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Fungal metabolites, PF1092 compounds and their derivatives, are nonsteroidal and selective progesterone receptor modulators

Yuji Tabata*, Yumiko Iizuka, Junko Kashiwa, Naomi Takei Masuda, Rie Shinei, Ken-ichi Kurihara, Tsuneo Okonogi, Shigeru Hoshiko, Yasushi Kurata

Drug Discovery, Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd., 760 Morooka-cho, Kohoku, Yokohama, 222-8567, Japan

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Abstract

The potential of new nonsteroidal progesterone receptor ligands, the derivatives of PF1092C ((4*aR*,5*R*,6*R*,7*S*)-6,7-dihydroxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one) discovered from fungal metabolites, was evaluated. PF1092A ((4*aR*,5*R*,6*R*,7*S*)-6-acetoxy-7-hydroxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one) showed good and moderate affinity for porcine and human progesterone receptors in in vitro receptor binding assays, respectively, and partial agonist activity for the progesterone receptor, as determined in assays of two types of progesterone-dependent enzymes in human mammary carcinoma T47D cells. The derivative of PF1092C, CP8481, ((4*aR*,5*R*,6*R*,7*S*)-6-(2-furancarbonyloxy)-7-hydroxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one) possessed better affinity for both progesterone receptors and showed less cross-reactivity for other steroid receptors, such as rat androgen receptor, human glucocorticoid receptor, and human estrogen receptor, and was a more potent modulator of the progesterone receptor than PF1092A. CP8400 ((4*aR*,5*R*,6*R*,7*S*)-6,7-diacetoxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one) and CP8401 ((4*aR*,5*R*,6*R*,7*S*)-6,7-dipropionyloxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one), other derivatives, were indicated to be progesterone receptor antagonists. These results suggest that PF1092 compounds can serve as a new pharmacophore for potent and specific nonsteroidal progesterone receptor modulators. © 2001 Published by Elsevier Science B.V.

Keywords: PF1092; Progesterone receptor modulator; Progesterone receptor antagonist; Progesterone receptor agonist; Nonsteroidal; T47D

1. Introduction

Progesterone, which is one of the steroid hormones, plays an important role in female reproduction. Its principal target organs are the uterus, ovaries, breasts, bone, and brain (Graham and Clarke, 1997). The biological actions of progesterone are mediated through the progesterone receptor, a member of the intracellular superfamily of ligand-dependent transcription factors. Owing to its central role in the menstrual cycle, progesterone and its synthetic analogues (progestins) have been used as a contraceptive since the 1960s. In addition, progestins combined with estrogen are widely prescribed in hormone replacement therapy as the average life expectancy of women continues to increase. Progestins are also used to treat gynecological disorders such as dysmenorrhea, endometriosis, and dysfunctional uterine bleeding caused by hormone imbalance (Editorial, 1996). Moreover, the possibility that progesterone promotes bone formation in ovariectomized rats (Barengolts et al., 1990) and protects against bone loss in breast-feeding women (Caird et al., 1994) has been reported. Thus, a progesterone receptor agonist could serve a positive function in the treatment of established osteoporosis.

Progesterone receptor antagonists may also be used potentially to treat various gynecological and obstetric diseases. Based on the initial results of clinical trials, certain reports have identified RU486, the only clinically available progesterone antagonist, as having potential therapeutic effects in the treatment of breast cancer (Klijn et al., 1994), endometriosis (Kettel et al., 1996), uterine leiomyomata (Murphy et al., 1995), and meningioma (Grunberg et al., 1991).

Nevertheless, all clinically available progesterone agonists and antagonists possess a steroidal moiety, which results in the appearance of side effects associated with

* Corresponding author. Tel.: +81-45-545-3113; fax: +81-45-545-3193.

E-mail address: yuji_tabata@meiji.co.jp (Y. Tabata).

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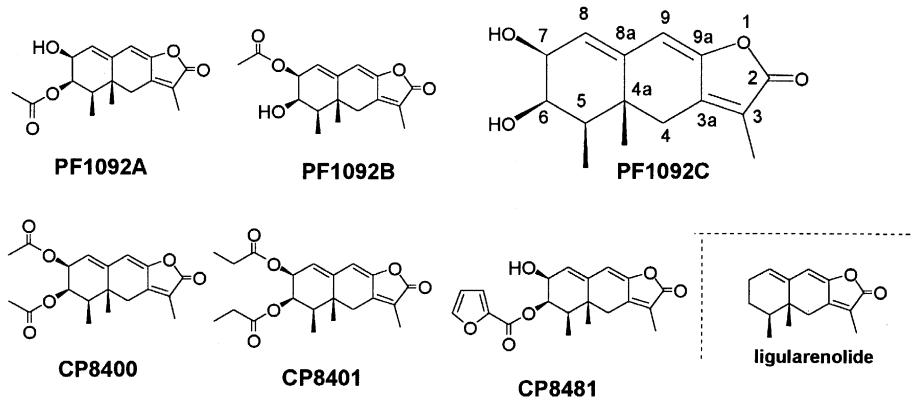

Fig. 1. PF1092 compounds, their derivatives and ligularenolide.

cross-reactivity for other steroid receptors (Editorial, 1996; Spitz and Bardin, 1993). Therefore, it is necessary to develop new progesterone receptor agonists and antagonists without a steroidal structure.

