Synthesis and Biological Activity of a Novel, Highly Potent Progesterone Receptor Antagonist

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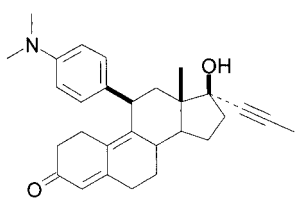
Herein we describe the chemical synthesis and pharmacological characterization of a novel, highly potent progesterone receptor (PR) antagonist, ZK 230211. The introduction of a 17α-pentafluorethyl side chain in the D-ring of the steroid skeleton allowed the combination of high antiprogestagenic activity with little or no other endocrinological effects. In contrast to many other antiprogestins, ZK 230211 did not convert to an agonist in the presence of protein kinase A (PKA) activators and showed high antiprogestagenic activity on both PR isoforms PR-A and PR-B. This high antiprogestagenic activity could also be demonstrated in several in vivo models. Furthermore, this compound displayed only marginal antiglucocorticoid effects. In tumor models ZK 230211 exhibited strong antiproliferative action. The pharmacological properties of ZK 230211 may prove useful in the treatment of endometriosis, leiomyomas, breast cancer, and in hormone replacement therapy.

Introduction

Progesterone plays a critical role in the maintenance and development of female reproductive functions. Its biological activity is mediated by the progesterone receptor (PR), which induces a cascade of transcriptional events after binding of hormone.[1] Blocking PR function by using a PR antagonist should allow the modulation of various reproductive processes. On this basis, antiprogestins were developed which disrupt the normal progesterone-induced signal transduction pathway by competitive binding to the PR. Therefore, antiprogestins have considerable potential as therapeutic drugs for numerous gynecological, obstetrical, and oncological indications. They were shown to have a contraceptive potential by blocking ovulation[2−4] and preventing implantation.[5−9] Concomitant with the latter pharmacological effect is the observation that antiprogestins induce profound alterations in endometrial morphology. This phenomenon is based on their antiproliferative action and is observed in several species.[10−13] In nonhuman primates the antiproliferative effect was convincingly demonstrated where, e.g., low doses of antiprogestin induced endometrial atrophy characterized by reduced mitotic activity of the glands, compaction of the stroma, and degradation of spiral arteries.[12,13] This antiproliferative action might also have been the basis for observations made in clinical studies regarding endometrial fertility control,[14] treatment of endometriosis,[15] uterine leiomyomas,[16] and breast cancer.[17,18]

The first antiprogestin, RU 38486 (**1**, known as RU 486), was synthesized by Philibert, Deraedt, and Teutsch in 1981.[19−21] RU 486 is a derivative of 19-nortestosterone and has an additional 4-(dimethylamino)-phenyl group at the 11*β*-position, a Δ^9-double bond, and a 1-propynyl chain at the 17α-position. Since the original discovery of RU 486, much effort has been devoted to optimizing antiprogestational structures with regard to steroid receptor selectivity. Several modifications of the steroid nucleus were studied: e.g., 13α-,[22] 14*β*-,[23] 9,10-dihydro-, 10,11-bridged-,[24] and 10*β*-methyl-antiprogestins[25] have been prepared. Moreover, replacing the dimethylamino function in the 11*β*-substituent of RU 486 by acetyl,[26] cyanophenyl, or heteroaryl substituents led to potent antiprogestins. Exchange of the propynyl 17-side chain for hydroxypropenyl or spiroether[27] moieties in some cases enhanced antiprogestagenic activity. In addition, unwanted hormonal partial activities, e.g., antiglucocorticoid activities, were reduced by these structural elements at C-17. Stimulated by these findings we continued the investigation of the C-17 substitution in order to find highly potent antiprogestins with considerably reduced endocrine side effects.



Mifepristone
RU 38486

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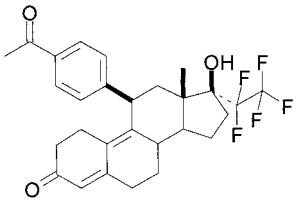

ZK 230211

2

In this paper we describe the chemical synthesis and pharmacological characterization of our new compound ZK 230211 (**2**) in several cell culture and animal models. We compared its antiprogestagenic potency and its

