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Novel, orally active selective progesterone receptor modulator CP8947 inhibits leiomyoma cell proliferation without adversely affecting endometrium or myometrium☆,☆☆

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ABSTRACT

Uterine leiomyomas are highly prevalent and often symptomatic, but current medical therapies are limited. A novel, potent, selective, orally active therapy is needed. The goal of these studies was to determine the progesterone receptor (PR) specificity and activation, endometrial response, and impact on leiomyoma cell proliferation and extracellular matrix (ECM) production of the novel non-steroidal selective progesterone receptor modulators (SPRMs) CP8863 and CP8947. In vitro progestational activity was assessed by alkaline phosphatase assay and ER-α expression. In vivo progestational activity was assayed by the McPhail assay. Proliferation and gene expression studies were performed in immortalized human leiomyoma and myometrial cells. Both CP8863 and CP8947 were highly selective for progesterone receptor (PR) but not for ER-α, AR, and GR. Both compounds induced alkaline phosphatase comparably to progesterone, while CP8947 induced ER-α in leiomyoma cells but not myometrial cells. CP8947 was progestational in rabbit endometrium. Nanomolar CP8947 treatment inhibited human leiomyoma but not myometrial cell proliferation. Extracellular matrix components were decreased in leiomyoma cells, including COL1A1 and COL7A1 at nanomolar concentrations. CP8947 was a potent novel non-steroidal SPRM that was selective for PR, demonstrated progestational activity in endometrium, inhibited leiomyoma cell proliferation and decreased ECM component production, without disrupting myometrial cell proliferation.

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1. Introduction

Uterine leiomyomas, or fibroids, are common uterine tumors found in over 70% of women by the age of 50 [1]. While a proportion of women with leiomyomas may remain asymptomatic, many women suffer from menorrhagia, dysmenorrhea, dyspareunia, infertility, miscarriage, placental abruption, malpresentation, and preterm labor [2–4]. Despite the high prevalence and symptomatic nature of these tumors, the etiology of leiomyomas is incompletely understood. Consequently, therapeutic medical options remain limited.

Currently, the definitive therapy for leiomyomas is hysterectomy [5]. However, for women who desire pregnancy or who wish to avoid surgery, hysterectomy is not a viable therapeutic option. Newer therapies, including uterine artery ablation and MRI-guided high frequency ultrasound ablation, are contraindicated for women who wish to preserve fertility [6,7]. Myomectomy, or the surgical removal of the leiomyomas while preserving the uterus, is the standard surgical procedure if future pregnancy is desired. However, myomectomy is associated with significant morbidity including hemorrhage, adhesion formation, leiomyoma recurrence, blood transfusion, bowel injury, and rarely hysterectomy [8]. Effective medical therapies are needed.

Leiomyoma growth is regulated in part by estrogen and progesterone. Growth with sex steroids is illustrated by the increase in leiomyoma size beginning with menarche and their regression after menopause [9]. Based upon this insight, various hormonal therapies have been developed for leiomyoma treatment.

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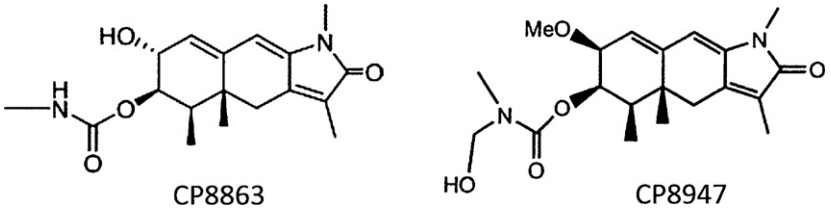

Fig. 1. Chemical structure of CP8863 and its metabolite CP8947. The novel compounds CP8863 (left) and CP8947 (right) are not structural derivatives of known steroids, but have a unique structure among the selective progesterone receptor modulators.

Selective progesterone receptor modulators (SPRMs) have shown promise for the treatment of leiomyomas. Examples of such SPRMs include mifepristone, and the mifepristone congeners CDB-2914, CDB-4124, and asoprisnil. Mifepristone itself is efficacious in reducing leiomyoma size [10], but due to its anti-progestational effect, this agent has been associated with endometrial hyperplasia [11]. Furthermore, mifepristone can also interact with the glucocorticoid receptor [12], potentially disrupting glucocorticoid function.

CDB 2914 and CDB 4124 are metabolites of mifepristone that exhibit less glucocorticoid activity and reduce leiomyoma size, but the anti-progestational effect in the endometrium resulted in endometrial abnormalities [12–14]. Asoprisnil, another mifepristone congener, also possesses limited anti-glucocorticoid activity, and causes a reduction in leiomyoma size [15]. However, investigation of asoprisnil was halted due to endometrial changes including abnormal vascular growth [16]. The greatest challenge in identifying an effective SPRM for the treatment of leiomyomas is to identify a compound with exquisite progesterone receptor selectivity that can selectively act as a progestin in the endometrium, while acting as an anti-progestin within the leiomyoma.

