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Endocrinological properties of two novel nonsteroidal progesterone receptor modulators, CP8816 and CP8863

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ABSTRACT

We have isolated PF1092A, B and C, novel nonsteroidal progesterone ligands with preferential affinity for the progesterone receptor, from fermentation broth of a fungus (Tabata et al., 1997a and 1997b). The original skeleton of PF1092, tetrahydronapthofuranone, was modified synthetically to produce a new skeleton, tetrahydrobenzindrone, and in the present study, biological activities of two derivatives, CP8816 and CP8863, were investigated. Both CP8816 and CP8863 demonstrated selective binding to progesterone receptor and partial agonistic activity in a progesterone-dependent endogenous alkaline phosphatase expression assay. In the Clauberg-McPhail test, progestational activity of CP8816 (0.1 mg/kg, s.c. or 10 mg/kg, p.o.) was comparable to that of progesterone (0.15 mg/kg, s.c.) and oral administration of CP8863 at more than 1.0 mg/kg also exerted similar effects. Anti-estrogenic (antiuterotropic) activity was confirmed on daily oral application of more than 0.1 mg/kg of CP8863 for 3 days by inhibition of estrogen-dependent uterine wet weight gain in ovariectomized rats. CP8816 also exerted antiuterotropic activity at doses of 10 mg/kg (s.c.) and 100 mg/kg (p.o.). These results indicate that our nonsteroidal progesterone ligands have affinity for the progesterone receptor with partial progestational activity *in vitro* and clear progestational effects *in vivo*. Thus these progesterone receptor modulator profiles suggest that CP8863 and CP8816 are good candidate compounds for treatment of hormone dependent gynecological disorders.

Introduction

Progesterone, as well as estrogen, is an essential ovarian steroid hormone playing important roles in female reproductive processes, including the menstrual cycle and maintaining pregnancy (Csapo et al., 1969). Synthetic steroidal progesterone receptor modulators (PRM: agonists, antagonists or mixed agonists and antagonists) have been widely used, alone or in combination with estrogen, in the gynecological field for female contraception, hormone replacement therapy, new fertility controls, as well as for treatment of hormone dependent cancer, endometriosis and/or uterine adenomyosis (Spitz and Chwalize, 2000). Crystallographic analyses of the ligand binding domains of the nuclear receptor have allowed structure based drug design and have generated renewed interest in the development of new PRM with high selectivity for the progesterone receptor .

A series of novel nonsteroidal progesterone receptor ligands, PF1092A, B and C, possessing a tetrahydronaphthofuranone structure, have been discovered in fermentation broth of a rare fungus, *Penicillium oblatum*. Among these, PF1092A showed the highest binding affinity for progesterone receptor in porcine uteri cytosol preparations, with an IC_{50} of 30 nM (Tabata et al., 1997a) and some derivatives demonstrated partial agonistic activities *in vitro* assays (Tabata et al., 2002). We modified synthetically the original skeleton of a tetrahydronaphthofuranone by a few steps to produce new analogue tetrahydrobenzindolone type compounds with agonistic and antagonistic activity *in vivo*. (Tabata et al., 2002 & 2003).

In this report, we describe biological activities of two new nonsteroidal progesterone receptor ligands, the tetrahydrobenzindolone derivatives CP8863 and

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CP8816 (Fig.1). Both exhibit preferential affinity for progesterone receptor in comparison to other steroid hormone receptors and display partial agonistic activity in alkaline phosphatase expression assays *in vitro* and progestational effects in rabbit uterus and antiuteotrophic (antiestrogenic) activity in rats.

Materials and Methods

Chemicals.