In the last several years, nonsteroidal progesterone receptor ligands have been reported (Pathirana et al., 1995; Combs et al., 1995; Hamann et al., 1996; Pooley et al., 1998; Zhi et al., 1999). In the course of screening for nonsteroidal progesterone receptor ligands, we discovered PF1092A, B, and C (Fig. 1) in extracts of cell cultures of a rare fungus, *Penicillium oblatum* PF1092 (Tabata et al., 1997a,b). PF1092 compounds structurally resemble ligularenolide (Fig. 1), one of sesquiterpenoids isolated from the Chinese herb, "San-Shion" (Ishizaki et al., 1970). PF1092A has a good affinity for the porcine progesterone receptor, with an affinity equivalent to that of progesterone (Tabata et al., 1997a).

In a further study, we modified the original structure of PF1092C to produce more potent progesterone receptor agonists and antagonists. This paper describes the potential of PF1092 compounds to be used as a new pharmacophore for potent and specific nonsteroidal progesterone receptor modulators.

2. Materials and methods

2.1. Materials

PF1092A ((4*aR*,5*R*,6*R*,7*S*)-6-acetoxy-7-hydroxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one), PF1092B ((4*aR*,5*R*,6*R*,7*S*)-7-acetoxy-6-hydroxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*] furan-2(4*H*)-one) and PF1092C ((4*aR*,5*R*,6*R*,7*S*)-6,7-dihydroxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho [2,3-*b*]furan-2(4*H*)-one) were purified from extracts of cell cultures of the rare fungus *P. oblatum* PF1092 at our laboratory. Their derivatives CP8400 ((4*aR*,5*R*,6*R*,7*S*)-6,7-diacetoxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one), CP8401 ((4*aR*,5*R*,6*R*,7*S*)-6,7-dipropionyloxy-4*a*,5,6,7-tetrahydro-3,4*a*,5-trimethylnaphtho[2,3-*b*]furan-2(4*H*)-one), and CP8481 ((4*aR*,5*R*,6*R*,7*S*)-6-(2-furancarbonyloxy)-7-hydroxy-4*a*,5,6,7 - tetrahydro - 3,4*a*,5 - trimethylnaphtho [2,3-*b*] furan-2(4*H*)-one) were synthesized from PF1092C at the same location. Progesterone (4-pregnene-3,20-dione) was purchased from Junsei Chemical. Testosterone (4-androsten-17β-ol-3-one), 17β-estradiol (3,17β-dihydroxy-1,3,5[10]-estratriene) and medroxyprogesterone acetate (MPA, 6α-methyl-17α-hydroxyprogesterone acetate) were purchased from Wako. Dexamethasone (9α-fluoro-16α-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione) was purchased from Sigma. RU486 (11β-[4-dimethylamino]phenyl-17β-hydroxy-17-[1-propynyl]estra-4,9-dien-3-one) was also synthesized at our laboratory. The purity of all the compounds we prepared was confirmed to be over 95% by HPLC analysis. [1,2,6,7,-^{3}H(*N*)]-Progesterone (specific activity: 3589 GBq/mmol), [17α-methyl-^{3}H]-mibolerone (4-esteren-7α,17α-dimethyl-17β-ol-3-one) (specific activity: 3145 GBq/mmol), and [2,4,6,7,-^{3}H(*N*)]-estradiol (specific activity: 2664 GBq/mmol) were purchased from NEN™ Life Science Products. [1,2,4-^{3}H]-Dexamethasone (specific activity: 1590 GBq/mmol) was purchased from Amersham Pharmacia Biotech.

2.2. Steroid receptor binding assay

The conditions and the reference for each steroid receptor binding assay are summarized in Table 1. Each assay was performed by a method modified from that indicated in the references (Tabata et al., 1997a; Schilling and Liao, 1984; Kloosterboer et al., 1988; Sheen et al., 1985). Cytosol-containing steroid receptors was prepared from different cell lines or organs of various species. Porcine uteri, for the isolation of progesterone receptors, were provided by a slaughterhouse. The experimental procedure for rat androgen receptors was in accordance with the Guidelines for Animal Experimentation approved by the Pharmaceutical Research Center of Meiji Seika Kaisha. Each cell and organ was homogenized in appropriate preservation buffer