Tabata et al. have developed several progesterone receptor inhibitors from the microorganism *Penicillium oblatum* that are based upon an eremophilane-type sesquiterpene carbon skeleton [17]. These compounds are unique because they are not derived from steroidal derivatives [18]. CP8863 is a semi-synthetic orally active derivative that demonstrates progestational activity similar to natural progesterone in the endometrium [19]. This compound also inhibits estradiol-mediated epithelial cell proliferation but does not influence stromal cell proliferation [20]. The major metabolite of CP8863 is CP8947. The effects and role of CP8947 in leiomyoma treatment have not previously been reported.

We hypothesized that two novel non-steroidal PR ligands, CP8863 and CP8947, would have PR-specific selectivity and would inhibit leiomyoma cell proliferation. Furthermore, since symptoms caused by leiomyomas are due to increasing bulk which is directly related to the excessive and disorganized extra cellular matrix production [21], we further hypothesized that these compounds regulate critical extracellular matrix components which define the leiomyoma phenotype [22].

2. Materials and methods

All studies were approved by the Institutional Review Board and Animal Review Board of the Uniformed Services University of the Health Sciences.

2.1. Receptor binding assays

Cultured cells were washed once with pre-warmed (37 °C) 1× PBS and then detached from substrate with pre-warmed (37 °C) cell dissociation buffer (3 mM EDTA in 1× PBS without Ca^{2+} and Mg^{2+}). The cell pellet was recovered by centrifugation at 500 rpm in an Eppendorf 5804 R centrifuge for 10 min at 4 °C. The cells were resuspended in 5 packed volumes of 1× lysis buffer (20 mM Tris–HCl (pH 7.4), 1.0 mM EDTA, 10 mM sodium molybdate, 10% glycerol, 1.0 mM DTT, and complete protease inhibitors (Roche, Branchburg, NJ)). Cells were disrupted by brief sonication and centrifuged at 100,000 × *g* for 60 min at 4 °C. Supernatants containing steroid receptors were aliquoted and stored at −80 °C. Cell lines used as sources of steroid receptors were LNCaP prostate carcinoma cells (androgen receptor; American Type Culture Collection, Manassas, VA), Ishikawa endometrial adenocarcinoma cells (estrogen receptor; generous gift of Dr. Erlio Gurpide), T-47D mammary ductal carcinoma cells (progesterone receptor; ATCC), and HeLa cervical adenocarcinoma cells (glucocorticoid receptor; ATCC). Ishikawa cells were cultured in DMEM/F12 containing 10% charcoal-stripped fetal bovine serum. HeLa cells were cultured in DMEM containing 10% charcoal-stripped fetal bovine serum and T-47D cells were cultured in RPMI1640 containing 10% charcoal-stripped fetal bovine serum.

Aliquots of the respective lysates were incubated with [6,7-^{3}H(N)]-dexamethasone (Amersham, Quebec, Canada) for 24 h to detect binding of glucocorticoid receptors, [17α-methyl-^{3}H]-mibolerone (Amersham) for 24 h to detect specific binding of androgen receptor, [1,2,6,7-^{3}H(N)]-progesterone (Amersham) for 1 h to detect binding of progesterone receptor, or [2,4,6,7-^{3}H(N)]-estradiol (Amersham) for 20 h to detect binding of estrogen receptor. Progesterone, estradiol, hydrocortisone, and dexamethasone were obtained from Sigma–Aldrich (St. Louis, MO). Mibolerone was from Perkin Elmer (Waltham, MA). CP8863 and CP8946 were generous gifts from Tokai Pharmaceuticals, Inc. (Cambridge, MA; Fig. 1). Binding reactions were then treated with dextran-coated charcoal to remove unbound steroids, centrifuged, and binding of radiolabeled steroid was determined by scintillation counting. Nonspecific binding was that observed in the presence of a molar excess of unlabeled steroid.

2.2. Cell proliferation studies

Immortalized myometrial and leiomyoma cells [22] maintained in DMEM-F12 supplemented with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2 were sub-cultured in DMEM-F12 phenol red-free media containing 10% charcoal-stripped FBS to 50% confluence. We have demonstrated previously that our immortalized cultures maintain both estrogen and progesterone receptors, and maintain the molecular phenotype of surgical specimens, including alterations in critical biomarkers [23] (data not shown). Cells were trypsinized, plated in 96-well plates, and monolayer cultures of 50% confluence were treated with graded concentrations of CP8863 and CP8947 in serum-free, phenol red-free DMEM-F12 for times indicated. The plates were collected each day for up to five days. The final concentration of ethanol vehicle in culture medium or control cultures was <0.01%. Cell proliferation was measured using sulforhodamine-B method (Sigma–Aldrich) according to manufacturer's protocol and repeated in triplicate.

Table 1
Binding affinities of CP8863 and CP8947 for steroid receptors.

Compounds	IC_{50}			
	Progesterone receptor (nM)	Estrogen receptor (nM)	Glucocorticoid receptor (nM)	Androgen receptor (nM)
CP8863	57	>1000	>1000	>1000
CP8947	8	>1000	>1000	>1000
MPA	2			

2.3. RNA and protein protocol

Immortalized myometrial and leiomyoma cells maintained in DMEM-F12 supplemented with 10% FBS were plated in 6-well plates and allowed to reach 20% confluence before media was replaced with DMEM-F12 phenol-free media containing 10% charcoal-treated FBS. Cells reached 60% confluence before treatment with CP8863 and CP8947 at 10^{-9} to 10^{-5} M. Experiments were repeated in triplicate. After 24 h treatment with the agents, cells were collected for RNA and protein analysis.

