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Synthesis and Biological Activity of 5-Methylidene 1,2-Dihydrochromeno[3,4-*f*]quinoline Derivatives as Progesterone Receptor Modulators

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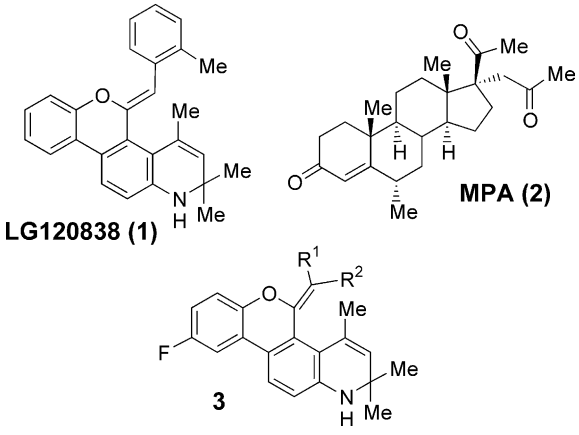
Abstract—A series of 5-methylidene 1,2-dihydrochromeno[3,4-*f*]quinoline derivatives were synthesized and tested in biological assays to evaluate scope and limitations of the nonsteroidal SPRM pharmacophore (**3**). A number of orally available highly potent nonsteroidal modulators were identified by modification of the substituents at 5-methylidene position.

The 5-substituted 1,2-dihydrochromeno[3,4-*f*]quinoline has been demonstrated to be a robust pharmacophore for human progesterone receptor (hPR) modulators.[1] One of the appealing series developed from the pharmacophore contains an unique achiral 5-benzylidene moiety (i.e., LG120838, **1**). This compound exhibited potent progestational activity similar to medroxy-progesterone acetate (MPA, **2**) in a rodent model via oral administration.[2] A newer analogue in the series demonstrated tissue selective progesterone receptor modulator (SPRM) activities, as evidenced by antagonism of estradiol-induced proliferation in uterus and vagina but with less stimulation of breast tissues.[3] To further explore the structure–activity relationship (SAR) around the methylidene region, we synthesized a number of novel analogues (**3**) and evaluated their biological activities in cotransfection[4] and competitive binding assays as well as in an in vivo model.

Most of the new analogues of general structure **3** were prepared by the synthetic routes as shown in Scheme 1. Treatment of the lactone **4**[1b] with an alkyl lithium reagent to afford hemiacetal **5** and the acid catalyzed dehydration gave compounds of structure **3** as *Z*-isomer when R^2 is hydrogen and as mixture of *Z*/*E*-isomers when R^2 is an alkyl in 80+% yield. Reaction of the lactone **4** with Tebbe Reagent[5] afforded the unsubstituted 5-methylidene compound **6** in 50% yield. Selective bromination of methylidene **6** with NBS provided compound **7** with the *Z*-olefin configuration (51%) and no *E*-isomer was observed. Compound **18** was prepared by treatment of lactone **4** with the dithiane anion.[6]



Scheme 2 describes the modification of the 5-methylidene side chain from compound **8**, which was prepared by the route depicted in Scheme 1 (R^1=CN, R^2=H). Reduction of the cyano methylidene **8** with DIBAL-H afforded the aldehyde analogue **9**. Addition of isopropyl-magnesium bromide to aldehyde **9** followed by TsOH catalyzed dehydration provided the 5-dienyl analogue **10** in 70% yield. Direct addition of methyllithium