Table 1
Experimental conditions

Receptor	Tissue (cell)	Species	Ligand	Incubation (h/°C)	Reference
PR	T47D	Human	[^{3}H]-progesterone	1/4	Tabata et al., 1997a
	Porcine uteri	Porcine	[^{3}H]-progesterone	1/4	Tabata et al., 1997a
AR	Rat prostate	Rat	[^{3}H]-mibolerone	24/4	Schilling et al., 1984
GR	IM9	Human	[^{3}H]-dexamethasone	24/4	Kloosterboer et al., 1988
ER	MCF7	Human	[^{3}H]-estradiol	20/4	Sheen et al., 1985

and centrifuged at 100,000 × *g* for 30 min. The resulting supernatant (cytosol) was stored at −80 °C until use. Cytosol was incubated with the corresponding radioligand in the corresponding assay buffer for the corresponding incubation time indicated in Table 1. Following the incubation, the indicated concentration of dextran-coated charcoal solution was added and further incubated at 4 °C for 10 min. The mixture was then centrifuged at 1800 rpm for 5 min. The radioactivity of 100 μl of the supernatant was determined in 2 ml of Aquasol-2 (Packard Instrument) using a liquid scintillation counter (Beckman LS6500). Non-specific binding was defined as the binding observed when 10 μg/ml of corresponding cold steroid was added to the reaction mixture.

2.3. Progesterone-dependent luciferase expression assay

Progesterone-dependent modulation of gene transcription was studied using the luciferase assay with stable transfected cells, T47D-pMAMneo-LUC. T47D, a human breast cancer cell, was purchased from American Type Culture Collection (ATCC). The plasmid, pMAMneo-LUC (CLONTECH), was transfected into T47D cells using lipofect amine, and transformants were collected through the addition of G418 (600 μg/ml, Meiji Seika Kaisha). Transformant cells were then cloned individually, and their cellular luciferase activity was determined with or without progesterone. One of the transformants, T47D-pMAMneo-LUC, was selected for its high luciferase activity following the addition of progesterone. This stable transformed cell line was used for further studies. The growth medium (Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 600 μg/ml G418) was replaced with DMEM containing 5% fetal bovine serum treated with dextran-coated charcoal and 600 μg/ml G418. After 24 h of incubation at 37 °C, the cells were plated in 96-well plates at 50,000 cells/well in phenol red-free DMEM (Gibco) containing 5% fetal bovine serum treated with dextran-coated charcoal. After 8 h of incubation at 37 °C under 5% CO_2, test compounds dissolved in dimethyl sulfoxide (DMSO) and diluted with 50% MeOHaq and PBS(−) were added to each well to achieve the appropriate compound concentration. The same volume of methanol/DMSO/PBS(−) solution was added to control cells. The final concentrations of DMSO and MeOH were under the concentration at which they affected the assay results (DMSO < 0.01%, MeOH < 0.5%). After 16 h of incubation at 37 °C, 100 μl of Luc Lite (Packard) solution was added to each well and mixed. After 15 min of incubation at room temperature, luminescence was measured using a luminometer, LUMIstar (SLT Labinstruments), or ARVO (Wallac). All data points were measured in triplicate.

2.4. Progesterone-dependent alkaline phosphatase expression assay

The assay was performed using the modified method described by Markiewicz and Gurpide (1994). The progesterone-dependent modulation of alkaline phosphatase expression was studied using T47D. The growth medium (DMEM containing 10% fetal bovine serum) was replaced with phenol red-free DMEM (Gibco) containing 5% fetal bovine serum treated with dextran-coated charcoal. After 24 h of incubation at 37 °C under 5% CO_2, the cells were plated in 96-well plates at 25,000 cells/well in phenol red-free DMEM (Gibco) containing 5% fetal bovine serum treated with dextran-coated charcoal. After 24 h of incubation at 37 °C, test compounds dissolved in DMSO and diluted with 50% MeOHaq and PBS(−) were added to each well to achieve the appropriate compound concentration. The same volume of methanol/DMSO/PBS(−) solution was added to control cells. The final concentrations of DMSO and MeOH were under the concentration at which they affected the assay results (DMSO < 0.01%, MeOH < 0.5%). After 20 h of incubation at 37 °C, the medium was removed and the cells were washed with 200 μl of PBS(−). The plates were kept at −80 °C for 15 min, followed by thawing at room temperature. This freeze-and-thaw cycle was performed twice. PBS(−) (50 μl) was then added to the plates at room temperature. After 5 min, 30 μl of CSPD™ chemiluminescent substrate solution (final 0.06125 mM, 1.25 mM × 20 dilution with chemiluminescence enhancer, Great EscApe™ SEAP Detection kit (CLONTECH)) was added to each well and mixed. After 30 min of incubation at room temperature, luminescence was measured with a luminometer, ARVO (Wallac). All data points were measured in triplicate. Sigmoid fitting of the results expressed as alkaline phosphatase induction (as 100% with 10 nM progesterone) or inhibition (against alkaline phosphatase induction by 5 nM progesterone) by the test compounds was achieved using KaleidaGraph software. EC_{50} and IC_{50} values were deter-

mined from the parameters of the curve obtained by sigmoid fitting.