CP8816, (4aR,5R,6R,7R)- 6-(N,N-dimethylaminocarbonyl) oxy-7-methoxy-4a,5,6,7- tetrahydro-1,3,4a,5- tetramethylbenz[f]indol- 2(4H)-one, and CP8863, (4aR,5R,6R,7R)-7-hydroxy-6-(N-methylcarbamoyl)oxy- 4a,5,6,7- tetrahydro- 1,3,4a,5-tetramethylbenz[f]indol-2(4H)-one, were synthesized from PF1092C in our laboratory. Their structures are shown in Fig.1. The purity of both compounds was 99 % by HPLC analysis. Progesterone for *in vitro* and *in vivo* tests was obtained from Junsei Chemical (Tokyo, Japan) and Teikoku Hormone (Tokyo, Japan), respectively. Testosterone, 17β-estradiol and medroxyprogesterone acetate (MPA) were purchased from Wako (Osaka, Japan), dexamethasone from Sigma (St. Louis, Mo.), promegestone (R5020) [1,2,6,7-^{3}H(N)]-progesterone (specific activity: 3589 GBq/mmol), [17α-methyl-^{3}H]-mibolerone (specific activity: 3145 GBq/mmol), and [2,4,6,7,-^{3}H(N)]-estradiol (specific activity: 2664 GBq/mmol) from NEN (Boston, MA), and [1,2,4-^{3}H]-dexamethasone (specific activity: 1590 GBq/mmol) from Amersham (Little Chalfont, England)

Cell cultures.

T47D human breast carcinoma cells, IM-9 human lymphoma cells, and MCF-7 human breast carcinoma cells were purchased from the American Type Culture Collection (ATCC). The T47D and MCF-7 lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL) and the IM-9 cells in RPMI 1640, containing 10 % fetal bovine serum. All cell lines were cultured at 37 ºC with 5 % CO_2.

Receptor binding assays.

Each compound was dissolved in DMSO at a concentration of 10 mM. The solutions were diluted with methanol and distilled water to appropriate concentrations for assays with a final concentration of methanol in the assay buffer of less than 0.5 %. The steroid receptor binding assay methods were as described previously (Tabata et al., 2001). In brief, cytosol containing steroid receptors was prepared from different cell lines or organs of various species (human progesterone receptor from T47D, rat androgen receptor from rat prostate, human glucocorticoid receptor from IM9 and human estrogen receptor from MCF7). Experimental procedures for rat androgen receptor were in accordance with the Guidelines for Animal Experimentation approved by the Pharmaceutical Research Center of Meiji Seika Kaisha. Cells and organs were homogenized in appropriate preservation buffer and centrifuged at 100,000xg for 30 min. The resulting supernatant (cytosol) was stored at -80 ℃ until use. Aliquots were then incubated with radioligands for appropriate incubation times: ([1,2,6,7, -^{3}H(N)]-progesterone for 1 h for the human progesterone receptor; [17α-methyl-^{3}H]- mibolerone for 24 h for the rat AR; [1,2,4-^{3}H]- dexamethasone for 24 h for the human

glucocorticoid receptor; and [2,4,6,7-^{3}H(N)]-estradiol for 20 h for the estrogen receptor). At the end of the incubation period, cold dextran-coated charcoal solution was added and following incubation at 4 ℃ for 10 min and centrifugation at 5,000 x g, the radioactivity of the supernatant was determined with Aquasol-2 (Perkin-Elmer, Boston, MA) using a liquid scintillation counter LS6500 (Beckman). Non-specific binding was defined as that observed when 10 μM of corresponding non-labelled steroid was added to the reaction mixture. Experimental conditions for receptor binding assays were summarized in Table 1.

Progesterone-dependent endogenous alkaline phosphatase expression assay.

Each compound was dissolved in DMSO at a concentration of 10 mM, and then 50 % aqueous methanol was added to give a 100 μM concentration. The resultant solutions were then diluted to appropriate concentrations in PBS (-) and added to the assay medium. The final concentration of methanol in the assay medium was less than 0.5 %.