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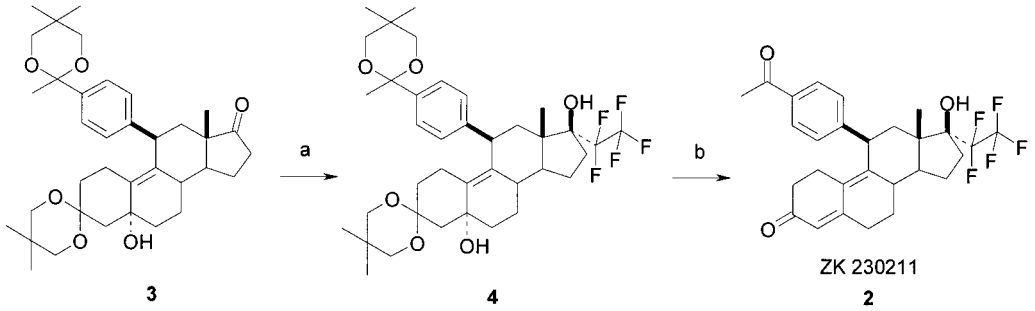
Scheme 1[a]



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[a] Reagents: (a) C_2F_5I, MeLi·LiBr, Et_2O; (b) 50% H_2SO_4, MeOH.

Table 1. Relative Binding Affinities of ZK 230211 to Steroid Hormone Receptors[a]

RBA (%)			
PR (rabbit)	AR (rat)	GR (rat)	ER (rat)
140*	3.1*	22.2**	nc**

[a] nc = no competition, *24 h incubation, **2 h incubation. The reference compounds were progesterone for PR, R1881 for AR, dexamethasone for GR, and estradiol for ER. The RBA values of the respective reference compounds were arbitrarily designated 100%.

Table 2. Transactivation Profile of ZK 230211

	ZK 230211	RU 486
PR-A agonist efficacies (%)[a]	ne	ne
PR-A antagonist potency (IC_{50}, nM)[b]	0.0036	0.028
PR-B agonist efficacies (%)[a]	ne	ne
PR-B antagonist potency (IC_{50}, nM)[b]	0.0025	0.025
AR agonist efficacies (%)[a]	4	6
AR antagonist potency (IC_{50}, nM)[b]	54	10
GR agonist efficacies (%)[a]	ne	ne
GR antagonist potency (IC_{50}, nM)[b]	16	2.2
ER agonist efficacies (%)[a]	ne	nd
ER antagonist potency (IC_{50}, nM)[b]	ne	nd

[a] Agonist efficacies were compared to those of the respective reference compounds which were set at 100%.The reference compounds were R5020 for PR-A and PR-B, R1881 for AR, dexamethasone for GR, and estradiol for ER. [b] IC_{50} values for antagonistic potency were determined from full dose−response curves ranging from 10^{-12} to 10^{-6}. ne = no effect; nd = not determined.

endocrine side effects with RU 486 and its antitumor potency with onapristone (ZK 98299[22]).

Chemistry

Beside position 11, ring D of the steroid is a common site for chemical variation of antiprogestins.[21−23,27] This site offers the opportunity of various modifications without loss of activity in many cases. Thus, position 17 was used for a more detailed analysis of the structure−activity relationship (SAR). Among a variety of modifications leading to active compounds, the 17α-pentafluoroethyl side chain was found to combine high antiprogestagenic with low undesirable hormonal activities. To our knowledge, this is the first report on steroidal perfluoroalkyl substitution higher than trifluoromethyl at C-17. The introduction of the side chain is depicted in Scheme 1. It was performed following a modified Gassman protocol.[28] In the presence of 17-ketone **3**,[29] an 8.5-fold excess of 1,1,1,2,2-pentafluoro-2-iodoethane was subjected to an iodide lithium exchange using methyllithium−lithium bromide complex. The protecting groups of pentafluoroethyl adduct **4** were removed using 50% sulfuric acid in methanol to furnish ZK 230211 (**2**).

Results and Discussion

Receptor Binding Profile. Table 1 presents the relative binding affinities (RBA) of ZK 230211 to PR, the glucocorticoid receptor (GR), the androgen receptor (AR), and the estrogen receptor (ER). This comparative study was performed using cytosol fractions of steroid hormone receptor-containing tissue of rabbit (PR) or rat (GR, AR, ER). The RBA values were determined in competition experiments using radioactively labeled standard compounds as reference. ZK 230211 showed a highly selective receptor profile with strong binding to PR, considerably weaker binding to both GR and AR, and no binding to ER (Table 1).

Transactivation Profile. To determine the antiprogestagenic and progestagenic potency of ZK 230211 in vitro, transactivation assays were carried out with cells stably transfected with human PR-A or PR-B. ZK 230211 showed strong antiprogestagenic activity on both PR isoforms with IC_{50} values of 0.0036 nM (PR-A) and 0.0025 nM (PR-B), respectively (Table 2). In comparison to RU 486, ZK 230211 is a 10 times stronger antagonist at both PR-A and PR-B. ZK 230211 did not show any progestagenic activity similar to RU 486 (Figure 1).