3. Results

3.1. Affinity of the derivatives of PF1092 for steroid receptors

We prepared the derivatives of PF1092C through modification at the 6- and/or 7-position(s). The structures of the derivatives are shown in Fig. 1. The relative binding affinities of these compounds are summarized in Table 2. PF1092A had good affinity for the porcine progesterone receptor, similar to that of progesterone, but its affinity for the human progesterone receptor was less than that of progesterone. CP8481, in which a 2-furancarbonyloxy group replaces the acetoxy group at the 6-position, had a better affinity than progesterone for both porcine and human progesterone receptors. Most of the derivatives of PF1092C had a much weaker affinity for the rat androgen receptor and no affinity for the human glucocorticoid receptor and the human estrogen receptor in comparison to steroidal ligands. These results indicated that PF1092 derivatives are specific ligands for the progesterone receptor.

3.2. Modulation of progesterone-dependent enzyme expression by PF1092 derivatives

We evaluated PF1092 derivatives in the progesterone-dependent alkaline phosphatase expression assay using T47D human breast cancer cell line, and in the progesterone-dependent luciferase expression assay using T47D transfected with the plasmid, pMAMneo-LUC. In both assays, PF1092 derivatives were evaluated in the absence (agonist format) and presence (antagonist format) of progesterone. In the progesterone-dependent luciferase expression assay, PF1092A and B stimulated luciferase expression in the agonist format and suppressed luciferase expression in the antagonist format (Fig. 2). CP8400 and CP8401 showed slightly stimulated luciferase expression at only 10^{-6} M in the agonist format and inhibited progesterone dependent luciferase expression by up to 85–95% in the antagonist format.

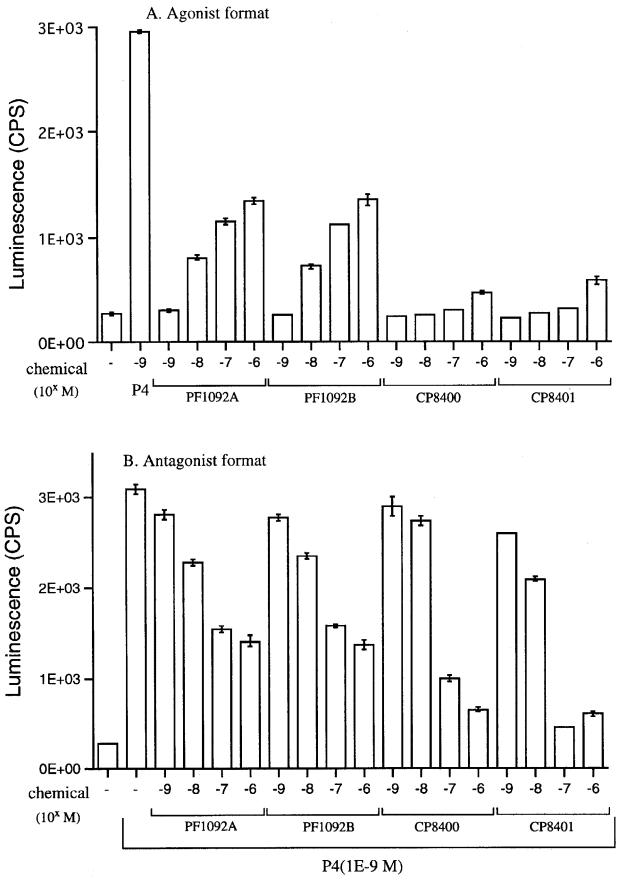

Fig. 2. Modulation of luciferase expression in T47D-pMAMneo-LUC by PF1092A, B, CP8400 and CP8401. The indicated concentrations of test compounds were added to T47D-pMAMneo-LUC and incubated at 37 °C for 16 h with ((B) Antagonist format) or without ((A) Agonist format) progesterone (P4, 1 nM). Luciferase expression was estimated by measuring luminescence with Luc Lite (Packard). The results represent the means ± S.E. of three replicates.

In the progesterone-dependent alkaline phosphatase expression assay, PF1092A and B also caused alkaline phosphatase expression in the agonist format and suppression of alkaline phosphatase expression in the antagonist format, as seen in the luciferase expression assay (Fig. 3). CP8400 and CP8401 did not cause alkaline phosphatase expression in the agonist format and almost completely prevented 5 nM progesterone-dependent alkaline phos-

