A Tissue-Selective Nonsteroidal Progesterone Receptor Modulator: 7,9-Difluoro-5-(3-methylcyclohex-2-enyl)-2,2,4-trimethyl-1,2-dihydrochromeno[3,4-*f*]quinoline

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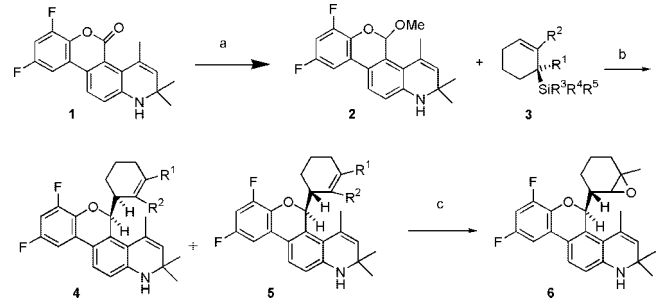
Abstract: The progesterone receptor plays an important role in the female reproductive system. Here we describe the discovery of a new selective progesterone receptor modulator (SPRM). In rats, the lead compound, 7,9-difluoro-5-(3-methylcyclohex-2-enyl)-2,2,4-trimethyl-1,2-dihydrochromeno[3,4-*f*]quinoline (**5c**), inhibited ovulation and showed full efficacy in uterine and vaginal tissue but was a mixed partial agonist/antagonist in breast tissue. The compound also suppressed ovulation in monkeys, but in contrast to currently approved steroidal PR agonists, it did not suppress estradiol levels.

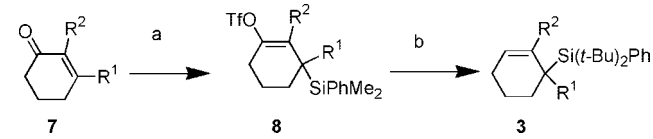
The progesterone receptor (PR[a]) is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Two major isoforms of the progesterone receptor, PR-A and PR-B, have been identified.[1] The progesterone receptor is mainly expressed in female reproductive tissues, and the actions in these tissues have been the target of drugs acting on PR. Synthetic steroidal PR agonists, in combination with estrogens, have been widely used in oral contraception (OC), female hormone replacement therapy (HRT), and the treatment of a number of female reproductive disorders for decades. PR antagonists, like mifepristone, effectively terminate pregnancy and offer therapeutic promise for endometriosis, uterine fibroids, and breast cancer.[2] Recently, clinical data on asoprisnil, a selective PR modulator, has been reported, targeting endometriosis.[3] Like the natural ligand progesterone, all of the currently marketed progestins possess some degree of cross-reactivity with other steroid hormone receptors, especially androgen and glucocorticoid receptor, which can cause potential side-effects. Addition of a progestin in estrogen replacement therapy has proven to significantly reduce endometrial cancer risk due to its antiestrogenic activity in the uterus. However, in breasts, progestins demonstrate stimulative activity in addition to the proliferative effect of estrogens. This has raised some concern about a potential breast cancer risk for women with long-term use of progestins in OC and HRT in combination with estrogens even though the clinical results have been controversial. The latest results from the Women's Health Initiative confirmed the increased breast cancer risk for women who used combined hormone preparation of conjugated equine estrogens and medroxyprogesterone acetate (MPA). Selective progesterone receptor modulators (SPRMs) can potentially alleviate some of the risks associated with currently available progestins. In an effort to further optimize a series of selective PR modulators,[4] we investigated 5-cycloalkenyl substituted chromenoquinolines that have been reported as very efficacious and potent glucocorticoid receptor (GR) modulators developed from the PR template.[5] Here we report results on the series of SPRMs that demonstrated tissue selectivity in various animal models. The lead compound **5c** is a highly potent and efficacious selective PR modulator and demonstrated full efficacy in a rat ovulation inhibition assay. The compound was a partial agonist/antagonist in breast tissue.

Scheme 1[a]



[a] Reagents: (a) (i) DIBAL-H, THF, −78°C, (ii) MeOH, p-TsOH, 71%; (b) $BF_3 \cdot OEt_2$, CH_2Cl_2 55−90%; (c) *m*-CPBA, CH_2Cl_2, 4%.

Scheme 2[a]



[a] Reagents: (a) (i) $Me_2PhSiLi$, CuCN, MeLi, THF, (ii) $PhN(Tf)_2$, 45−78%; (b) $Pd(OAc)_2$, PPh_3, Et_3N, HCO_2H, DMF, 65−75%.