Since ZK 230211 binds to AR and GR, the effects of this antiprogestin on AR- and GR-mediated transcription were investigated. The results were compared with those of RU 486, which is known to be a partial agonist at the AR and a potent antiglucocorticoid. As shown in Table 2, ZK 230211, like RU 486, is a partial agonist in the AR transactivation assay, exhibiting antiandrogenic activity and only marginal androgenic activity. In the GR transactivation assay ZK 230211 displayed low antiglucocorticoid activity and no glucocorticoid activity (Table 2). In comparison to the strong antiglucocorticoid action of RU 486, ZK 230211 showed about 10 times less antiglucocorticoid potency. Although ZK 230211 did not show any detectable binding to ER, an ER transactivation assay was carried out to definitely exclude any estrogenic or antiestrogenic activity. As expected ZK 230211 showed neither estrogenic nor antiestrogenic activity (Table 2). Taken together, these in vitro results indicate that ZK 230211 is a highly potent PR antagonist exhibiting partial agonistic activity at the AR with a strong focus on the antiandrogenic side, very low antiglucocorticoid activity, and no activity at the ER.

Transactivation Behavior of ZK 230211-Liganded PR in the Presence of 8-Br-cAMP. Several years

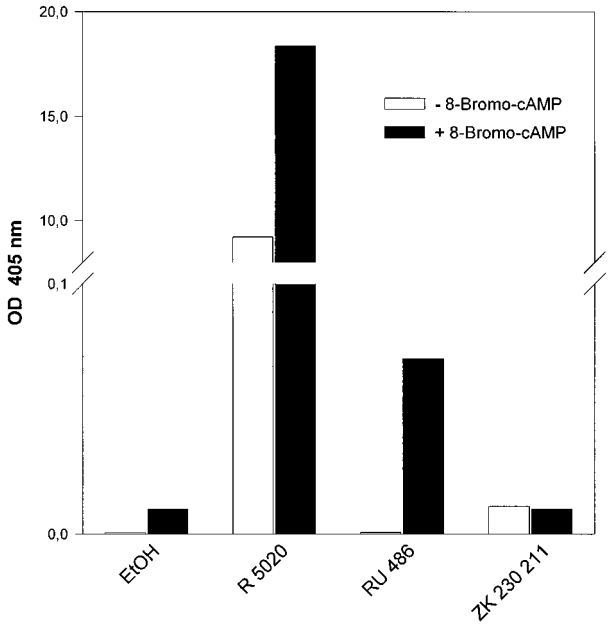

Figure 1. ZK 230211-bound PR does not show agonistic activity upon 8-Br-cAMP treatment. T47D cells stably transfected with MMTV-CAT were cultured in the absence (EtOH control) or presence of 1 μM/L R5020, 1 μM/L RU 486, and 1 μM/L ZK 230211, respectively, either without 8-Br-cAMP (white bars) or with 8-Br-cAMP (black bars). After 48 h cells were harvested and duplicate dishes were assayed for the amount of CAT enzyme with a CAT ELISA.

ago it was shown that the antiprogestin RU 486 can become an agonist in vitro in the presence of protein kinase A (PKA) activators such as cyclic adenosine monophosphate (cAMP).[30] Therefore, a transactivation assay in PR-positive T47D cells was carried out with 8-Br-cAMP to test the behavior of ZK 230211-bound PR in the presence of PKA activators. As reported in the literature, the positive control R5020 showed stronger transactivation levels in the presence of 8-Br-cAMP compared to transactivation levels in the absence of this PKA activator.[30] As expected RU 486-bound PR showed a switch to agonistic activity (Figure 1). In contrast to RU 486, ZK 230211-bound PR did not show this switch to agonistic activity (Figure 1). These results show that ZK 230211 is a pure PR antagonist.

Antiprogestagenic Activities in Vivo. The antiprogestagenic activity of ZK 230211 in vivo was assessed in juvenile estradiol/progesterone-stimulated rabbits. In this assay, ZK 230211 showed almost complete antagonism of the progesterone-induced differentiation of the endometrial glands using a dose of 0.3 mg/kg/day po and a maximal effect at a dose of 3 mg/kg/day po (Figure 2). In a separate experiment, RU 486 was tested using three doses (1, 3, and 10 mg/kg po). Only the highest dose of 10 mg/kg displayed a full antagonistic effect, whereas the lower doses showed only slight antiprogestagenic effects (data not shown). The ED_{50} values were ~4 mg/kg for RU 486 and about 0.3 mg/kg for ZK 230211. Thus, in this model, ZK 230211 was about 10 times more potent than RU 486. By analyzing the intrinsic progestagenic activity of ZK 230211, no effect was found up to a dose of 3 mg/kg/day po (Figure 2). These findings are in good agreement with the results of the transactivation assays carried out with PR-A and PR-B.