Table 2
Relative binding affinity of PF1092 derivatives for steroid receptor

Ligand	pPR (progesterone = 100)	hPR (progesterone = 100)	rAR (testosterone = 100)	hGR (dexamethasone = 100)	hER (17β-estradiol = 100)
PF1092A	144 ± 11	16 ± 3	1.7 ± 0.2	< 0.017	< 0.0095
PF1092B	3.4 ± 0.2	0.62 ± 0.1	0.48 ± 0.02	< 0.017	< 0.0095
CP8400	21 ± 15	3.9 ± 0.2	0.21 ± 0.02	< 0.017	< 0.0095
CP8401	83 ± 4	15 ± 1	0.15 ± 0.00	< 0.017	< 0.0095
CP8481	217 ± 20	150 ± 4	0.30 ± 0.02	< 0.017	< 0.0095
MPA	ND	150 ± 10	33 ± 2	16 ± 2	< 0.0095

Relative binding affinity (RBA) was calculated as follows: (IC_{50} value of the standard steroidal compound/IC_{50} value of the test compound) × 100. IC_{50} values were calculated from three independent experiments. RBA represents the mean ± S.E.
ND: not determined.

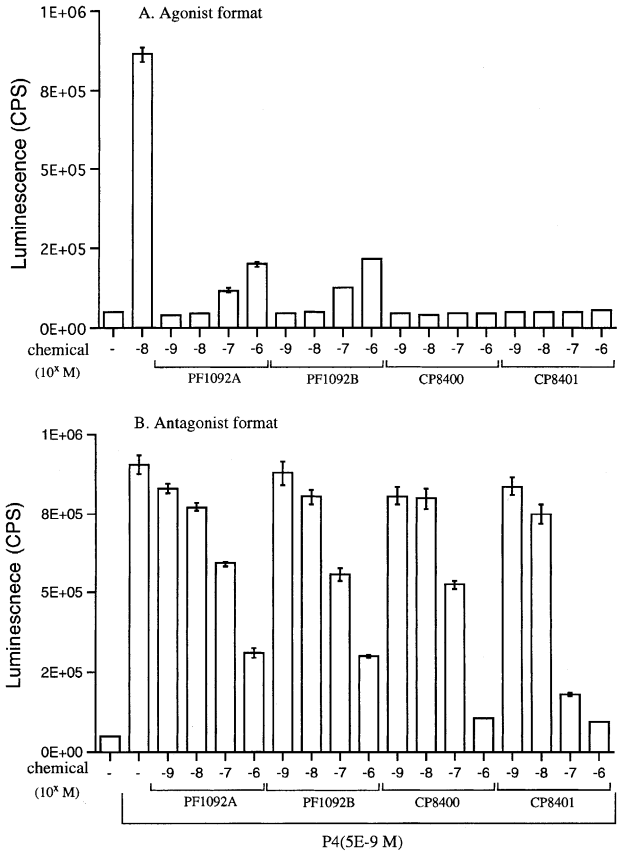

Fig. 3. Modulation of alkaline phosphatase expression in T47D by PF1092A, B, CP8400 and CP8401. The indicated concentrations of test compounds were added to T47D and incubated at 37 °C for 20 h with ((B) Antagonist format) or without ((A) Agonist format) progesterone (P4, 5 nM). Alkaline phosphatase expression was estimated by measuring luminescence with the Great EscApe™ SEAP Detection kit (CLONTECH). The results represent the means ± S.E. of three replicates.

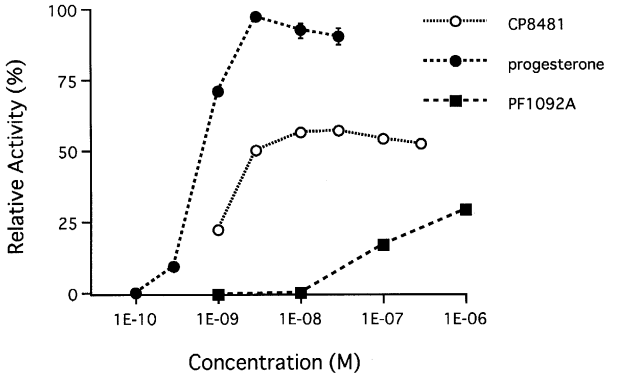

Fig. 5. Modulation of alkaline phosphatase expression in T47D by PF1092A, CP8481 and progesterone. The indicated concentrations of test compounds were added to T47D and incubated with 37 °C for 20 h. Alkaline phosphatase expression was estimated by measuring luminescence with the Great EscApe™ SEAP Detection kit (CLONTECH). Relative activity was calculated as 100% of that of 10 nM of progesterone. The results represent the means ± S.E. of three replicates.

phatase expression at 10^{-6} M in the antagonist format. However, the antagonist activity of CP8400 and CP8401 was less potent than that of RU486 (Fig. 4). The IC_{50} values of these compounds were 149 ± 31, 40 ± 8 and 1.2 ± 0.2 nM, respectively (mean ± S.E. from three independent experiments).