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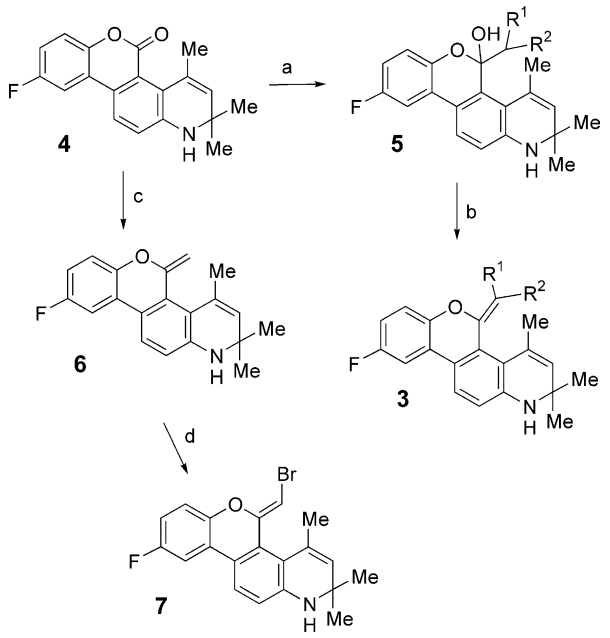
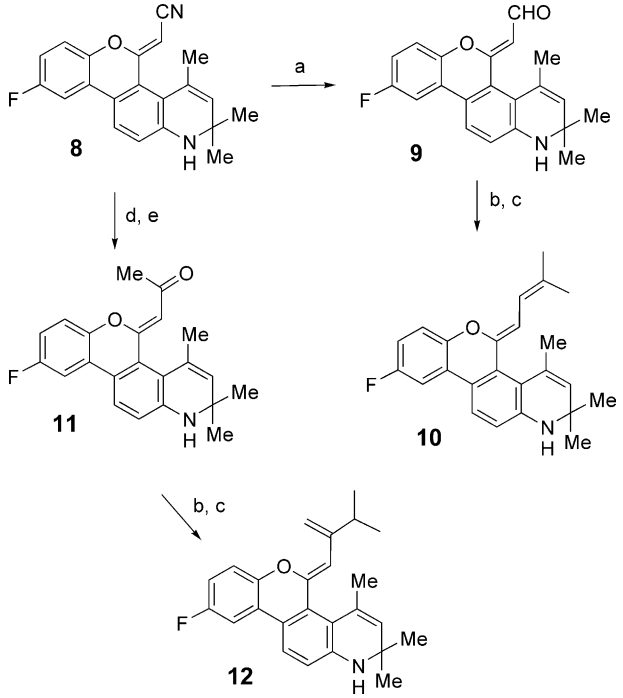


Scheme 1. (a) R^1R^2CHLi, THF, −50 °C; (b) TsOH, CH_2Cl_2, rt; (c) Tebbe reagent, THF/toluene, −40 °C to rt; (d) NBS, DMF, rt.

to compound **8** followed by workup with aqueous base gave the acetyl methylidene **11**. Similar treatment of compound **11** with isopropylmagnesium bromide/TsOH afforded unexpected compound **12** in low yield.

Results and Discussion

The affinities of the new analogues **3** and hPR were measured by a competitive binding assay.[7] The ability



Scheme 2. (a) DIBAL-H, ether, rt; (b) *i*PrMgBr, THF, rt; (c) TsOH, CH_2Cl_2, rt; (d) MeLi, THF; (e) aqueous NaOH.

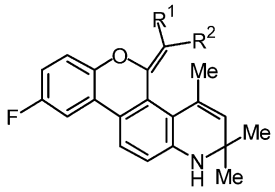
of the new compounds to modulate the transcriptional activity of hPR in a cellular context was determined by using the cotransfection assay in CV-1 cells. The assay results of the new analogues are depicted in Table 1. Progesterone and MPA were used as the standard steroidal hPR agonists and mifepristone as a standard hPR antagonist. 5-Methylidene analogue **6** was a weak hPR agonist in the cotransfection assay despite of its excellent binding affinity. Introduction of a propyl group on the 5-methylidene (R^1 = propyl, R^2 = H, **13**) significantly improved the agonist activity. Compound **14**, prepared to test the necessity of an aromatic substituent at the methylidene moiety, had much weaker activity in both assays. The vinylic analogues **10** and **12** demonstrated super progestational activity, which implies that the olefin can serve as a bioisostere of an aryl as part of the 5(*Z*)-side-chain. The 5(*Z*)-bromo analogue **7** unexpectedly behaved as a highly potent super agonist, which suggests that bromine behaved similar to an olefin rather than an alkane in this series. Introduction of a heteroatom into the 5(*Z*)-conjugated-olefin side chain converted the hPR agonist pharmacophore into antagonists (**8**, **9** and **11**). The cyano analogue **8** demonstrated decent hPR antagonist activity, which can be considered as an excellent lead for nonsteroidal antiprogestins.[8]

During our SAR study of the 5-benzylidene pharmacophore (i.e., **1**), we found that the 5(*Z*)-olefin analogues are light sensitive in solution. The pure *Z*-isomer obtained as a sole product from the acid catalyzed dehydration of compound **5** isomerized to a mixture of *Z*/*E*-isomers under prolonged exposure to white light.[9] The symmetric ($R^1 = R^2$) analogues **17** and **18** were designed to circumvent the *Z*/*E* isomerization issue and were found to be light-stable. Compound **18** behaved as an excellent nonsteroidal progestin in the bioassays. However, diaryl analogue **17** is inactive. Compounds **15** and **16** with two small alkyl groups at the 5-methyliene had biological activities similar to propyl analogue **13**, which indicates that a small R^2 group is well tolerated for the agonist activity.