2.4. Quantitative reverse transcriptase-polymerase chain reaction analysis

Real time reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to quantify expression of extracellular matrix (ECM) genes, COL1A1, COL7A1, versican, connective tissue growth factor, TGF-β3, and fibronectin, as described previously [23–25]. The 18S ribosomal RNA gene was used as an internal control and each sample was analyzed in triplicate. Bio-Rad iCycler software version 3.1 was used for data analysis.

2.5. Western blot

Protein was isolated using a RIPA lysis and extraction buffer (Pierce Biotech, Rockford, IL) containing 1× Halt protease inhibitor (Pierce Biotech) as described previously [25,26]. Briefly, aliquots of the proteins extracted from treated cultured cells were electrophoresed on a SDS-PAGE under reducing conditions, blotted onto nitrocellulose, and apoptosis related proteins were detected overnight with mouse monoclonal antibody against Caspase 3 (sc-7272; dilution 1:200) at 4 °C, rabbit polyclonal antibody against Caspase 7 (sc-33773, dilution 1:200), or TRAIL (sc-7877, dilution 1:200) as indicated. All antibodies were obtained from Santa Cruz Biotechnology, Santa Cruz, CA. Horseradish peroxidase (HRP)-conjugated secondary antibody (ImmunoPure, Pierce Biotech) in combination with the SuperSignal West Pico (Pierce Biotech) was used for detection of the proteins. Beta-actin (sc-1616; Santa Cruz Biotechnology) was an internal control for protein loading.

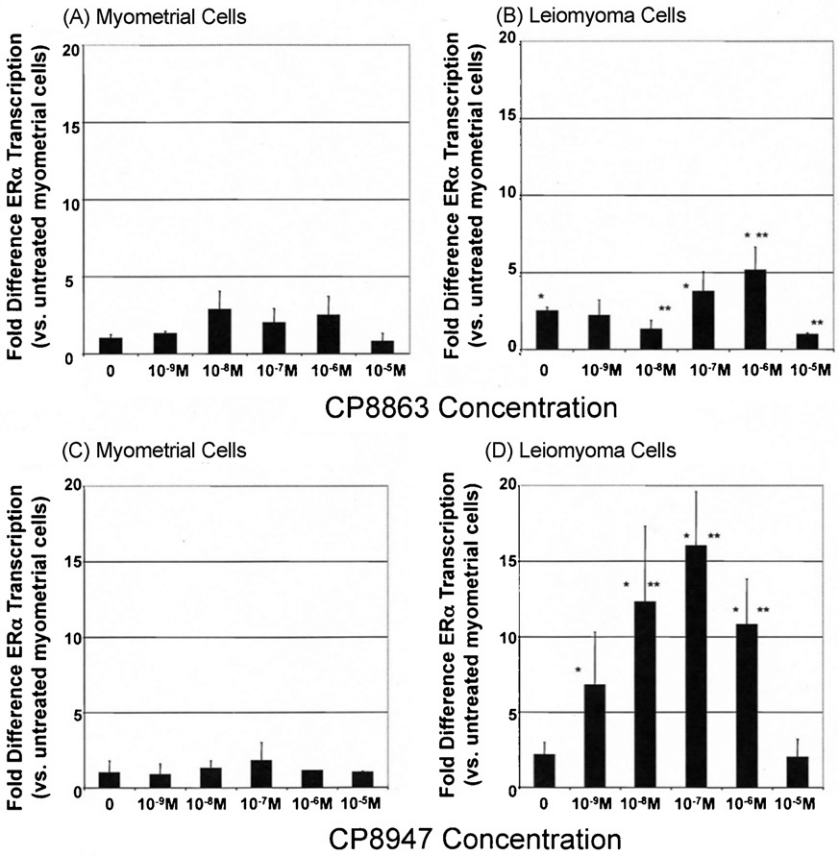

Fig. 2. Effects of graded concentrations of CP8863 and CP8947 on estrogen receptor-alpha (ER-α) mRNA levels in immortalized human leiomyoma and patient-matched myometrial cells as assessed by real time RT-PCR. (A) ER-α transcription in myometrial cells treated with graded doses of CP8863. (B) ER-α transcription in leiomyoma cells treated with graded doses of CP8863. (C) ER-α transcription in myometrial cells treated with graded doses of CP8947. (D) ER-α transcription in leiomyoma cells treated with graded doses of CP8947. *$p < 0.05$ vs. untreated myometrial cells; **$p < 0.05$ vs. untreated leiomyoma cells.