CP8481 was also evaluated in both expression assays to clarify its partial agonistic activity. The agonist activity of CP8481 was much stronger than that of PF1092A (Fig. 5), though it served as a partial agonist. The EC_{50} values and relative efficacy of CP8481 and progesterone were 1.8 ± 3 nM, $57 \pm 2\%$, and 1.0 ± 0.1 nM, 100%, respectively (mean ± S.E. from three independent experiments). The

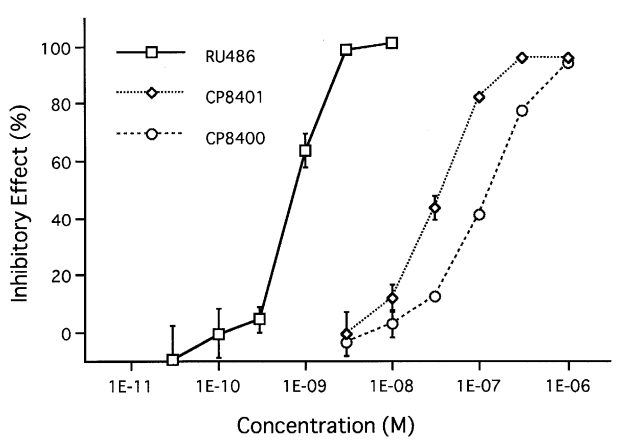

Fig. 4. Inhibitory effect of CP8400 and CP8401 on alkaline phosphatase expression. The indicated concentrations of test compounds were added to T47D and incubated at 37 °C for 20 h with progesterone (5 nM). Alkaline phosphatase expression was estimated by measuring luminescence with the Great EscApe™ SEAP Detection kit (CLONTECH). The results represent the means ± S.E. of three replicates.

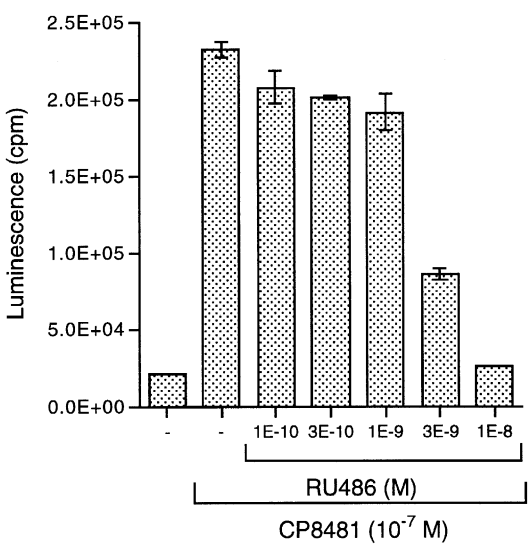

Fig. 6. Inhibition of CP8481-induced alkaline phosphatase expression by RU486. 10^{-7} M of CP8481 and indicated concentrations of RU486 were added to T47D and incubated at 37 °C for 20 h. Alkaline phosphatase expression was estimated by measuring luminescence with the Great EscApe™ SEAP Detection kit (CLONTECH). The results represent the means ± S.E. of three replicates.

agonist activity of PF1092A was so weak that its EC_{50} values and relative efficacy could not be calculated. The alkaline phosphatase expression induced by CP8481 in T47D was inhibited by RU486 in a dose-dependent manner (Fig. 6).

4. Discussion

Currently used steroidal progesterone receptor agonists such as 19-nor-teststerone and antagonists such as RU486 have been reported as possessing cross-reactivity for other steroid receptors, particularly androgen receptors and glucocorticoid receptors, which is thought to be one of the causes of their unwanted side effects (Editorial, 1996; Spitz and Bardin, 1993; Hackenberg et al., 1996). This report shows that the fungal product PF1092A and some of its derivatives possess selective affinity for both human and porcine progesterone receptors, and slight or no affinity for rat androgen receptors, human glucocorticoid receptors, and human estrogen receptors (Table 2).

There is evidence that the affinity for the progesterone receptor and the transcriptional activity of the derivatives can be changed when the 6-position of PF1092C is synthetically modified. CP8481, which differs from PF1092A in that it possesses a 2-furancarbonyloxy group in the 6-position, showed better affinity for both progesterone receptors (Table 2), had a greater effect on alkaline phosphatase expression in T47D than PF1092A (Fig. 5), and had its agonist activity antagonized by RU486 (Fig. 6). These results demonstrate that alkaline phosphatase expression induced by CP8481 occurred after CP8481 bound to progesterone receptor. The reason why only CP8481 was tested in combination with RU486 is that CP8481 is the strongest progesterone modulator in this paper. This experiment shown in Fig. 6 was performed to prove the progesterone receptor dependent-transcription activity of the compounds with this pharmacophore. These results suggest that the group at the 6-position of PF1092C might play an important role in binding to the progesterone receptor. Williams and Sigler (1998) reported the 3D-structure of the progesterone receptor-ligand binding domain as determined by X-ray analysis. From this data, we speculated that PF1092 derivatives bind to the progesterone receptor-ligand binding domain via hydrogen bonds between Arg766 on Helix 5, Gln725 on Helix 3 of the progesterone receptor-ligand binding domain, and the carbonyl group of γ-lactone of PF1092 derivatives, as well as via van der Waals contacts. The acyl group at 6- and/or 7-position(s) might interact with the progesterone receptor-ligand binding domain, as the methyl-ketone substituent at C17 of progesterone does.