The cross-reactivity of selected new nonsteroidal progestins with other steroid receptors was assessed using human androgen (hAR), glucocorticoid (hGR), estrogen (hER), and mineralocorticoid receptor (hMR) cotransfection assays (Table 2). No agonist activity was observed for any of the test compounds, but antagonist activities were detected, most notably on hAR and hGR. Compounds which displayed the most significant (<1000 nM) cross-reactivities are tabulated. The separation between hPR agonist and hAR antagonist activity was generally 100-fold or greater, particularly for the more active analogues.

To quickly assess the in vivo progestational activities of the new analogues, an immature female rat model was employed by utilizing a modification of several published methods.[10] Briefly, the sexually immature female rats were primed with an estrogen to induce proliferation of uterine luminal epithelium.[11] On day 2, animals were randomly assigned to control and treatment

Table 1. hPR agonist and antagonist activity in cotransfected CV-1 cells and binding affinitites to hPR[a]



No.	Compd		hPR cotransfection assay in CV-1 Cells[b]				hPR binding K_i (nM)
	R^1	R^2	Agonist Efficacy (%)	EC_{50} (nM)	Antagonist Efficacy (%)	IC_{50} (nM)	
	Progesterone (Pr.)		100	2.9±0.9	—[c]	—	3.5±0.2
	Mifepristone		—	—	99±0	0.30±0.04	1.1±0.3
1	LG120838		166±35	5.7±3.7	—	—	0.66±0.20
2	MPA		80±7	0.15±0.05	—	—	0.34±0.04
6	H	H	87±6	1000±300	—	—	6.1±2.6
7	Br	H	135±28	2.2±0.9	—	—	2.1±0.3
8	CN	H	70±10	2700±600	75±7	65±6	45±8
9	CHO	H	—	—	97±2	243±46	>100
10	Isobutenyl	H	172±16	1.7±0.6	—	—	2.5±0.4
11	Acetyl	H	—	—	91±2	124±42	>100
12	2-(3-Me-1-butenyl)	H	122±24	29±12	—	—	26
13	*n*-Pr	H	90±21	28±12	—	—	3.1
14	Cyclohexyl	H	28±4	48±10	70	800	>100
15	Et	Me	112±35	11±1	—	—	8.0±2.9
16	Me	Et	64±17	27±5	38	980	16.7±4.9
17	Ph	Ph	—	—	90±8	1930±320	>100
18	$S(CH_2)_3S$		151±26	2.1±1.0	—	—	6.3±5.7

[a]Efficacy for agonist assays is defined in % versus progesterone = 100. Efficacy for antagonist assays is % inhibition of transcriptional activity observed at an EC_{50} concentration of progesterone.
[b]Values are in nM, mean±SEM, $N>2$. If no SEM is noted, value is from $N<3$.
[c]'—' = not active (<20% efficacy and/or >10 μM potency).

Table 2. Antagonist cross-reactivities of selected new compounds with hAR, hGR, hER, and hMR[a,b]

Compd	hAR Eff. (%)	hAR IC_{50} (nM)	hGR Eff. (%)	hGR IC_{50} (nM)	hER Eff. (%)	hER IC_{50} (nM)	hMR Eff. (%)	hMR IC_{50} (nM)
Progesterone	46±7	37±2	—	—	—[d]	—	83±14	14±4
MPA	159±10[c]	6.1±1.0[c]	157±22[c]	10±1[c]	—	—	—	—
7	85	250	—	—	—	—	—	—
10	—	—	65±4	177±68	—	—	—	—
15	88±6	250±35	99	324	—	—	—	—
18	79±6	180±30	99±1	146±77	84	631	92	681

[a]Efficacy is % inhibition of transcriptional activity observed at an EC_{50} concentration of DHT for AR, dexamethasone for GR, estradiol for ER and aldosterone for MR.
[b]See Table 1 for legend.
[c]Agonist efficacy, EC_{50}.
[d]Efficacy <20% and/or potency >1000 nM.

groups ($n=5$) and administered orally the test compounds or vehicle. On day 3, 5-bromo-2′-deoxyuridine (BrdU) was injected intra-peritoneal 2 h before sacrifice for immunohistochemically labeling of proliferating cells. Animal uteri were isolated and subjected to routine histology using paraffin embedding and immunochemistry procedures. Number of BrdU-labeled nuclei was determined in a defined area of the luminal epithelium and the labeling index was calculated by expressing the number of BrdU-labeled nuclei per mm^2 of uterine luminal epithelium. For statistical data analysis, mean and standard error (SEM) were calculated for each experimental group, and the level of significance estimated by using Student's *t*-test. The results of MPA as a positive control and a representative analogue **18** are summarized in Figure 1. A single injection of 17β-estradiol benzoate (1 μg/rat in 0.1 mL of corn oil) induced a 10-fold proliferation of the luminal epithelium over the basal level (vehicle control). Treatment of the rats with MPA (0.15 mg/kg) in corn oil significantly suppressed the proliferation. Compound **18** was orally administrated in corn oil and demonstrated significant antiproliferative effect in both 0.05 and 0.15 mg/kg. The efficacy and potency of compound **18** in the assay is similar or superior to that of MPA.