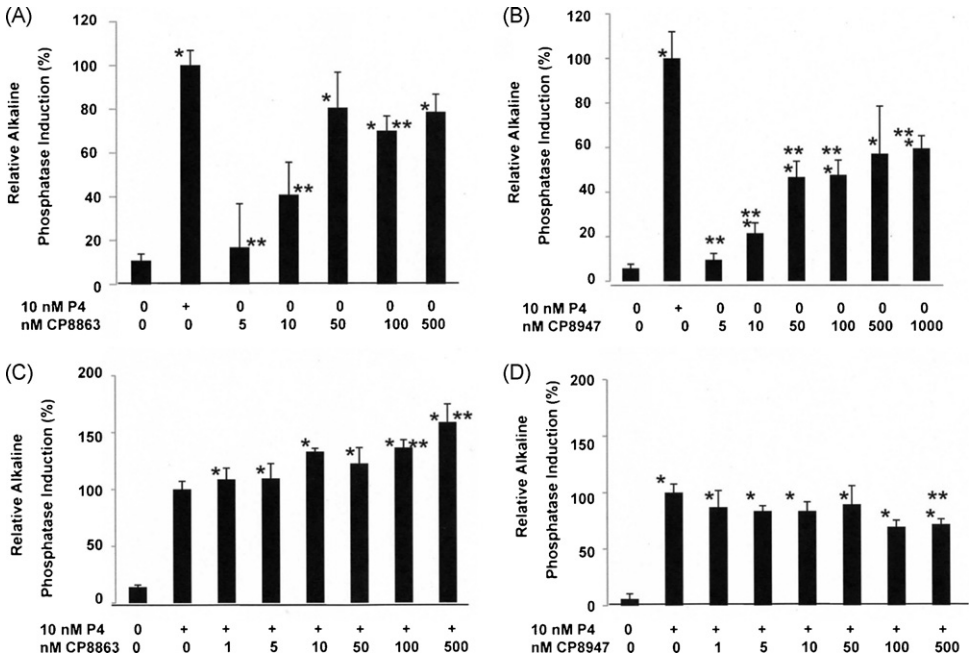

Fig. 3. Effects of CP8863 and CP8947 on expression of alkaline phosphatase activity in T47D breast cancer cells. (A) Alkaline phosphatase induction by MPA alone, or graded doses of CP8863. (B) Alkaline phosphatase induction by MPA alone, or graded doses of CP8947. (C) Alkaline phosphatase induction with 10 nM MPA plus graded doses of CP8863. (D) Alkaline phosphatase induction with 10 nM MPA plus graded doses of CP8947. $^{*}p < 0.01$ relative to untreated cells; $^{**}p < 0.01$ relative to progesterone-treated cells.

2.6. Alkaline phosphatase assay

Methods for determining progesterone-dependent induction of alkaline phosphatase activity were similar to those described by Madauss et al. [27]. Briefly, 8×10^4 T47D cells were plated per well in 48-well plates in DMEM/F12 medium containing 2% charcoal-stripped FBS and incubated at 37 °C overnight. The next day, progesterone, increasing concentrations of test compound alone, or 10 nM progesterone plus increasing concentrations of test compound were added to cells and incubation at 37 °C was continued overnight. After 20 h incubation, cells were washed with 1× Tris-buffered saline (TBS), lysed by addition of 100 μL 0.1 M Tris–HCl, pH 9.8 containing 0.2% Triton X-100 with shaking for 15 min at room temperature. 300 μL of 0.1 M Tris–HCl, pH 9.8 containing 4 mM p-nitrophenyl phosphate was then added to each well. V_{max} measurements were recorded at 405 nM during 2 min intervals over a 30 min period using a Bio-Rad Benchmark Plus microplate spectrophotometer.

2.7. Endometrial progestational activity: McPhail assay

Endometrial decidualization and agonist and antagonist activity of the two anti-progestins, CP8863 and CP8947 were determined in estrogen-primed immature rabbits. Immature female rabbits weighing 1.2–1.8 kg were purchased from Covance Research Products Inc. (Denver, PA). A stock solution of each drug, CP8863 and CP8947, and estradiol (Sigma–Aldrich, St. Louis, MO) was made in absolute alcohol. Medroxyprogesterone 17-acetate (MPA, Sigma) was dissolved in chloroform. Further dilutions for subcutaneous injections (Experiment 1) or oral delivery (Experiment 2) to the rabbits was performed using sesame oil (Sigma).

2.7.1. Experiment 1

Rabbits were primed with estrogen (5 μg/kg/day) for 6 days, and then treated with subcutaneous administration of either CP8863 or CP8947 (0, 0.1, 1.0 or 10 mg/kg/day) or MPA (0.15 mg/kg/day) for 5 consecutive days. Each group consisted of five rabbits.

2.7.2. Experiment 2

Five rabbits per group were primed with estrogen (5 μg/kg/day) for 6 days, and then orally administered the test compound (0, 0.04, 0.2, 1.0 or 3.0 mg/kg/day) or the test compound in combination with MPA (0.15 mg/kg/day) for 5 consecutive days.

All rabbits were sacrificed on the day following the final administration of test medication or progesterone. The uteri were excised and fixed in 10% buffered formalin. Six transverse sections, three each from proximal (closest to the uterus), medial and distal parts of the right and left uterine horns were stained with haematoxylin and eosin, and examined histologically. The grading of endometrial transformation was based on the previously described McPhail method [28].

2.8. Statistical analysis

For qRT-PCR data, results are reported as mean ± SEM. For each result the average expression of three replicates was calculated and normalized against 18S RNA. Relative expression was calculated based on Pfaffl method [29]. Mann–Whitney *U*-test was used for interval data statistical evaluation. For proliferation data, statistical significance was calculated by Student's *t*-test. For western blot analysis, calculations were done using QualityOne software from Bio-Rad. Data are presented as fold difference between relative density units of treated and untreated samples, normalized for loading. For each study, the primary outcome is defined, the experiment performed prospectively, and when applicable, dose dependency is determined.

