity of potential metabolites of the parent compounds was largely reduced, with respect to the 17α-ethynyl group, as shown later with the Allen-Doisy tests. For the five-membered-spiromethylene group a combination with ethynyl was most effective, for the six-membered-spiromethylene group ethenyl and ethynyl were the best compounds, while for the six-membered-spiromethylene lactone, the ethynyl substituent was the most potent one. This oral progestagenic activity scheme of these compounds in rats was slightly different from that found in rabbits. This difference is most likely due to differences in the metabolizing pathways between the two species, but not to intrinsic estrogenic activities of metabolites of five-and six-membered-spiromethylene (lactone) ethers. In general the 11-substituents with the exception of the 11-methylene addition showed in combination with their respective 17-derivatives a good resemblance and correlation with respect to McPhail and ovulation inhibition tests.

In the hormone screening test in rats, both glucocorticoidal and estrogenic side-effects can be assessed. For the compounds with the different groups on position

17, it appeared that the methyl and ethenyl substituents on position 11 in all groups induced glucocorticoidal activity at 1 mg/kg. This was in line with the data found with the transactivation assays. In case of the five- and six-membered-spiromethylene (lactone) ethers methylene and ethenyl substituents also induced glucocorticoidal activity at these high dose levels. In the transactivation assays these compounds were also identified as more glucocorticoidal. All compounds with the 17α-ethynyl group showed estrogenic activity independent of the 11-substitution. On the other hand, estrogenicity was only found in the five- and six-membered spiromethylene ethers with the 11β-ethyl substitution. This implies that ethynyl and methyl substituents induce glucocorticoid activity, while the ethyl addition leads to estrogenic effects. Moreover, all 17α-ethynyl compounds with 11 additions induced these estrogenic effects. This leaves the 2-propenyl and 1-propynyl additions and to a lesser extent the ethyl substituent the most promising 11-substituents in combination with the five- and six-membered-spiromethylene (lactone) ethers, as these compounds show the weakest glucocorticoidal side-effects.

In the Hershberger test, all the tested compounds showed only an androgenic side-effect at or above 5 mg/kg, whereas ETG was already active at 2 mg/kg. Despite the relatively high RBA value of the six-membered-spiromethylene lactone with 11β-1-propynyl, an androgenic side-effect could be excluded for this compound. Thus the low transactivation activity, instead of the high binding activity, of the six-membered-spiromethylene lactone with the 1-propynyl substituent correlates better with this in vivo activity.

Clear estrogenic effects, already found with the hormone screening test, were also identified in the Allen-Doisy test for compounds with a 17α-ethynyl group in combination with ethyl and ethenyl substituents and to a lesser extent with methylene and methyl substituents. In these cases the balance of metabolism was probably largely in favor of the estrogens. On the other hand, if the progestagenic potential prevails the compounds may become active as antiestrogens and this may lead to estrogenic effects at levels only above 0.5 mg/kg. If these progestagenic compounds are tested in combination with an antiprogestagen, the real intrinsic estrogenic effect of the compound can be visualised more thoroughly. Moreover, a chemical change of the 3-keto group into a 3β-hydroxy group with or without a change of the Δ^4 double bond into a 5α-reduced compound might also lead to improved estrogenic profiling. For several of the parent compounds of the 17α-ethynyl group a clear estrogenic profile was demonstrated with potential metabolites of these compounds in other studies [25]. This illustrates that not the parent compounds, but their metabolites are the active estrogenic compounds.