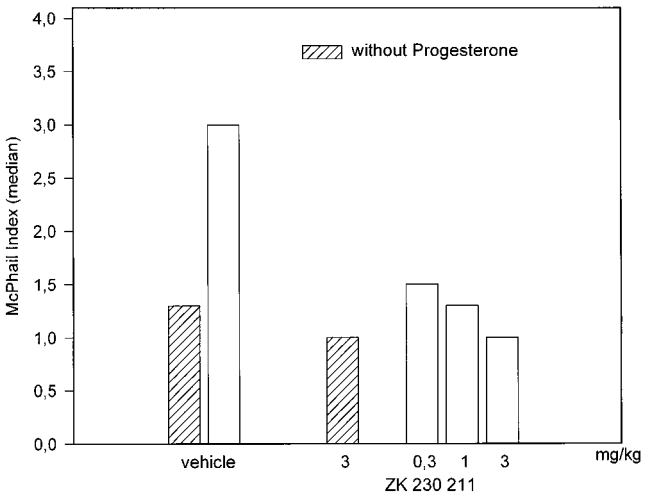

Figure 2. Antiprogestagenic and progestagenic activity of ZK 230211 after oral application in rabbits. This experiment was performed in juvenile female rabbits. From days 1−4 rabbits were primed with 5.0 μg/kg/day 17β-estradiol sc. From days 7−10 the test compound was applied po. The study groups in which progesterone was used as an inducer of endometrial differentiation received 0.2 mg/kg/day progesterone sc. As a negative control served a group which received only the vehicle. Controls received either vehicle or progesterone only. To study the progestagenic activity of ZK 230211, one treatment group received the antiprogestin (3 mg/kg/day) only. The McPhail index (degree of differentiation, scores: 1−4; 1 = no differentiation, 4 = maximal differentiation) served as the parameter for progestagenic and antiprogestagenic (inhibition of progesterone-induced endometrial differentiation) activity.

Table 3. Interruption of Early Pregnancy in Rats

compd	dose (mg/animal/day)	rate of abortion (%)[a] complete	incomplete
vehicle		0	0
ZK 230211	0.03	0	0
ZK 230211	0.1	83	17
ZK 230211	0.3	100	0

[a] Animals were treated with ZK 230211 sc from days 5−7 pc. Autopsy was performed on day 9. The absence of implantation sites was assessed as complete abortion, the presence of regressed and/or hemorrhagic implantation sites (pathological nidation sites) as incomplete abortion. RU 486 was fully effective only with a dose of 3 mg/animal/day sc.

Progesterone is essential for all phases of mammalian pregnancy. Therefore, we analyzed the potency of ZK 230211 to interrupt early stages of gestation in rats. Using various doses, ranging from 0.03 to 0.3 mg/animal/day, a full abortifacient effect was obtained with doses of 0.1 and 0.3 mg/animal/day administered sc, whereas the lower dose of 0.03 mg/animal/day sc did not result in termination of pregnancy (Table 3). RU 486 had a full effect only after treatment with 3 mg/animal/day sc (data not shown). Thus, in this in vivo assay ZK 230211 showed 10 times higher potency than RU 486.

Other Hormonal Activities in Vivo. To analyze the antiglucocorticoid activity of ZK 230211 in vivo, its ability of reversing the thymus involution induced by glucocorticoids (e.g., dexamethasone) was evaluated. ZK 230211 exhibited antiglucocorticoid activity in rats with an ED_{50} of 2 mg/animal/day and RU 486 with 1.1 mg/animal/day administered po (Table 4). Considering the 10 times higher antiprogestagenic activity in vivo,

Table 4. Antiglucocorticoid and Antiandrogenic Activity of ZK 230211 and RU 486 After Oral Application in Adrenalectomized or Orchidectomized Young Male Rats

	ED_{50} (mg/animal/day)[a]	
compd	antiglucocorticoid activity	antiandrogenic activity
ZK 230211	2.0	1.3
RU 486	1.1	1.6

[a] ED_{50} values were calculated from dose−response curves.