Progesterone-dependent modulation of alkaline phosphatase expression was examined using T47D cells as previously described (Tabata et al. 2001). In brief, growth medium was replaced with phenol red-free DMEM containing 5 % fetal bovine serum treated with dextran-coated charcoal. After 24 h of cultivation, the cells were plated in 96-well plates at 25,000 cells/well. After 24 h of cultivation, a test compound was added to each well at an appropriate concentration. After 20 h cultivation, the medium was removed and the cells were washed with 200 μl of PBS(-). The plates were kept at –80 ℃ for 15 min, and then thawed at room temperature. This freeze-and-thaw

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cycle was repeated once again. After 5 min incubation at room temperature, 50 µl of PBS(-) and 30 µl of CSPD™ chemiluminescent substrate solution (Great EscApe™ SEAP Detection kit, Clontech) was added to each well with agitation. After 30 min of incubation at room temperature, luminescence was measured with an ARVO luminometer (Perkin-Elmer).

Progestational activity in estrogen-primed immature rabbits.

The experimental procedure for the rabbit endometrial transformation test complied with the Guidelines for Animal Experimentation approved by the Pharmaceutical Research Center of Meiji Seika Kaisha.

CP8816: The aim of this assay was to determine which dose route is superior for agonist activity of this compound, either s.c. or p.o.. CP8816 was dissolved in miglyol oil (MIGLYOL® 812 Neutral Oil, Mitsuba Trading, Tokyo, Japan) at 100 ℃ with stirring to obtain a clear solution, then cooled to room temperature. The final concentration was 10 mg/ml. Immature female rabbits, weighing 800- 1100 g were used at 3 animals per group. All rabbits were injected subcutaneously with 17β-estradiol (estrogen: 5 μ g/kg/day) dissolved in sesame oil for six days and then treated with 0 (vehicle: miglyol oil), 0.1, 1.0 or 10 mg/kg/day (s.c.), or 1.0 or 10 mg/kg/day (p.o.) of CP8816, dissolved in miglyol oil, or 0.15 mg/kg/day (s.c.) of progesterone, dissolved in sesame oil, for 5 days. Rabbits were sacrificed on the day after the last administration of test compound or progesterone. The uteri were excised, weighed, fixed in buffered formalin and cut into 2-3 mm sections with a razor blade. Six transverse sections, three each (proximal, medial and distal parts) from the right and left uterine horns, were

prepared, stained with haematoxylin and eosin, and examined histologically by microscopic observation. The grade of endometrial transformation as a progestational effect was recorded according to the method described previously (McPhail, 1934).

CP8863: We estimated that this compound is more stable *in vivo* than CP8816. Therefore determination not only of agonist but also antagonist activity was performed.

CP8863 was dissolved using the same method as for CP8816. The final concentration was 10 mg/ml. Immature female rabbits, weighing 800-1040 g were used at 5 animals per group. They were injected subcutaneously with estrogen (5 μ g/kg/day) dissolved in sesame oil for six days and then treated with 0 (vehicle: miglyol oil), 0.04, 0.2, 1.0 or 5.0 mg/kg/day (p.o.) of CP8863 for 5 days (agonist format). In a separate experiment (antagonist format), estrogen-primed rabbits were administered the test compounds at the same doses as for agonist format in combination with progesterone (0.15 mg/kg/day, dissolved in sesame oil) for 5 days. The uteri were collected on the day following the last administration of test compounds or progesterone and prepared for histological examination as described in the assay of CP8816.

Antiuterotrophic effects in estrogen-primed ovariectomized rats.

The experimental procedure for the rat antiuterotrophic test complied with the Guidelines for Animal Experimentation approved by the Pharmaceutical Research Center of Meiji Seika Kaisha. This assay was used to detect antiuterotrophic (anti-estrogenic) activity of CP compounds in estrogen-primed ovariectomized rats.