In both enzyme expression assays, PF1092A and B acted as partial agonists (Figs. 2 and 3). The activity of both compounds was at the same level, though their respective affinity for the progesterone receptor was different (Table 2), which explains why they were mutually converted in the medium during incubation until the ratio of their respective concentrations became constant (PF1092A/PF1092B = 2:1) (data not shown).

CP8400 and CP8401 showed progesterone receptor antagonist activity in alkaline phosphatase expression assay (Fig. 3). They differ from PF1092A and CP8481 in that they possess the acyl group at the 7-position of PF1092C. These results provide evidence that the functional groups at the 6- and 7-positions play an important role in modulating progesterone receptor-dependent transcription.

We feel that the alkaline phosphatase expression assay is a specific progesterone-dependent expression assay, because we confirmed that other steroids (particularly testosterone) at high concentrations (10^{-7}–10^{-6} M) stimulated enzyme expression to a much greater extent in the luciferase expression assay than in the alkaline phosphatase expression assay (data not shown). CP8400 and CP8401 caused slight enzyme expression at a high concentration (10^{-6} M) in the agonist format of the luciferase expression assay (Fig. 2A), but not in the alkaline phosphatase assay (Fig. 3A). Furthermore, in the antagonist format of the luciferase expression assay, CP8401 suppressed luciferase expression at 10^{-7} M but reactivated it at 10^{-6} M (Fig. 2B). From these results, we feel that luciferase expression caused by CP8400 and CP8401 at 10^{-6} M is not related to a progesterone receptor-dependent mechanism, because the effects were not detected in the alkaline phosphatase expression assay and it is difficult to explain their contradictory effects in the antagonist format of the luciferase expression assay by a progesterone receptor-dependent mechanism.

We conclude from these results that fungal products, PF1092 compounds, are a useful pharmacophore with good affinity and selectivity for the progesterone receptor, and that it is possible to synthesize progesterone receptor-selective agonists and antagonists from this pharmacophore. Thus far, our group has reported on some chemical synthetic efforts related to PF1092 compounds (Kurihara et al., 1997, 1999; Tatsuta et al., 1997; Harimaya et al., 1997), and further work is being conducted in our laboratory to synthesize derivatives from this pharmacophore and to identify their biological activity.

References

Barengolts, E.I., Gajardo, H.F., Rosol, T.J., D'Anza, J.J., Pena, M., Botsis, J., Kukreja, S.C., 1990. Effects of progesterone on postovariectomy bone loss in aged rats. J. Bone Miner. Res. 5, 1143–1147.

Caird, L.E., Reid-Thomas, V., Hannan, W.J., Gow, S., Glasier, A.F., 1994. Oral progestogen—only contraception may protect against loss of bone mass in breast-feeding women. Clin. Endocrinol. 41, 739–745.

Combs, D.W., Reese, K., Phillips, A., 1995. Nonsteroidal progesterone receptor ligands: 1. 3-Aryl-1-benzoyl-1,4,5,6-tetrahydropyridazines. J. Med. Chem. 38, 4878–4879.

Editorial, 1996. Progestins: present and future. J. Steroid Biochem. Mol. Biol. 59, 357–363.

Graham, J.D., Clarke, C.L., 1997. Physiological action of progesterone in target tissues. Endocr. Rev. 18, 502–519.

Grunberg, S.M., Weiss, M.H., Spitz, I.M., Ahmadi, J., Sadun, A., Russell, C.A., Lucci, L., Stevenson, L.L., 1991. Treatment of unresectable meningiomas with the antiprogesterone agent mifepristone. J. Neurosurg. 74, 861–866.

Hackenberg, R., Hannig, K., Beck, S., Schmidt-Rhode, P., Scholz, A., Schulz, K.D., 1996. Androgen-like and anti-androgen-like effects of antiprogestins in human mammary cancer cells. Eur. J. Cancer 32, 696–701.

Hamann, L.G., Farmer, L.J., Johnson, M.G., Bender, S.L., Mais, D.E., Wang, M.W., Crombie, D., Goldman, M.E., Jones, T.K., 1996. Synthesis and biological activity of novel nonsteroidal progesterone receptor antagonists based on cyclocymopol monomethyl ether. J. Med. Chem. 39, 1778–1789.

Harimaya, K., Magome, E., Tabata, Y., Sasaki, T., Tsuruoka, T., 1997. Chemical conversion of progesterone receptor toxin into new eremophilanolides as progesterone receptor ligands. Sci. Rep. Meiji Seika Kaisha 36, 19–27.