Scheme 1 describes the synthesis of the target compounds **4**, **5**, and **6**. Reduction of the lactone **1**[5] with diisobutylaluminium hydride followed by treatment with methanol in the presence of a catalytic amount of *para*-toluenesulfonic acid afforded the stable methoxyacetal **2** as a racemic mixture. Nucleophilic addition of cycloalkenylsilanes **3** to the oxycarbenium ion generated from this acetal was used to prepare 5-cycloalkenyl compounds **4** and **5**. Racemic substituted cyclohexenylsilanes **3** were prepared in a two-step procedure as described in Scheme 2. Copper mediated conjugate addition of $PhMe_2SiLi$ to a cyclohexenone (**8**) and trapping the enolate with phenyltriflimide afforded the enol triflate (**9**).[6] The triflate was reduced to the cyclohexenyl silane (**3**) with palladium acetate and formic acid/triethylamine.[7] Addition of the substituted allylic silanes (**3**) furnishes products with two new chiral centers, and generally a mixture of two racemic diastereomers was obtained. The *anti* (**4**) and *syn* (**5**) diastereomers were separated by chromatographic methods initially. When tested in various biological assays (vide infra), it was found that the *syn* diastereomer of 5-(3-methylcyclohex-2-enyl)-chromenoquinolines was much more active than the *anti* diastereomer. Therefore we sought to improve the diastereoselectivity in the addition reaction in favor of the *syn* diastereomers (**5**). When we initially prepared these compounds via a Lewis acid catalyzed reaction of methoxy acetal **2** with dimethyl(1-methylcyclohex-2-enyl)phenylsilane, the *syn*/*anti*

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[a] Abbreviations: PR, progesterone receptor; SPRM, selective progesterone receptor modulator; MPA, medroxyprogesterone acetate; OC, oral contraception; HRT, hormone replacement therapy; MCPBA, *meta*-chloroperbenzoic acid; OVX, ovariectomized.

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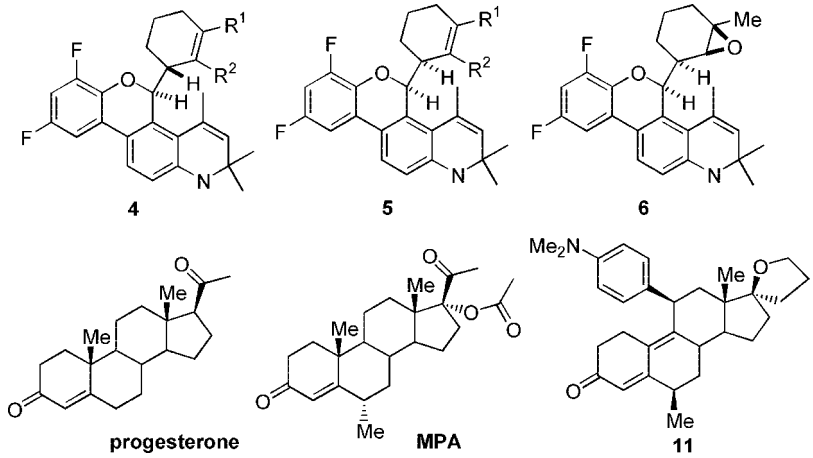
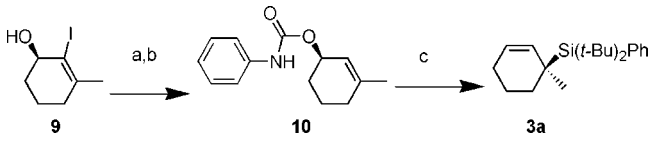
Table 1. Cotransfection, T47D Alkaline Phosphatase Assay, and Competitive Binding Data for SPRMs, Progesterone, and MPA



compound	R^1	R^2	hPR agonist EC_{50} (nM)	hPR agonist[a] Eff (%)	hPR T47D assay agonist EC_{50} (nM)	hPR T47D assay agonist Eff (%)	hPR binding K_i (nM)
5a (*rac, syn*)	H	H	3.4 ± 0.4	171 ± 32	27 ± 3	66 ± 4	4.8 ± 0.6
5b (*rac, syn*)	CH_3	H	0.6 ± 0.2	162 ± 12	3.5 ± 0.9	54 ± 3	3.7 ± 0.5
4b (*rac, anti*)	CH_3	H	5.4 ± 0.7	100 ± 16	33 ± 4	63 ± 3	14
(−)**-5c** (*syn*)	CH_3	H	0.5 ± 0.1	145 ± 12	4.3 ± 0.9	55 ± 2	4.4 ± 1.8
(+)**-5d** (*syn*)	CH_3	H	50.8 ± 3.1	11 ± 5			>1000
5e (*rac,syn*)	CH_3	CH_3	10.7 ± 1.6	97 ± 17	96 ± 9	66 ± 5	n.d.
(−)**-6**			0.4 ± 0.1	177 ± 15	2.8 ± 0.3	59 ± 6	1.2 ± 0.2
progesterone			0.5 ± 0.0	100	2.2 ± 0.1	100	3.4 ± 0.1
MPA			0.5 ± 0.3	98 ± 11	0.4 ± 0.1	98 ± 6	1.4 ± 0.3