CP8816: Three or four-week-old female SD rats for the s.c. and p.o dosing studies were ovariectomized bilaterally under pentobarbital sodium anesthesia. One week

thereafter, the rats were divided into 11 groups. In the s.c. dosing study, rats in groups 1-4 (5 rats each) were subcutaneously injected with estrogen (2 μ g/kg/day) and simultaneously received daily s.c. administration of 0, 0.1, 1 and 10 mg/kg of CP8816 dissolved in miglyol oil respectively for three days. Rats in groups 5 (5 rats) were given 10 mg/kg of CP8816 without estrogen administration for evaluation of uterotrophic effects. Rats in group 6 (5 rats) were given vehicle (miglyol oil) alone. In the p.o. dosing study, animals in groups 7-10 (6 - 8 rats) were given orally 0, 1.0, 10 and 100 mg/kg CP8816 plus estrogen (2 μ g/kg/day, s.c.) for three days. Rats in group 11 were given 10 mg/kg MPA plus estrogen for three days as a positive control. We set a 10 times higher dose for the p.o. dosing study in consideration of the first pass effect *in vivo*. Autopsy was performed the day after the last administration. Uterine horns were excised and weighed.

CP8863: Four-week-old female SD rats were ovariectomized bilaterally under pentobarbital sodium anesthesia. One week thereafter, the rats were divided into 13 groups. Rats in groups 1-5(5 rats each) were subcutaneously injected with estrogen (2 μ g/kg/day) and simultaneously received daily p.o. administration of 0, 0.01, 0.1, 1.0 and 10 mg/kg of CP8863 dissolved in miglyol oil respectively, for three days. Rats in group 6 (6 rats) were given 10 mg/kg of CP8863 without estrogen administration, and served for assessment of uterotrophic effects. Rats in group 7 (6 rats) were given vehicle (miglyol oil) alone. Animals in groups 8-12 (5 rats each) were given orally MPA plus estrogen (s.c.) for an antiuterotrophic study at the same doses as in groups 1-5. Group 13 (6 rats) served for an uterotrophic study of 10 mg/kg MPA alone. Autopsy was performed the day after the last administration. Uterine horns

were excised and weighed.

Statistical analysis.

Statistical analysis of the mean values of uterine weights was performed using the unpaired t-test. *P* values less than 0.05 were considered significant.

Results

Binding affinities for steroid receptors.

The results for binding affinities of CP8816 and CP8863 for various steroid receptors are shown in Table 2. IC_{50} values of CP8816 and CP8863 for progesterone binding to human progesterone receptor were 25 ± 15 and 40 ± 7 nM, respectively. The IC_{50} of CP8816 for human progesterone receptor was comparable to values for other synthetic progesterone receptor agonist, MPA (13 ± 1 nM). Little cross reactivity of CP8863 or CP8816 was observed in the assays with other steroid receptors, such as rat androgen receptor, human glucocorticoid receptor and human estrogen receptor.

Progesterone-dependent alkaline phosphatase expression assay.

The results of the progesterone-dependent alkaline phosphatase expression assay using the T47D human breast cancer cell line are shown in Fig. 2 and Table 3. The EC_{50} value with CP8816 (1.6 ± 0.8 nM) was comparable to that for progesterone (1.6 ± 0.8 nM), while the value for CP8863 (13 ± 3 nM) was approximately ten times lower. The relative efficacies of CP8816 and CP8863 were 49 ± 3% and 57 ± 6%, respectively (mean ± S.D.) as percentages of the response induced by progesterone at 10 nM . Therefore, both were found to possess partial agonistic activity.

Progestational activity in estrogen-primed immature rabbits.

The results for progestational action of CP8816 (s.c. or p.o.) are presented in Table 4 and for the progestational (agonist format) or antiprogestational (antagonist format) action of CP8863 in Table 5.

Dose-related increase in McPhail units (endometrial transformation by progestational activity) was clearly demonstrated in the uteri of estrogen-primed rabbits treated with CP8816. Largest scores were observed at a dose of 1 mg/kg, s.c. or more, while rather weak progestational activity was noted after oral dosing with CP8816.

Oral administration of CP8863 in the agonist format induced dose-related progestational activity. The McPhail score (3.1 ± 0.1) at 5 mg/kg was comparable to that for progesterone (3.3 ± 0.3, 0.15 mg/kg, s.c.). In the antagonist format, anti-progestational effects of CP8863 vs 0.15 mg/kg progesterone were not detected at any of the doses used.