3. Results

Binding affinities for CP8863 and CP8947 for AR, ER-alpha, PR, and GR in LNCaP, Ishikawa, T-47D, and HeLa lysates are shown in Table 1. The IC_{50} value for CP8863 was 57 nM and for CP8947 was 8 nM compared to the progesterone receptor ligand and agonist medroxyprogesterone acetate (MPA) value of 2 nM. The IC_{50} values for the two compounds were greater than 1000 nM for all

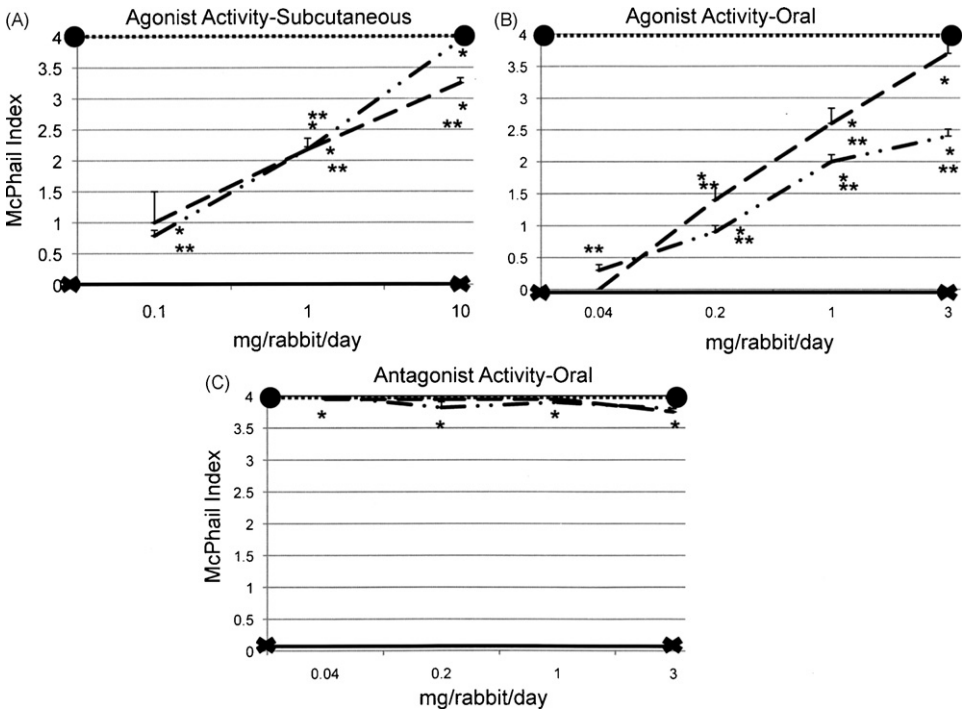

Fig. 4. Progestational effects of CP8863 and CP8947 on rabbit endometrium assayed by McPhail Index after estradiol priming. In the absence of compound, no progestational influence was noted (X, solid). In the presence of progesterone, maximal progestational histologic changes were noted (●, dashed line). (A) CP8863 (long dash) or CP8947 (short and long dash) provided subcutaneously. (B) CP8863 (long dash) or CP8947 (short and long dash) provided orally. (C) Competition studies using 10 nM progesterone and increasing oral concentrations of either CP8863 or CP8947. $^{*}p < 0.01$ relative to untreated animals; $^{**}p < 0.01$ relative to progesterone-treated animals.

other receptors. Both CP8863 and CP8947 showed high specificity for progesterone receptor binding.

Progestational activity was assessed by induction of ER-α mRNA transcripts in human leiomyoma and myometrial cells. CP8863 increased steady-state levels of ER-alpha transcript compared to the untreated cells in both leiomyoma and myometrial cell lines at 100 nM (Fig. 2A and B). There was a 5.2-fold increase in expression of ER-α in leiomyoma cells treated with CP8863 at 1 μM (10^{-6} M). In contrast, CP8947 increased ER-α transcripts in leiomyoma cells in a dose-dependent manner but did not affect myometrial cell steady-state levels of ER-α mRNA (Fig. 2C and D). A 6.9-fold increase in mRNA expression was noted at a concentration of 1 nM (10^{-9} M), which was significantly greater than the expression seen in CP8863 treated cells. At potentially cytotoxic concentrations (10 μM, 10^{-5} M), ER-α transcripts were decreased.

To confirm progesterone receptor specific activation, we also performed alkaline phosphatase assays in T47D human breast cancer cells. As a positive control, the progesterone receptor ligand MPA at 10 nM concentration significantly increased alkaline phosphatase activity (Fig. 3A and B). Both CP8863 and CP8947 increased alkaline phosphatase production in a dose-dependent fashion; both CP8863 and CP8947 demonstrated significant progestational activity at 10–50 nM ($(1–5) \times 10^{-8}$ M) treatment concentrations (Fig. 3A and B). When these cells were treated with both CP8863 and MPA, alkaline phosphatase production was increased to levels greater than with MPA alone with CP8863 concentrations of 100 nM (10^{-7} M) or greater (Fig. 3C). At high concentration (500 nM; 5×10^{-7} M), CP8947 inhibited MPA-mediated alkaline phosphatase induction (Fig. 3D), but maintained induction at rates greater than untreated leiomyoma cells. These results suggest that CP8947 acts as a less potent progestin relative to progesterone, obtaining 70% efficacy at 50× concentration relative to progesterone.