[a] Agonist efficacies were determined relative to progesterone (100%), a hyphen indicates efiicacy <10%.

Scheme 3[a]



[a] Reagents: (a) Phenylisocyanate, Et_3N, DMAP, Et_2O, 0°C, 70%; (b) *n*-Butyllithium, Et_2O −85°C, 50%; (c) $(t\text{-Bu})_2PhSiLi$, CuI, PPh_3, THF, 80%.

products were obtained in a 2:1 ratio. Changing the reaction conditions, by varying temperature, Lewis acid, stoichiometry, solvent, concentration, order of addition, and rate of addition, led only to minor improvements in the *syn*/*anti* ratio of the product. It was found later that the *syn*/*anti* ratio of the product was highly dependent on the size of the silane substituent of the 1-methylcyclohex-2-enylsilanes and using the bulky diphenyl-*t*-butylsilane improved the diastereoselectivity of the reaction significantly to furnish the addition product with an 8:1 *syn*/*anti* ratio. As can be seen in Table 1, the (5*S*)-enantiomer (−)**5c** had much greater hPR activity than the (5*R*)-enantiomer (+)**5d**. As a result, we focused our attention on developing an enantioselective synthesis of product (−)**5c**. We were pleased to find that the Lewis acid promoted reaction of the chiral silane **3a** with the racemate methoxyacetal **2** is highly stereospecific. In fact, when enantiomerically enriched silane was used, the products **4** and **5** that were obtained had the same enantiomeric ratio as the starting silane, and we were not able to detect any other enantiomer by analytical chiral HPLC when enantiomerically pure allylsilane was used. The required enantiomerically pure cyclohexenylsilane **3a** was obtained from the enantiomerically pure 2-iodo-3-methylcyclohex-2-enol[8] **9** following literature procedures for similar systems (Scheme 3). Conversion of the alcohol to the carbamate with phenylisocyanate, followed by an S_N2 displacement with the silylcuprate was regio- and enantiospecific[9] and afforded the enantiomerically pure cycloalkenylsilane **3a**. The epoxide **6** was obtained from treatment of the alkene (−)**5c** with MCPBA, but oxidation mainly took place at the dihydroquinoline double bond and the epoxide **6** was only a minor product and isolated in 4% yield.

Table 1 summarizes the SAR results of several cycloalkenyl substituted dihydrochromeno-quinolines in cotransfection and competitive binding assays. In line with previous findings on D-ring SAR,[10] it was found that 7,9-difluoro substituents were superior to other D-ring substitution patterns tested,[5b] and the results shown here are for compounds with a 7,9-difluoro substituted D-ring. As can be seen from the data, the substituents on the cycloalkene ring have a significant influence on the activity of the compounds. The unsubstituted cyclohexenyl compound **5a** showed quite good efficacy and potency, whereas the 2,3 dimethylcyclohexenyl analog, **5e**, was less efficacious and potent. The 3-methylcyclohexenyl (**5b**) is most optimal and showed the best activity with more than 5-fold EC_{50} (0.6 nM) improvement over **5a** (3.4 nM). As we have pointed out, the stereochemistry of the compounds affects their biological activity, the *anti*-isomer **4b** is much less active (EC_{50} of 5.4 nM) than the *syn*-isomer **5b**, although there is no difference between their binding affinities. For the compounds that were tested in enantiomerically pure form, it was clear that almost all of the activity resides in one of the enantiomers, which is consistent with the results of all other related series. As can be seen, eutomer (−)**5c** has activity almost identical to racemate compound **5b** and distomer (+)**5d** is inactive. Epoxide **6** showed very good in vitro biological activity, indicating the epoxide can be used as a bioisostere of olefin that is an essential piece of the activity. However, we did not pursue this compound further because of its complexity of synthesis.