Antiuterotrophic effects in estrogen-primed ovariectomized rats.

Antiuterotrophic activities of CP8816 are shown in Table 6. Subcutaneous injection of 10 mg/kg CP8816 with estrogen pretreatment significantly decreased uterine wet weight gain when compared with values for the estrogen alone (group 1). However, CP8816 orally administered at a 10 times higher dose (100 mg/kg) was needed as compared with s.c administration.

Uterine wet weight gains for each group in the CP8863 are shown in Table 7. Uterotrophic effects were clear in group 1 rats treated with 2 μg/kg (s.c.) estrogen followed by vehicle (estrogen alone). Average uterine wet weight gains of rats

administrated orally with doses above 0.1 mg/kg of CP8863 plus estrogen were significantly lower than with estrogen alone. Antiuterotrophic effects of MPA were observed at doses more than 1 mg/kg. Uterotrophic effects were not observed in any of the groups treated with CP8863 alone. On the other hand, although oral doses of MPA (1, 10 mg/kg) inhibited uterine wet weight gain, uterotorophic effects were evident at the dose of 10 mg/kg, p.o.

Discussion

In the present studies, *in vitro* and *in vivo* assays revealed that CP8816 and CP8863 exert specific partial agonistic activity for progesterone receptor, with clear progestational effects in the estrogen-primed rabbit uterus. Furthermore, the treatment caused inhibition of uterine weight gain due to estrogen in ovariectomized rats.

In our previous study, CP8661, a tetrahydrobenzindolone type derivative, and CP8754, tetrahydronaphthofuranone type derivative, exerted antagonist activity and anti-progestational effects without an agonist in the rabbit assay (Tabata et al., 2002). In addition CP8668, a preliminary tetrahydrobenzindolone type derivative, exerted agonist effects in the rabbit assay (Tabata et al., 2003). Thus, the biological activities of our compounds range from agonist to antagonist with synthetic structural modification and their lead structures may have further potential for development of new nonsteroidal progesterone modulators. Furthermore, these compounds from two core structures, although showing different alkaline phosphatase expression *in vitro*, consistently demonstrate selective binding affinity for progesterone receptors. These endocrine features with a nonsteroidal structure may have great advantages for clinical use without side effects.

Antiuterotrophic activity, i.e. anti-estrogenic effects, is a key action for clinical benefit of PRM for hormone dependent diseases. Treatments with CP compounds in estrogen-primed ovariectomized rats have demonstrated inhibition of uterine wet weight gain. Progesterone inhibits proliferation in the estrogen-primed endometrium of ovariectomized mice, but reciprocally acts as a mitogen in stromal cells of the endometium (Tachi and Tachi, 1974; Moyer and Felix, 1998; Martin et al., 1971). We

have already reported that, CP8816 exerts anti-proliferative effects on both epithelial cells and stromal cells in estrogen-primed mice, while both CP8816 and CP8863 inhibit the development of adenomyosis induced experimentally by pituitary grafting (Mori et al., 2002). Thus, the mode of anti-proliferative action of CP8816 on the cell components of the endometrium differs from that of progesterone. In the current experiment, direct anti-estrogenic activity of CP8816 and CP8863 was also revealed in the assay of antiuterotrophic effects (Tables 6 and 7), while treatment with the derivatives alone did not induce any estrogenic action in the uterus of ovariectomized rats. Furthermore, CP8863 at high doses did not change the estrus cycle in treated rats (unpublished data). We confirmed that CP8816 and CP8863 might inhibit development of adenomyosis through direct antiestrogenic actions and partial agonist activity on the endometrium.

CP8863 showed good progestational activity in *in vivo* assays, though its in vitro activity was milder than that of CP8816. One of the causes is thought to be the metabolic stability of CP8863. On testing of metabolic stability with female rat and human liver S9 fractions, CP8863 proved more stable than CP8816 (unpublished data).