One limitation of SPRM therapy is the anti-progestational activity on the endometrium. We therefore assessed the in vivo endometrial progestational activity of CP8863 and CP8947 in estrogen-primed rabbits. Treatment with MPA resulted in maximal endometrial decidualization (Fig. 4). Both CP8863 and CP8947 showed a dose-dependent increase in the McPhail index, demonstrating a progestational effect on the uterine lining with both subcutaneous and oral dosing (Fig. 4). A greater progestational effect was observed with oral CP8947 compared to subcutaneous CP8947 and oral CP8863 (Fig. 4A and B). Concomitant treatment with MPA demonstrated a McPhail score equivalent to MPA alone, suggesting that neither CP8863 nor CP8947 exhibited anti-progestational effects in rabbit endometrium (Fig. 4C).

Next we evaluated the influence of CP8863 and CP8947 treatment on immortalized human leiomyoma and patient-matched myometrial cells at doses ranging from 0.01 nM to 10 μM (Fig. 5).

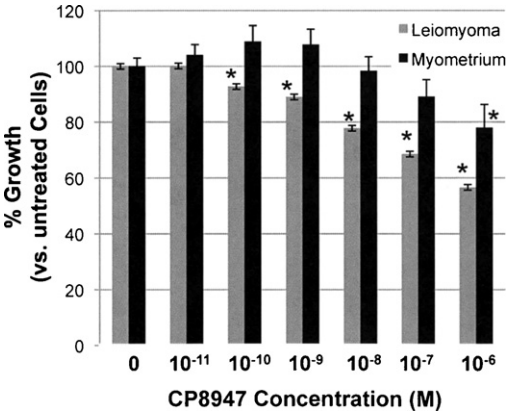

Fig. 5. Effects of graded concentrations of CP8947 on the number of viable cultured human myometrial and leiomyoma cells. Myometrial cell proliferation (black bars), leiomyoma cultures (gray bars). Results represent the mean + SEM of at least three independent experiments performed in triplicate. $^{*}p < 0.025$ relative to untreated cells.

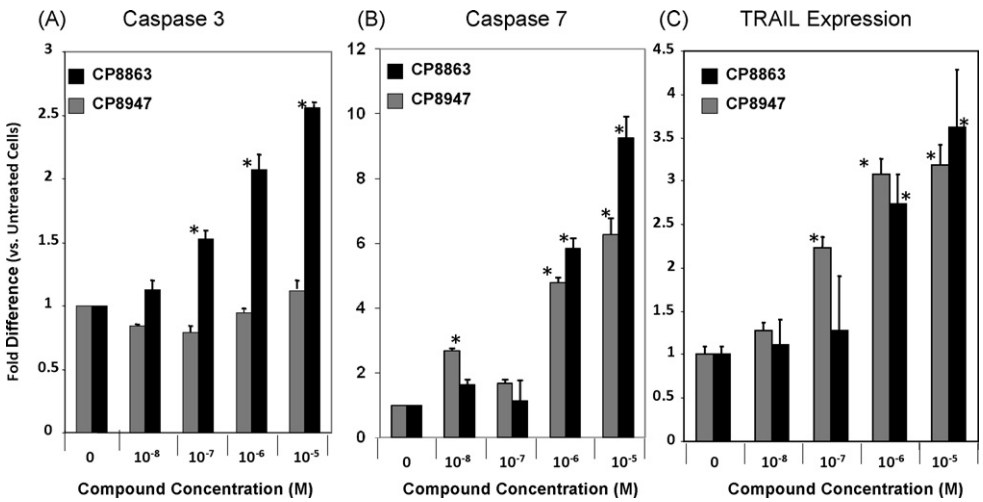

Fig. 6. Effects of graded concentrations of CP8863 and CP8947 on apoptosis markers in cultured human leiomyoma cells. Caspase-3 expression (A), caspase-7 expression (B), and TRAIL induction (C). Results represent the mean + SEM of at least three in triplicate. $^{*}p < 0.01$ relative to untreated cells.

Both estradiol and MPA stimulated leiomyoma cell proliferation (data not shown). Neither leiomyoma nor myometrial cell proliferation was influenced by CP8863 treatment, even at concentrations as high as 10 μM (10^{-5} M; data not shown). Treatment with CP8947 demonstrated efficacy at 0.1 nM (10^{-10} M) concentrations in leiomyoma cells, while not significantly impacting myometrial cell proliferation at 1000-fold greater concentrations (Fig. 5). These results suggest that CP8947 selectively inhibited leiomyoma cell proliferation at 0.1–1 nM concentration (10^{-9} to 10^{-10} M) while having negligible effects on myometrial cell growth at these concentrations.