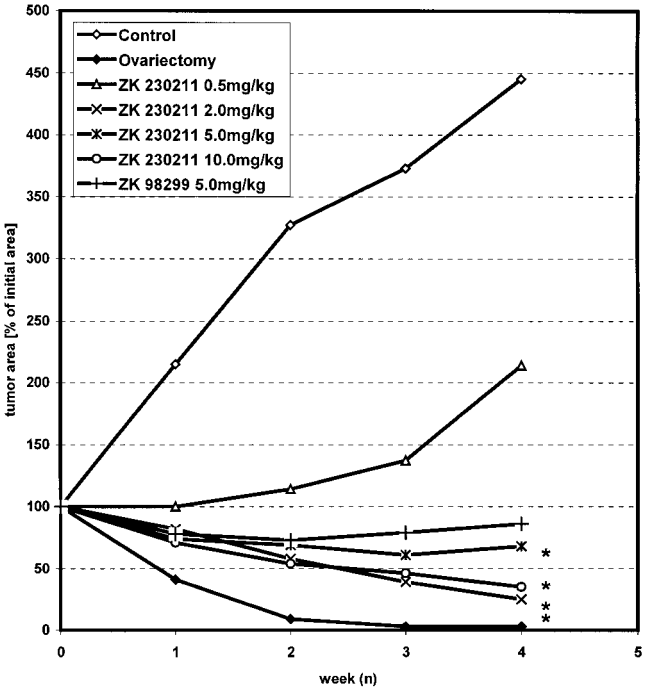

Figure 3. Antitumor effect of ZK 230211 and onapristone (ZK 98299) in the DMBA-induced mammary tumor model in the rat. Mammary tumors were induced by a single oral administration of 10 mg of DMBA. Rats with at least one established tumor were treated for 4 weeks, and the tumor growth was measured. *Statistical significant difference ($p < 0.05$) among control and experimental therapy groups, Kruskal−Wallis statistical analysis based on tumor weights at the end of the experiment.

ZK 230211 is an antiprogestin with a markedly reduced antiglucocorticoid activity compared to RU 486.

The androgenic and antiandrogenic activity of ZK 230211 was investigated in castrated male rats. In this assay neither ZK 230211 nor RU 486 showed androgenic activity (data not shown). ZK 230211 exhibited an antiandrogenic activity with an ED_{50} value of 1.3 mg/animal/day (Table 4). The ED_{50} value for RU 486 was 1.6 mg/animal/day (Table 4). In conclusion, ZK 230211 is an antiprogestin devoid of any androgenic activity in vivo and considerable antiandrogenic activity.

The intrinsic estrogenic activity of ZK 230211 was analyzed in ovariectomized rats. No amplification of uterine weight and luminal epithelial height, the classical estrogenic parameters, was observed when a high dose of 10 mg/animal/day was used (data not shown). These data indicate that ZK 230211 has no intrinsic estrogenic activity in vivo which is consistent with the results of the in vitro studies.

Antitumor Activity. The antitumor activity of ZK 230211 was analyzed in rats with DMBA-induced mammary tumors. In control animals, progressive tumor growth was observed, whereas ovariectomy caused a complete tumor regression in 90% of the animals. A dose of 0.5 mg/kg administered sc led to an inhibition of tumor growth, although this was not statistically significant. A maximal statistically significant growth inhibition was achieved with doses ≥ 2 mg/kg (Figure 3). In the group treated with 2 mg/kg, 50% of the animals showed a complete tumor regression. Higher doses of ZK 230211 (5 and 10 mg/kg) revealed a tumor growth inhibition comparable to the dose of 2 mg/kg. Onapristone, a pure PR antagonist, also showed a growth inhibitory effect but with no statistical significance at the dose used (5 mg/kg). Taken together, in the DMBA-induced mammary tumor model in the rat, ZK 230211 completely suppressed tumor growth in intact animals and was more potent than onapristone.

Conclusion

Our results clearly demonstrate that ZK 230211 is a very potent and pure PR antagonist which is highly receptor-selective. In contrast to RU 486, ZK 230211 does not induce agonistic activity of PR in the presence of PKA activators. Furthermore, we were successful in obtaining a compound which has only low antiglucocorticoid properties. In addition, ZK 230211 shows antiproliferative activity in tumor models. This new compound has considerable potential in estrogen replacement therapy and in the treatment of endometriosis, leiomyomas, and breast cancer.

Experimental Section

Chemical Methods. Melting points were determined on a Mettler FP 62 melting point apparatus and are uncorrected. Compounds were recrystallized from the solvent indicated in parentheses. Optical rotations were determined on a Perkin-Elmer polarimeter 241. IR spectra were recorded on a Bruker FT-IFS 25 spectrometer. Mass spectra were measured with a Fisons Instruments VG 70-70 E spectrometer at 70 eV ionizing voltage (EI). ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300 (300 MHz/75 MHz), and δ values are given in ppm relative to tetramethylsilane as internal standard (J values in Hz). Microanalyses were provided by Schering analytical department and are within ±0.4% of the theoretical values. TLC analyses were performed on Merck F_{254} silica gel plates. Spots were visualized by soaking plates with a diethyl ether solution containing vanillin (0.25%) and sulfuric acid (5%) and heating by means of a heat gun. Column chromatography was carried out on Merck silica gel 60, 70−230 mesh, using ethyl acetate/hexane as eluent. Reactions were run under nitrogen atmosphere. Solvents were reagent grade and dried prior to use.