Ishizaki, Y., Tanahashi, Y., Takahashi, T., Tori, K., 1970. The structure of ligularenolide : a new sesquiterpene lactone of eremophilane type. Tetrahedron 26, 5387–5393.

Kettel, L.M., Murphy, A.A., Morales, A.J., Ulmann, A., Baulieu, E.E., Yen, S.S.C., 1996. Treatment of endometriosis with the antiprogesterone mifepristone (RU486). Fertil. Steril. 65, 23–28.

Klijn, J.G.M., Setyono-Han, B., Sander, H.J., Lamberts, S.W.J., de Jong, F.H., Deckers, G.H., Foekens, J.A., 1994. Progesterone receptor-clinical and clinical treatment of breast cancer with antiprogestins. Hum. Reprod. 9, 181–189.

Kloosterboer, H.J., Deckers, G.H.J., van der Heuvel, M.J., Loozen, H.J.J., 1988. Screening of anti-progestagens by receptor studies and bioassays. J. Steroid Biochem. 31, 567–571.

Kurihara, K., Tanabe, K., Shinei, R., Okonogi, T., Ohtsuka, Y., Omoto, S., Yasuda, S., Tatsuta, K., 1997. Synthesis of (±)-PF1092A, B, and C: new nonsteroidal progesterone receptor ligands. J. Antibiot. 50, 360–362.

Kurihara, K., Tanabe, K., Yamamoto, Y., Shinei, R., Ajito, K., Okonogi, T., 1999. Synthesis and structure–activity relationships of new nonsteroidal progesterone receptor ligands. Bioorg. Med. Chem. Lett. 9, 1837–1842.

Markiewicz, L., Gurpide, E., 1994. Estrogenic and progestagenic activities coexisting in steroidal drugs: quantitative evaluation by in vitro bioassays with human cells. J. Steroid Biochem. Mol. Biol. 48, 89–94.

Murphy, A.A., Morales, A.J., Kettel, L.M., Yen, S.S.C., 1995. Regression of uterine leiomyomata to the antiprogesterone RU486: dose–response effect. Fertil. Steril. 64, 187–190.

Pathirana, C., Stein, R.B., Berger, T.S., Fenical, W., Ianiro, T., Mais, D.E., Torres, A., Goldman, M.E., 1995. Nonsteroidal human progesterone receptor modulators from the marine alga *Cymopolia barbata*. Mol. Pharmacol. 47, 630–635.

Pooley, C.L.F., Edwards, J.P., Goldman, M.E., Wang, M.W., Marschke, K.B., Crombie, D.L., Jones, T.K., 1998. Discovery and preliminary Sandorogen receptor studies of a novel, nonsteroidal progesterone receptor antagonist pharmacophore. J. Med. Chem. 41, 3461–3466.

Schilling, K., Liao, S., 1984. The use of radioactive 7 α, 17 α-dimethyl-19-nortestosterone (mibolerone) in the assay of androgen receptors. Prostate 5, 581–588.

Sheen, Y.Y., Ruh, T.S., Mangel, W.F., Katzenellenbogen, B.S., 1985. Antiestrogenic potency and binding characteristics of the triphenylethylene H1285 in MCF-7 human breast cancer cells. Cancer Res. 45, 4192–4199.

Spitz, I.M., Bardin, C.W., 1993. Clinical pharmacology of RU 486—an antiprogestin and antiglucocorticoid. Contraception 48, 403–444.

Tabata, Y., Miike, N., Hatsu, M., Kurata, Y., Yaguchi, T., Someya, A., Miyadoh, S., Hoshiko, S., Tsuruoka, T., Omoto, S., 1997a. PF1092A, B and C, new nonsteroidal progesterone receptor ligands produced by *Penicillium oblatum*: I. Taxonomy of producing strain, fermentation, isolation and biological activities. J. Antibiot. 50, 304–308.

Tabata, Y., Hatsu, M., Kurata, Y., Miyajima, K., Tani, M., Sasaki, T., Kodama, Y., Tsuruoka, T., Omoto, S., 1997b. PF1092A, B and C, new nonsteroidal progesterone receptor ligands produced by *Penicillium oblatum*: II. Physico-chemical properties and structure elucidation. J. Antibiot. 50, 309–313.

Tatsuta, K., Yasuda, S., Kurihara, K., Tanabe, K., Shinei, R., Okonogi, T., 1997. Total synthesis of progesterone receptor ligands, (−)-PF1092A, B, and C. Tetrahedron Lett. 38, 1439–1442.

Williams, S.P., Sigler, P.B., 1998. Atomic structure of progesterone complexed with its receptor. Nature 393, 392–396.

Zhi, L., Tegley, C.M., Marschke, K.B., Mais, D.E., Jones, T.K., 1999. 5-Aryl-1,2,3,4-tetrahydrochromeno[3,4-*f*]quinolin-3-ones as a novel class of nonsteroidal progesterone receptor agonists: effect of A-Ring modification. J. Med. Chem. 42, 1466–1472.