To determine the mechanism of anti-proliferative effect of CP8947, we evaluated markers of apoptotic activity. Western blots demonstrated an increase in caspase 7, and TRAIL expression in cells treated with CP8947, without an increase in caspase 3 (Fig. 6). Caspase 7 expression was consistently increased by treatment with CP8947 at concentrations at 1 μM (10^{-6} M; Fig. 6B). TRAIL expression was increased significantly with treatment concentrations of 100 nM CP8947 (Fig. 6C). Protein concentration of caspase 3, caspase 7, and TRAIL increased with CP8863 treatment at 0.1–1 μM (10^{-7} to 10^{-6} M) concentrations. The micromolar concentrations required to alter apoptotic markers suggests that apoptosis induction is not the mechanism of leiomyoma cell inhibition, which occurs at nanomolar concentrations.

Ultimately, symptoms from leiomyomas result from increasing bulk due to overproduction of a disorganized extracellular matrix

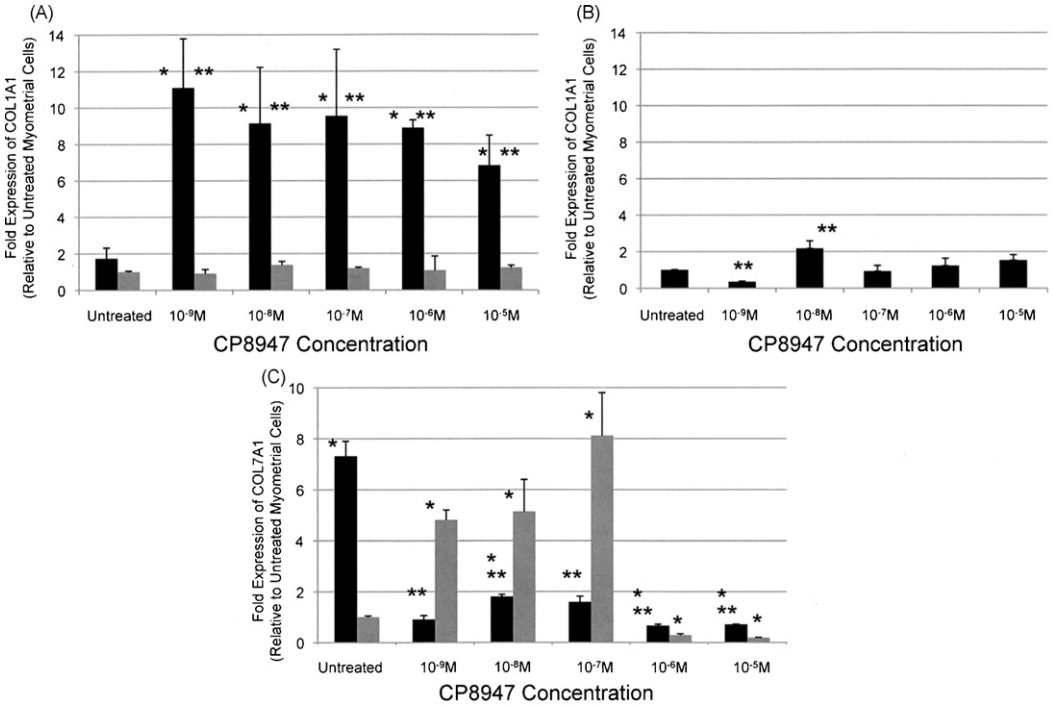

Fig. 7. Effects of graded concentrations of CP8947 on COL1A1 and COL7A1 expression in human leiomyoma and patient-matched myometrial cells, as assessed by real time RT-PCR analysis. COL1A1 expression in human myometrial cells (black bars; A) and leiomyoma cells (gray bars) treated with CP8947 at 24 h (A) and 48 h (B). (C) COL7A1 expression in human myometrial cells (gray bars) and human leiomyoma cells (black bars). Results represent the mean + SEM of at least three in triplicate. $^{*}p < 0.01$ relative to untreated myometrial cells; $^{**}p < 0.01$ relative to untreated leiomyoma cells.

Table 2
Summary of gynecologic effects of SPRMs CP8863 and CP8942 relative to MPA.

Compound	Leiomyoma	Myometrium	Endometrium
MPA	Stimulated proliferation	Inhibitory at high concentration	Induced decidualization
CP8863	No effect on proliferation	No effect on proliferation	Induced decidualization
CP8947	Inhibited proliferation	Inhibitory at high concentration	Induced decidualization

[21,30]. We therefore evaluated the influence of CP8947 on extracellular matrix genes known to be altered in human leiomyomas [23]. CP8947 at 1 nM or greater concentration had a no effect on myometrial expression of COL 1A1 (Fig. 7A). In leiomyoma cells, COL 1A1 initially increased by up to 12-fold at 24 h with 1 nM CP8947 treatment, but returned to expression levels identified in untreated myometrial cells at 48 h after treatment (Fig. 7B). COL 7A1 expression was induced in myometrial cells by CP8947 concentrations of 1 nM (10^{-9} M), but inhibited at 1 μM (10^{-6} M) or greater concentrations (Fig. 7B). In leiomyoma cells, COL 7A1 expression was reduced with CP8947 at 1 nM (10^{-9} M). These findings demonstrate that CP8947 decreased transcripts encoding ECM components in leiomyoma cells at nanomolar concentrations. Neither CP8863 nor CP8947 altered expression of fibronectin, connective tissue growth factor, nor TGFβ3 (data not shown).