5,17α-Dihydroxy-17α-(1,1,2,2,2-pentafluoroethyl)-11β-[4-(2,5,5-trimethyl-1,3-dioxan-2-yl)phenyl]-5α-estr-9-en-3-one Cyclic 2,2-Dimethylpropane-1,3-diyl Acetal (4). At −30 °C, 1,1,1,2,2-pentafluoro-2-iodoethane (1.9 mL, 16 mmol) was condensed. A solution of 5-hydroxy-11β-[4-(2,5,5-trimethyl-1,3-dioxan-2-yl)phenyl]-5α-estr-9-ene-3,17-dione cyclic 3-(2,2-dimethylpropane-1,3-diyl acetal) (**3**; 1.08 g, 1.87 mmol) in diethyl ether (19 mL) was added at −78 °C. A 1.5 M solution of methyllithium−lithium bromide complex in diethyl ether (8.7 mL, 13 mmol) was added slowly, keeping the internal temperature below −65 °C. The reaction mixture was stirred for 1 h at −78 °C. The reaction mixture was then poured into saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate. The organic portions were combined, washed with brine, dried over sodium sulfate, filtered, and evaporated. Chromatography of the residue over silica gel using hexane/ethyl acetate yielded 644 mg (0.92

mmol, 49%) of the pentafluoroethyl adduct **4** as a colorless foam: mp 242.7 °C (ethyl acetate); $[\alpha]_D = +2.8°$ ($CHCl_3$; c = 0.490); MS (m/z) 698 (M^+, 2%), 680 ($M^+ - H_2O$, 48%), 129 (100%); ^{1}H NMR ($CDCl_3$) δ 7.29 (d, 2H, J = 9, aryl), 7.23 (d, 2H, J = 9, aryl), 4.42 (s, 1H, 5-OH), 4.35 (br d, 1H, J = 7, H-11), 3.58 (br d, 1H, J = 11.4, 3-acetal), 3.54 (br d, 1H, J = 11.4, 3-acetal), 3.52 (br d, 1H, J = 11.2, 3-acetal), 3.47 (br d, 1H, J = 11.2, 3-acetal), 3.38 (m, 4H, aryl acetal), 1.52 (s, 3H, aryl acetal), 1.26 (s, 3H, aryl acetal), 1.04 (s, 3H, 3-acetal), 0.89 (s, 3H, 3-acetal), 0.57 (s, 3H, aryl acetal), 0.51 (s, 3H, H-18). Anal. ($C_{38}H_{51}F_5O_6$) C, H, F.

11β-(4-Acetylphenyl)-17β-hydroxy-17α-(1,1,2,2,2-pentafluoroethyl)estra-4,9-dien-3-one (ZK 230211, 2). 50% aqueous sulfuric acid (0.4 mL) was added to a solution of 5,17β-dihydroxy-17α-(1,1,2,2,2-pentafluoroethyl)-11β-[4-(2,5,5-trimethyl-1,3-dioxan-2-yl)phenyl]-5α-estr-9-en-3-one cyclic 2,2-dimethylpropane-1,3-diyl acetal (**4**; 635 mg, 0.91 mmol) in methanol (9 mL). The reaction mixture was stirred at room temperature for 2 h. It was then cautiously poured into saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate. The organic portions were combined, washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Chromatography of the residue over silica gel using hexane/ethyl acetate afforded 428 mg of **2** (0.84 mmol, 92%) as a colorless foam: mp 260.4 °C (diisopropyl ether); $[\alpha]_D = +181.3°$ ($CHCl_3$; c = 0.535); IR (KBr, cm^{-1}) 1680 s (C=O), 1665 s (C=O); MS (m/z) 508 (M^+, 60%), 91 (100%); ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 2H, J = 9, aryl), 7.31 (d, 2H, J = 9, aryl), 5.81 (s, 1H, H-4), 4.50 (br d, 1H, J = 7, H-11), 2.59 (s, 3H, acetyl), 0.58 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 199.2 (C-3), 197.8 (acetyl), 155.9 (C-5), 150.4 (aryl), 143.3 (C-9), 135.0 (aryl), 130.3 (C-10), 128.8 (2C, aryl)), 127.1 (2C, aryl), 123.5 (C-4), 119.5 (CF_3), 117.2 (tq, J = 293, 35, CF_2), 84.1 (t, J = 23, C-17), 51.6 (C-14), 50.5 (C-13), 41.0 (C-11), 39.3 (C-8), 38.8 (C-12), 36.7 (C-2), 33.2 (C-16), 31.0 (C-6), 27.7 (C-7), 26.4 (acetyl), 25.8 (C-15), 25.0 (C-1), 16.6 (C-18). Anal. ($C_{28}H_{29}F_5O_3$) C, H, F.