Compound (−)**5c** was one of the most efficacious (145% of progesterone) and potent compounds tested in the cotransfection assay, and it also had excellent binding affinity for the human progesterone receptor. When tested for PR regulated alkaline phosphatase activity in the T47D breast cancer cell line, which

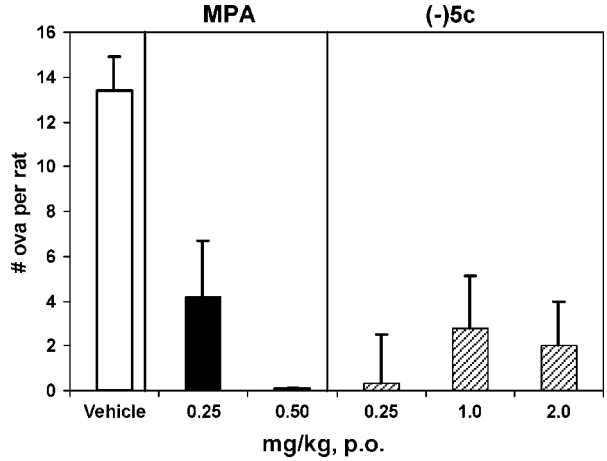


Figure 1. Ovulation inhibition by MPA (black bar) or (−)**5c** (striped bar) in rats. Rats ($n = 6$) were dosed with vehicle or progestin for 5 days. The number of ova per rat and the number rats ovulating were determined.

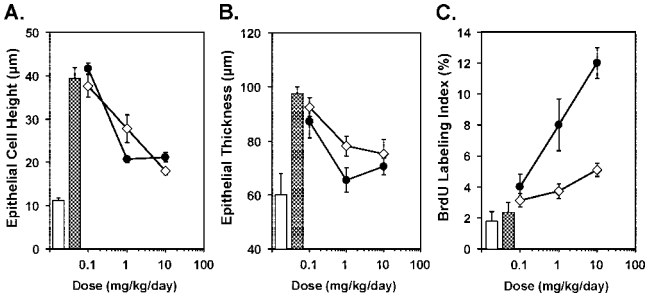


Figure 2. Progestational effects of MPA (black dots) or (−)**5c** (open diamonds) in combination with estrone on uterus (A), vagina (B), and breast (C) epithelium in ovariectomized rats. Open bar represents effect of vehicle and hatched bar represents effect of estrone treatment.

was used as a surrogate marker for breast activity, the compound showed only partial efficacy (55%), whereas the steroidal reference compounds (progesterone, MPA) showed full efficacy in this assay. Cross-reactivity of (−)**5c** with other steroidal hormone receptors was also tested in a cotransfection assay, and the compound was found to exhibit more than 100-fold selectivity toward the hPR versus human androgen receptor (hAR), human mineralocorticoid receptor (hMR), and human estrogen receptor alpha (hERα), with weak antagonist activity on the human glucocorticoid receptor (hGR), but no in vivo antiglucocorticoid activity was observed at doses up to 30 mg/kg.[11] (cotransfection assay data in Supporting Information).

In the classic Clauberg−McPhail assay to read in vivo progestational activity in rabbit uterine epithelium, compound (−)**5c** was active at an oral dose of 0.10 mg/kg for 3 days. (data not shown).

(−)**5c** was tested to determine its ability to inhibit ovulation in the female rat (Figure 1). (−)**5c** was able to dramatically reduce the number of ova at doses of 0.25, 1.0, or 2.0 mg/kg. To determine tissue selectivity of (−)**5c**, the effects on uterine epithelium cell height, vaginal epithelial thickness, and mammary alveolar bud proliferation in estrogen treated ovariectomized (OVX) rats were measured. While (−)**5c** shows similar efficacy as MPA in the uterus and vagina, it demonstrated significantly reduced efficacy and potency on breast alveolar bud proliferation compared to MPA (Figure 2).

The reduced efficacy seen in breast tissue prompted us to run the same experiment in antagonist mode. In this experiment MPA was dosed at its ED_{50} of 1 mg/kg, and (−)**5c** was given

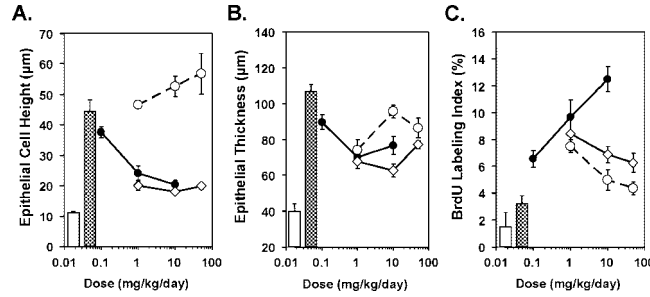


Figure 3. Effect of (−)**5c** (open diamonds) and **11** (open circles) in combination with MPA (1 mg/kg) and estrone on uterus (A), vagina (B), and breast (C) epithelium in ovariectomized rats. Effect of MPA and estrone is shown as black circles. Open bar represents effect of vehicle and hatched bar represents effect of estrone treatment.