So far, two progesterone receptor isoforms have been reported, namely progesterone receptor-A (PR-A) and progesterone receptor-B (PR-B). PR-A is 94-kDA in size, whereas PR-B is a 114-kDa protein that contains an additional 164 amino acids at its amino terminal (Schrader et al., 1981). These isoforms are due to alternative sites in the same messenger RNA (mRNA) or transcription from alternative promoters (Kastner et al., 1990). PR-B tends to be a stronger activator of progesterone target

genes, whereas PR-A has been shown to act as a dominant repressor of PR-B and other steroid hormones receptors such as those for androgen, glucocorticoid and estrogen receptors (Vegeto et al., 1993). Progestins and antiprogestins have been shown to inhibit estrogen-stimulated uterine proliferation (Medlock et al., 1994). These anti-estrogenic effects could be due to a dominant negative influence through PR-A and/or essential progestational effects through PR-B. In the present study, the discrepancy between partial agonist activities of CP8863 in the alkaline phosphatase expression assay and full agonist potential in the estrogen primed-immature rabbits could not be explained. However, it may depend on variation in progesterone receptor isoform expression in different species. The relationship between modulation of transcription by progesterone isoforms and specific clinical applications remains to be elucidated. However, the present results show that they are good candidates as nonsteroidal PRM for treatment of gynecological disorders.

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Legends of Figures

Fig.1. Structures of CP8816 and CP8863.

Fig.2. Effects of CP8816 and CP8863 in the progesterone dependent alkaline phosphatase expression assay. The indicated concentrations of test compounds were added to T47D with incubation at 37 ºC for 20 h. Alkaline phosphatase expression was estimated by measuring luminescence. The results represent activity relative to 10 nM of progesterone.

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TABLE 1

Experimental conditions for receptor binding assays

Receptor	Cell (tissue)	Species	Ligand	Incubation
Progesterone receptor	T47D	Human	^{3}H-progesterone	1 hr/4 ºC
Androgen receptor	Prostate	Rat	^{3}H-mibolerone	24 hr/4 ºC
Glucocorticoid receptor	IM9	Human	^{3}H-dexamethasone	24 hr/4 ºC
Estrogen receptor	MCF7	Human	^{3}H-estradiol	20 hr/4 ºC

Cytosol was used as the receptor source.

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TABLE 2
Binding affinity of CP8816 and CP8863 for steroid receptors

	IC_{50} (nM)			
Test compounds	Progesterone receptor (human)	Androgen receptor (rat)	Glucocorticoid receptor (human)	Estrogen receptor (human)
CP8816	25 ± 15	990 ± 460	>5000	>10000
CP8863	40 ± 7	9700 ± 3000	>10000	>10000
MPA	13 ± 1	19 ± 2	43 ± 8	>10000
Testosterone		6.3 ± 1.0		
Dexamethasone			6.8 ± 0.9	
17β-Estradiol				0.83 ± 0.36

The results represent means ± S.D. (three independent experiments).

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TABLE 3

Effects of CP8816 and CP8863 on progesterone dependent alkaline phosphatase expression

Test compounds	EC_{50} (nM)	Relative efficacy (%)
CP8816	1.6 ± 0.8	49 ± 3
CP8863	13 ± 3	57 ± 6
Progesterone	1.6 ± 0.8	100
Promegestone	0.46 ± 0.22	80 ± 6
MPA	0.19 ± 0.07	79 ± 6

The results are means ± S.D. Relative efficacy was estimated as 100% at 10nM progesterone.