Receptor Binding Assays. The preparation of rabbit PR and rat AR, GR, and ER and the competition experiments were carried out as described previously.[31] The reference compounds were progesterone for PR, R1881 for AR, dexamethasone for GR, and estradiol for ER.

Transactivation Assays. All transactivation experiments were carried out in cell lines stably expressing the respective steroid receptor and a reporter gene linked to a hormone-responsive promoter. Steroid receptor-free SK-NM-C cells (human neuroblastoma cells) stably expressing either the human PR-A or PR-B and the mouse mammary tumor virus promoter linked to the LUC reporter gene and steroid receptor-free CV-1 cells (green monkey kidney cells) stably transfected with the rat AR and a MMTV-LUC reporter gene were constructed as described in Fuhrmann et al.[32] NIH 3T3 cells with endogenous GR and stably transfected MMTV-CAT were a generous gift of A. Cato (Karlsruhe Research Center, Germany). MVLN cells (human breast adenocarcinoma cells) with endogenous ER and stably transfected MMTV-LUC were kindly provided by M. Pons (Karlsruhe Research Center). To study the effect of hormones, the stable cell lines were seeded onto 96-well dishes and cultured in medium supplemented with 3% charcoal stripped FCS. After 48 h hormones were added and incubation was continued for 24 h. To determine agonistic activity, cells were cultured in the presence of ZK 230211 or RU 486. LUC expression is given as normalized response value relative to the maximal LUC expression produced by a reference agonist: e.g., R5020 for PR, R1881 for AR, dexamethasone for GR, and estradiol for ER. As a negative control for reporter gene induction, cells were cultured in 1% ethanol. For the determination of antagonistic activity, cells were treated with the respective reference compound and, in addition, with increasing amounts of ZK 230211 or RU 486. For the determination of agonistic activity of RU 486 and ZK 230211 in the presence of 8-Br-cAMP, PR-positive T47D cells stably transfected with MMTV-CAT were treated with the respective compounds with and without 1.0 mM 8-Br-cAMP. After 24 h a luciferase or CAT assay was carried out. Transactivation assays were carried out at least three times. In Table 2 and Figure 2 data from one representative experiment are shown.

In Vivo Assays. All animals experiment were carried out according to German animal protection laws. Animals were kept under conventional housing and feeding conditions. The vehicles for po and sc administration were 0.085% Myrj/0.9% NaCl (w/v) and benzyl benzoate/castor oil (1:5, v/v), respectively.

Antiprogestagenic Effects on Rabbit Endometrium. This experiment was performed in juvenile female rabbits (New Zealand white, 30−35 days old; Schriever Germany). From days 1−4 all rabbits were primed with 5.0 μg/kg/day 17β-estradiol (sc, 0.5 mL/kg/day) to induce proliferation of the endometrium. From days 7−10 the test compound was applied orally (po, 0.5 mL/kg/day) at doses of 0.3, 1, and 3 mg/kg/day. The study groups in which progesterone was used as an inducer of endometrial differentiation received 0.2 mg/kg/day progesterone (sc, 0.25 mL/kg/day). A group which received only vehicle after estradiol priming served as negative control. A second group which received only progesterone to induce endometrial differentiation after estradiol priming was used as positive control. To study the progestagenic activity of ZK 230211, one treatment group received after estradiol priming antiprogestin only. Autopsy was performed on day 11. As a parameter for progestagenic and antiprogestagenic (inhibition of progesterone-induced endometrial differentiation) activity the McPhail index (degree of differentiation) was determined by two independent investigators using a light microscope (scores: 1−4; 1 = no glandular differentiation, 4 = maximal differentiation).