Taken all these data into consideration the following conclusion can be drawn. The binding and transactivation data show that progestagenic efficacy is improved with the introduction of the five- and six-membered spiromethylene ethers and is most effective with the six-membered-spiromethylene lactone. Androgenic and estrogenic binding and transactivation activities were very low or even absent. As shown by glucocorticoidal binding and transactivation potencies, the six-membered-spiromethylene (lactone) ethers in combination with methyl and ethynyl and to a lesser degree with methylene, ethyl and ethynyl might induce glucocorticoidal side-effects at high dose levels. With respect to the oral and subcutaneous McPhail tests, the introduction of the five- and six-membered-spiromethylene (lactone) ethers also increased the progestagenic efficacy, being more pronounced with the six-membered-spiromethylene lactone and especially in combination with a 11β-1-propynyl substituent. Although these increases in progestagenic potential were not fully reflected in the ovulation inhibition test, there were advantages for the combination of two and three carbon 11-side chains and the five- and six-membered-spiromethylene (lactone) ethers. Again the six-membered-spiromethylene (lactone) ethers in combination with 2-propenyl and 1-propynyl, but also ethynyl, have a very favorable profile. In the Hershberger tests all tested compounds were less potent than ETG, making them even less androgenic at the biopotency levels. Some compounds with a particular 11-substituent, like methyl and ethynyl and less so for methylene, ethyl and ethenyl, induce more glucocorticoidal side-effects at 1 mg/kg than others. Although these side-effects are only found at relatively high dose levels, these might be considered prohibitive for further development. Consequently, 2-propenyl and 1-propynyl 11-substituents appear to be the most favorable side chains for the five- and six-membered-spiromethylene (lactone) ethers. Moreover with the Allen-Doisy test, it becomes clear that the activity of the five- and six-membered-spiromethylene (lactone) ethers are mediated via the progesterone receptor in both McPhail and ovulation inhibition tests. However, for the methylene, methyl, ethyl and ethenyl derivatives in combination with the 17α-ethynyl group, it appears that metabolites are also involved in the induction of estrogenic effects. This implies that in case of ovulation inhibition the combined effect of progestagens and estrogens can induce ovulation inhibition. Such a combination might also lead to very good control of contraception. Therefore the 17α-ethynyl combined with ethyl and ethenyl might be very interesting as an oral contraceptive. Finally, it appears that there is a very good resemblance between the in vitro and in vivo data of progestagenic, androgenic, estrogenic and glucocorticoidal tests.

Based on the high efficacy and low side-effects, the following compounds show a high selectivity: 17α-ethynyl with ethyl, ethenyl and 2-propenyl substituents, six-membered spiromethylene ether with ethyl and six-membered-spiromethylene lactone with ethyl, 2-propenyl or 1-propynyl substituents. These compounds have relatively high binding and transactivation values for PR, and have high biopotencies in the McPhail and ovulation inhibition tests, while showing very weak androgenic and glucocorticoid activities. These compounds may be very useful for contraception for either oral and/or subcutaneous administration.

References

[1] M.L.E. Orme, D.J. Back, A.M. Breckenridge, Clinical pharmacokinetics of oral contraceptive steroids, Clin. Pharmacokin. 8 (1983) 95–136.

[2] D.J. Back, S. Ward, M.L.E. Orme, Recent pharmacokinetic studies of low-dose oral contraceptives, Adv. Contracept. 7 (suppl. 3) (1991) 164–179.

[3] R.W. Rebar, K. Zeserson, Characteristics of the new progestogens in combination oral contraceptives, Contraception 44 (1991) 1–10.

[4] G.M. Shenfield, J.M. Griffin, Clinical pharmacokinetics of contraceptive steroids — an update, Clin. Pharmacokinet. 20 (1991) 15–37.

[5] J. Spona, Androgenic action of progestins and possible synandrogenic properties of antiandrogens used in oral contraceptives, Gynecol. Obstet. Invest. 17 (1984) 66–72.

[6] D.J. Dorflinger, Relative potency of progestins used in oral contraceptives, Contraception 31 (1985) 557–570.

[7] A. Phillips, D.W. Hahn, S. Klimek, J.L. McGuire, A comparison of the proteins and activities of progestagens used in contraceptives, Contraception 36 (1987) 181–192.

[8] J. van der Vies, J. de Visser, Endocrinological studies with desogestrel, Arzneim.-Forsch. 33 (1983) 231–236.

[9] G.H. Deckers, W.G.E.J. Schoonen, H.J. Kloosterboer, Influence of the substitution of 11-methylene, Δ^{15}, and/or 18-methyl groups in norethisterone on receptor binding, transactivation assays and biological activities in animal experiments, J. Steroid Biochem. Mol. Biol. 74 (2000) 83–92.