TABLE 4

Progestational activity of CP8816 in estrogen primed-immature rabbits

Test compounds	Dose (mg/kg)	No.of Rabbits	Route	McPhail Score (0-4) (means ± S.D.)
CP8816	0	3	s.c.	0.2 ± 0.4
	0.1	3	s.c.	3.1 ± 0.1
	1.0	3	s.c.	4.0 ± 0.0
	10	3	s.c.	4.0 ± 0.0
CP8816	1.0	3	p.o.	2.8 ± 0.3
	10	3	p.o..	3.2 ± 0.2
Progesterone	0.15	3	s.c.	3.2 ± 0.3

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TABLE 5

Progestational or antiprogestational activity of CP8863 in estrogen primed-immature rabbits

Test compounds	Dose (mg/kg)	No.of Rabbits	Route	McPhail Score (0-4) (means ± S.D.)
CP8863	0	5	p.o	0.1 ± 0.3
	0.04	5	p.o.	0.4 ± 0.3
	0.2	5	p.o.	0.8 ± 0.4
	1.0	5	p.o.	2.2 ± 0.4
	5.0	5	p.o.	3.1 ± 0.1
CP8863+0.15mg/kg Progesterone	0	5	p.o.	3.3 ± 0.4
	0.04	5	p.o.	3.3 ± 0.5
	0.2	5	p.o.	3.3 ± 0.4
	1.0	5	p.o.	3.4 ± 0.2
	5.0	5	p.o.	3.2 ± 0.3

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TABLE 6
Antiuterotrophic activity of CP8816 in ovariectomized rats

Group	Test compounds	Dose (mg/kg)	No. of rats	Route	Estrogen-Primed (2μg/kg)	Uterine Weight (mg) (means ± S.D.)
1	CP8816	0	5	s.c.	+	120.0 ± 6.0
2		0.1	5	s.c.	+	111.9 ± 14.6
3		1.0	5	s.c.	+	109.6 ± 12.5
4		10	5	s.c.	+	100.7 ± 7.5 [a)]
5		10	5	s.c.	—	46.3 ± 12.4
6		0	5	s.c.	—	53.6 ± 15.2
7	CP8816	0	6	p.o.	+	245.4 ± 30.0
8		1.0	7	p.o	+	202.7 ± 41.0
9		10	7	p.o	+	214.1 ± 64.0
10		100	6	p.o.	+	164.0 ± 32.4 [b)]
11	MPA	10	8	p.o.	+	145.4 ± 12.7 [b)]

a) Significantly different from group 1 (the estrogen alone) at $p<0.01$.
b) Significantly different from group 7 (the estrogen alone) at $p<0.001$.

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TABLE 7
Antiuterotrophic activity of CP8863 in ovariectomized rats

Group	Test compounds	Dose (mg/kg)	No. of rats	Route	Estrogen-Primed (2μg/kg)	Uterine Weight (mg) (means ± S.D.)
1	CP8863	0	5	p.o.	+	186.4 ± 23.5
2		0.01	5	p.o.	+	163.8 ± 13.0
3		0.1	5	p.o.	+	135.2 ± 20.1 [a)]
4		1.0	5	p.o.	+	148.1 ± 16.9 [a)]
5		10	5	p.o.	+	141.5 ± 10.9 [a)]
6		10	6	p.o.	—	54.5 ± 10.5
7		0	6	p.o.	—	40.3 ± 6.9
8	MPA	0	5	p.o.	+	174.4 ± 21.6
9		0.01	5	p.o.	+	165.9 ± 38.8
10		0.1	5	p.o.	+	144.4 ± 7.7
11		1.0	5	p.o.	+	95.0 ± 10.6 [a)]
12		10	5	p.o.	+	121.7 ± 10.8 [a)]
13		10	6	p.o.	—	85.6 ± 45.1 [b)]

a) Significantly different from group 1 (the estrogen alone) at $p<0.01$.
b) Significantly different from group 7 (the vehicle control) at $p<0.01$.

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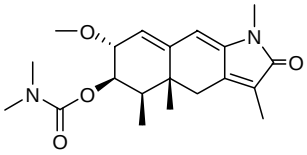
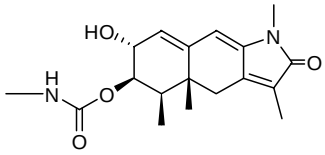


CP8816



CP8863

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