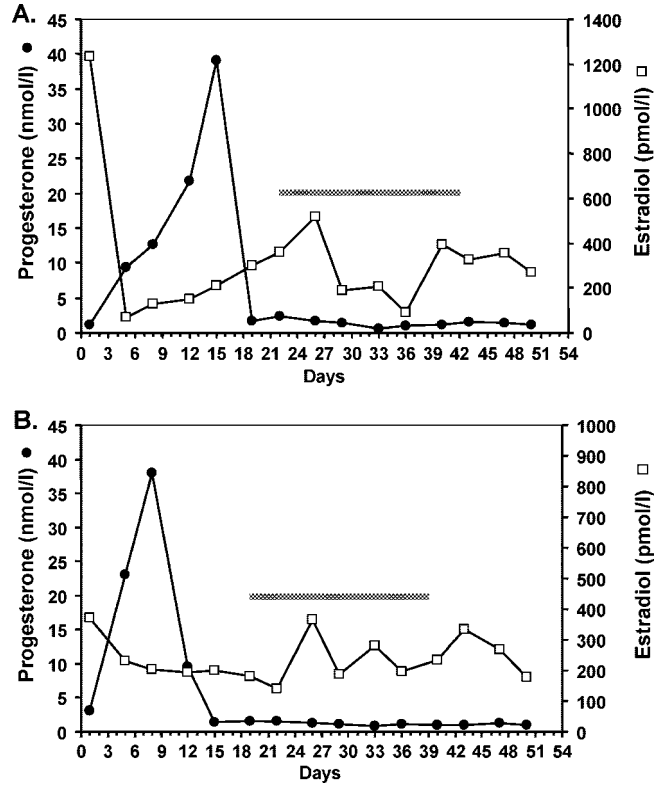


Figure 4. Ovulation inhibition by (−)**5c** in cynomolgus monkeys. Estradiol (open squares) and progesterone (closed circles) were measured in a cynomolgus monkey before and after 3 weeks of daily treatment with 0.06 mg/kg (A) or 0.3 mg/kg (B) of (−)**5c** (stippled bar).

in a dose response from 1 to 30 mg/kg. The results are shown in Figure 3. As can be seen in this figure, (−)**5c** was able to partially (~60% efficacy) antagonize the effect of MPA in breast tissue similar to a typical steroidal PR antagonist **11.**[11] (−)**5c** had no impact on the effect of MPA on the uterine epithelium cell height or vaginal epithelial thickness, whereas **11** totally reversed the effect of MPA, thereby demonstrating the unique tissue dependent partial agonist/antagonist activity of this compound.

To demonstrate the ability of (−)**5c** to inhibit ovulation in nonhuman primates, the compound was dosed orally in cynomolgus monkeys for 3 weeks. Four monkeys were given either 0.06 mg/kg or 0.3 mg/kg. Estradiol and progesterone levels were measured as indicators of the ovulatory cycle. As can be seen in Figure 4, daily dosing of (−)**5c** at 0.06 mg/kg (Figure 4A) or 0.3 mg/kg (Figure 4B) resulted in suppression of progesterone

levels, indicating inhibition of ovulation. Similar results to those shown in Figure 4 were seen in all monkeys ($n = 4$). Interestingly the estradiol levels in these animals were not fully suppressed and followed a cyclic pattern during treatment. This is in contrast to known steroidal progesterone agonists, where a suppression of estradiol levels is observed. These results are another indication of the unique activity of (−)**5c** in vivo.

In summary, (−)**5c** is a potent nonsteroidal selective hPR modulator. The compound exhibits tissue selectivity, with full suppression of estrogen induced uterine wet weight in rats but partial agonistic/antagonistic effects on alveolar bud proliferation in the same animals. Compound (−)**5c** is a potent inhibitor of ovulation in cynomolgus monkeys but does not fully suppress estrogen levels in contrast with steroidal progesterone agonists, which provides an opportunity to develop progestin alone contraceptives.

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Supporting Information Available: Synthetic procedures, chemical characterization data for compounds **4**−**6** and a description of the biological assays, including ovulation inhibition results in cynomolgus monkeys. This material is available free of charge via the Internet at http://pubs.acs.org.

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