[10] R.T. Burkman, L.P. Shulman, Oral contraceptive practice guidelines, Contraception 58 (1998) 35S–43S.

[11] M.F. Fathalla, A. Rosenfeld, C. Indriso, Oral hormonal contraception, in: A. Rosenfeld, M.F. Fathalla (Eds.), The FIGO Manual of Human Reproduction. Family Planning, vol. 2, Parthenon, Carnforth, UK, 1990, pp. 32–65 Chapter 2.

[12] E. Hirkoven, Progestins, Maturitas 23 (suppl.) (1996) S13–S18.

[13] E.W. Bergink, H.J. Kloosterboer, Structural requirements for optimal receptor binding of oral contraceptive progestagens, Adv. Contracept. 1 (1985) 256–257.

[14] H.-O. Hoppen, P. Hammann, The influence of structural modification on progesterone and androgen receptor binding of norethisterone. Correlation with nuclear magnetic resonance signals, Acta Endocrinol. 115 (1987) 406–412.

[15] W.G.E.J. Schoonen, J.W.H. Joosten, H.J. Kloosterboer, Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: I. MCF-7 cell lines, J. Steroid Biochem. Mol. Biol. 55 (1995) 423–437.

[16] W.G.E.J. Schoonen, R. Dijkema, R.J.H. Ries, J.L. Wagenaars, J.W.H. Joosten, M.E. de Gooyer, G.H. Deckers, H.J. Kloosterboer, Human progesterone receptor A and B isoforms in CHO cells. II. Comparison of binding, transactivation and ED_{50} values of several synthetic (anti)progestagens in vitro in CHO and MCF-7 cells and in vivo in rabbits and rats, J. Steroid Biochem. Mol. Biol. 64 (1998) 157–170.

[17] W.G.E.J. Schoonen, R.J.H. Ries, G.J.W. Mommers, M.E. de Gooyer, E. van der Struijk, R. Dijkema, The human glucocorticoid receptor: comparison of binding and transactivation modulation with CHO hGR-MMTV-LUC cells versus binding with IM-9 cells: steroid/thyroid/retinoic acid gene family. Keystone symposium, Lake Tahoe, CA, March 17–23, 1996, Abstract 151.

[18] R. Dijkema, W.G.E.J. Schoonen, R. Teuwen, E. van der Struijk, R.J.H. Ries, S.A.T. Kar, W. Olijve, Human progesterone receptor A and B isoforms in CHO cells. I. Stable transfection of receptor and receptor-responsive reporter genes: transcription modulation by (anti)progestagens, J. Steroid Biochem. Mol. Biol. 64 (1998) 147–156.

[19] D.J. Finney, Statistical Method in Biological Assay, third ed, Griffin, London, 1978.

[20] M.K. McPhail, The assay of progestin, J. Physiol. 83 (1934) 145–156.

[21] L.G. Hershberger, E.G. Shipley, R.K. Meyer, Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator and muscle method, Proc. Soc. Exp. Biol. Med. 83 (1953) 175–180.

[22] A. Allen, E.A. Doisy, An ovarian hormone. Preliminary report on its location, extraction and partial purification and action in test animals, J. Am. Med. Assoc. 81 (1923) 819–821.

[23] H.J. Kloosterboer, C.A. Vonk-Noordegraaf, E.W. Turpijn, Selectivity in progesterone and androgen receptor binding of progestagens used in oral contraceptives, Contraception 38 (1988) 325–332.

[24] E.W. Bergink, A.D. Hamburger, E. de Jager, J. van der Vies, Binding of a contraceptive progestagen Org 2969 and its metabolites to receptor proteins and human sex binding globulin, J. Steroid Biochem. 14 (1981) 175–183.

[25] W.G.E.J. Schoonen, G.H. Deckers, M.E. de Gooijer, R. de Ries, H.J. Kloosterboer, Hormonal properties of norethisterone, 7α-methyl-norethisterone and their derivatives, J. Steroid Biochem. Mol. Biol. (2000) (in press).