4. Discussion

We found that the novel compounds CP8863 and CP8947 are highly selective and potent selective progesterone receptor modulators, with minimal activity on estrogen, glucocorticoid, or androgen receptors. These compounds can bind to and activate the progesterone receptor as demonstrated by the alkaline phosphatase assay at nanomolar concentrations. Furthermore, both compounds are orally active, and induced progestational changes in the rabbit endometrium. They were unable to block progesterone-mediated endometrial changes. In human leiomyoma cells, CP8947 specifically inhibited proliferation while not influencing myometrial cell proliferation at nanomolar to micromolar concentrations (summarized in Table 2). Inhibited proliferation is unlikely to involve apoptosis, as demonstrated by alterations in apoptotic genes only at micromolar compound concentrations. Finally, CP8947 altered expression of critical extracellular matrix genes involved in the fibrotic phenotype of leiomyomas, inhibiting both COL1A1 and COL7A1 expression. Collectively, these results show that CP8947 is a highly potent, highly selective, orally active selective progesterone receptor modulator that selectively inhibited both leiomyoma cell proliferation and extracellular matrix gene expression without disrupting myometrial cell growth or acting as an anti-progestin in the endometrium. As such, CP8947 represents a compound that could have therapeutic efficacy in human leiomyomas.

Current medical therapy for uterine leiomyomas is based upon the finding that human leiomyomas are hormonally dependent tumors and involves the use of gonadotropin releasing hormone agonists or antagonists [31,32]. While these therapies are effective in reducing leiomyoma size from 30 to 50% [33], they therapies also induce a hypoestrogenic state, with side effects of hot flashes, vaginal dryness, and bone loss [34]. In addition, these therapies cannot be dosed orally. As a result of the significant side effects, therapy is often limited to 3–6 months, with longer treatment requiring a complex regimen of hormonal add-back medication [35].

As an alternative to GnRH analogue therapy, SPRMs have been developed for leiomyoma therapy: mifepristone, and the congeners of mifepristone, asoprisnil, CDB-4124 and CDB-2914 [11,14,16]. While each of these compounds showed promise, two innate characteristics of these compounds limited their applicability: interaction with glucocorticoid receptor and endometrial hyperplasia. Progesterone is able to interact with and activate the glucocorticoid receptor [36], due to the similarities in structure between the two hormones. Mifepristone and mifepristone congeners, which have a similar structure, exhibit glucocorticoid binding activity [37]. Furthermore, while mifepristone and congeners act as anti-progestins on the leiomyoma, they also act as anti-progestins on the endometrium. As a result, therapy results in endometrial abnormalities. After several months, the estrogen-induced endometrial changes may lead to hyperplasia and atypia [38].

The novel compounds CP8863 and CP8947 are semi-synthetic compounds that were isolated from the fermentation broth of *P. oblatum*. Among the prototypical compounds isolated, PF1092A possessed an IC_{50} of 30 nM for the porcine uterine progesterone receptor [17]. Further compound development resulted in CP8863, which inhibited estradiol-mediated epithelial cell proliferation [20]. The CP8863 IC_{50} for the progesterone receptor was 40 nM, while the IC50 was >9 μM for the glucocorticoid receptor, estrogen receptor, and androgen receptor [20]. Furthermore, oral dosing of CP8863 exerted a progestational action in the rabbit endometrium [19]. Analysis of CP8863 metabolism resulted in the identification of CP8947, which we show here to be a more potent and efficacious selective progestin receptor modulator.

It was interesting to note that regulation of ECM genes was not uniformly altered by CP compounds. Specifically, COL1A1 mRNA concentration initially increased in the first 24 h by CP8947 treatment, but ultimately decreased relative to untreated leiomyoma cells by 48 h. Such a 'flare' effect has been described with luteinizing hormone releasing hormone agonists, which initially activate receptor but ultimately abolish function [39]. Given the demonstrated agonist activity of CP8947 in other tissue types such as human leiomyoma and T47D human breast cancer cells, one can speculate that CP8947 functions by binding and initial receptor activation, followed by either inactivation or down-regulation of the progesterone receptor. This finding was not consistent among all ECM genes analyzed, however, suggesting that the transcriptional complex for COL1A1 differs from other ECM genes tested.

Given the different responses between leiomyoma and myometrial cells in the same patient, we cannot rule out the possibility that the CP compounds work via mechanisms aside from the progesterone receptor, particularly at micromolar concentrations. However, our data are certainly consistent with the hypothesis that these compounds are selective progesterone receptor modulators, in which they act as progestins or anti-progestins depending upon the cell type. Previous work demonstrated similar findings for compounds such as tamoxifen, which acts as an anti-estrogen at the breast but as an estrogen ligand at the endometrium [40,41]. One proposed mechanism involves cell-specific promoter complexes that regulate the level of transcription induced by a particular ligand [42].

In conclusion, we have characterized a novel selective progesterone receptor modulator, CP8947, which exhibits characteristics favorable for further development as a human leiomyoma therapy. In particular, CP8947 was active at nanomolar concentrations, was selective to the progesterone receptor, was orally active, had progestational activity in the endometrium, and inhibited leiomyoma cell proliferation and extracellular matrix gene expression while having no appreciable effect on myometrial cell proliferation. Clin-

ical trials will be necessary to determine the promise of CP8947 as an orally active therapy for uterine leiomyomas.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsbmb.2010.05.005.

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