Interruption of Early Pregnancy in Rats. In this experiment pregnant Wistar rats (200−220 g, day 5−7 post-coitum pc, 4−6 rats/group; Schering) were used. After successful mating, pregnant animals (presence of sperms in vaginal smears on day 1 of pregnancy = day 1 pc) were randomized to treatment or control groups. The animals received the test compound or vehicle sc from days 5−7 pc; autopsy was performed on day 9 pc. As a parameter for the progesterone antagonistic activity the uteri were inspected during autopsy. The absence of implantation sites was assessed as complete abortion, the presence of regressed and/or hemorrhagic implantation sites (pathological nidation sites) as incomplete abortion. The doses tested were 0.03, 0.1, and 0.3 mg/animal/day.

Antiglucocorticoid Activity in Rats. Male Wistar rats (100−150 g; Schering and Moellegard Breeding Center Ltd., Ejby, Denmark; experiment with RU 486) were adrenalectomized under slight ether anaesthesia at day 1 of the experiment. The rats were randomly allocated to treatment or vehicle control groups. The animals were treated sc for 4 days with 0.01 mg dexamethasone and po with the test compound ZK 230211 (1, 3, and 10 mg/animal/day; n = 5 animals/group) and the reference compound RU 486 (1, 3, 10, and 30 mg/animal/day; n = 7 animals/group). Two control groups were used. One control group was treated with the vehicle only, and the second control group received dexamethasone (0.01 mg/animal/day) and the vehicle of the test compound. The autopsy was performed 24 h after the last application (day 5 of experiment). During autopsy the thymus was removed and its relative wet weight was determined (mg thymus/100 g body weight). Treatment with 0.01 mg dexamethasone alone reduces the thymus weight by about 75% in comparison to the vehicle control group. Concomitant treatment with a compound having antiglucocorticoid activity reverses this effect. The antiglucocorticoid activity of the test compounds was expressed as the ED_{50} in mg/animal/day which was calculated from a dose−response curve where the reduction of the thymus weight induced by dexamethasone was taken as the bottom line.

Androgenic and Antiandrogenic Activity in Rats. Juvenile male Wistar rats (70−90 g; Schering) were anesthetized with ether, orchidectomized at day 1, and randomized to treatment or control groups. In the assay to test the

androgenic activity, the animals received ZK 230211 at doses of 1 and 3 mg/animal/day po for 7 days beginning at day 7 after orchidectomy. The control groups were vehicle only and testosterone propionate groups. Testosterone propionate was given at a dose of 0.1 mg/animal sc (0.2 mL/animal). At day 14 after orchidectomy the animals were sacrificed with CO_2 and the prostate and the musculus levator ani were prepared. To test the antiandrogenic activity ZK 230211 (0.1, 0.3, 1, and 3 mg/animal/day po) was given together with testosterone propionate (0.1 mg/animal sc) and instead of the musculus levator ani seminal vesicles were prepared. The antiandrogenic effect on prostate weight (the same was seen in seminal vesicles) was used as the basis for determination of the ED_{50} which was calculated from a dose−response curve.

Estrogenic Activity in Rats. Female Wistar rats (∼200 g; Schering) were anesthetized with ether, ovariectomized at day 1, and randomized to treatment or control groups. At day 11 after ovariectomy ZK 230211 was administered for 3 days po (0.5 mL/animal) using a dose of 10 mg/animal/day. Groups which received only vehicle or estradiol served as controls. Estradiol was given at a dose of 0.3 μg/animal sc for 3 days. This is sufficient for a maximal stimulation of uterine proliferation. During substance application the vaginal smears were controlled and assayed. At day 14 after ovariectomy the animals were sacrificed with CO_2 and the uteri were prepared. Increases in uterine weight and luminal epithelial height and the status of cell proliferation and keratinization of the vaginal smear served as parameters for estrogenic activity. The stimulation of the uterine weight and the luminal epithelial height was calculated as follows: $[(\text{mean weight}_{\text{test compound}} - \text{mean weight}_{\text{vehicle control}})/(\text{mean weight}_{\text{reference compound}} - \text{mean weight}_{\text{vehicle control}})] \times 100\%$.

Tumor Models. Immature female Sprague−Dawley rats (49−51 days old; 10 animals/group) were used in this study. Mammary tumors were induced by a single oral administration of 10 mg of 7,12-dimethylbenz[*a*]anthracene (DMBA; Serva/Heidelberg). Rats with at least one established tumor with a size of more than 150 mm^2 were treated for 4 weeks by: solvent control, ovariectomy (at treatment start), ZK 230211 (0.5, 2, 5, and 10 mg/kg sc, daily), and onapristone (ZK 98299; 5 mg/kg sc, daily). As parameter for tumor growth the percent change of tumor area was determined. At treatment start the total area of all tumors in each animal was termed 100%. Changes in tumor area were calculated in terms of this value. Tumor area was measured once weekly and is the product of the longest diameter and its perpendicular.

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