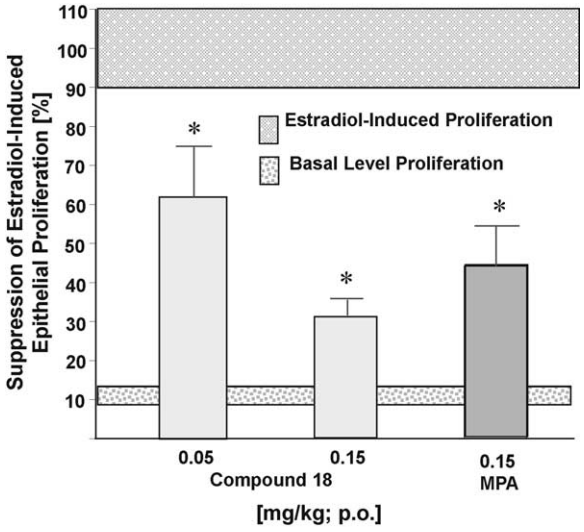


Figure 1. Effects of progestational compounds on suppression of estradiol-induced proliferation of uterine epithelium in immature female rats (* = $p < 0.01$ vs estradiol group).

Summary

The new 5-methylidene nonsteroidal series exhibited potent hPR modulating activity, especially as progestins. The present SAR study expanded the scope of the original 5(*Z*)-benzylidene pharmacophore and generated a number of analogues with high potency and desired selectivity for hPR over other related steroid hormone receptors.

References and Notes

1. (a) For SAR information of the related analogues and clinical opportunities of nonsteroidal progestins, see: Zhi, L.; Tegley, C. M.; Kallel, E. A.; Marschke, K. B.; Mais, D. E.; Gottardis, M. M.; Jones, T. K. *J. Med. Chem.* **1998**, *41*, 291. (b) Edwards, J. P.; West, S. J.; Marschke, K. B.; Mais, D. E.; Gottardis, M. M.; Jones, T. K. *J. Med. Chem.* **1998**, *41*, 303. (c) Edwards, J. P.; Zhi, L.; Pooley, C. L. F.; Tegley, C. M.; West, S. J.; Wang, M.-W.; Gottardis, M. M.; Pathirana, C.; Schrader, W. T.; Jones, T. K. *J. Med. Chem.* **1998**, *41*, 2779. (d) Zhi, L.; Tegley, C. M.; Edwards, J. P.; West, S. J.; Marschke, K. B.; Gottardis, M. M.; Mais, D. E.; Jones, T. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3365. (e) Zhi, L.; Tegley, C. M.; Marschke, K. B.; Mais, D. E.; Jones, T. K. *J. Med. Chem.* **1999**, *42*, 1466.

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6. Preparation of compound **18** [structure **3** of Scheme 1, where R^1, $R^2 = –S(CH_2)_3S–$]: To a solution of 1,3-dithiane (0.24 g, 2.0 mmol) in THF (10 mL) at −70 °C was added *n*BuLi (1.6 M in hexane, 1.3 mL) and the resulting mixture was stirred at −10 °C for 2 h. To the reaction mixture at −70 °C was added lactone **4** (0.12 g, 0.40 mmol) in THF (1 mL). The dark red solution was slowly warmed to −30 °C till the red color fade away and quenched immediately with water. Extraction with EtOAc and chromatography afforded the intermediate **5**, which was treated in CH_2Cl_2 (10 mL) with catalytic amount of TsOH for 15 h. The reaction was quench with aqueous carbonate and extracted with EtOAc. Chromatography provided compound **18** (70 mg, 42%) as a yellow solid, mp 120–122 °C, ^{1}H NMR (400 MHz, $CDCl_3$) 7.34 (d, $J = 8.3$ Hz, 1H), 7.32 (dd, $J = 9.7$ and 2.9 Hz, 1H), 7.07 (dd, $J = 8.7$ and 4.9 Hz, 1H), 6.84 (td, $J = 8.4$ and 2.8 Hz, 1H), 6.62 (d, $J = 8.3$ Hz, 1H), 5.48 (s, 1H), 4.17 (s, 1H), 3.02 (ddd, $J = 13.4$, 8.2 and 5.1 Hz, 1H), 2.91–2.79 (m, 2H), 2.68 (dt, $J = 13.4$ and 5.5 Hz, 1H), 2.20–2.04 (m, 2H), 1.99 (s, 3H), 1.41 (s, 3H) and 1.28 (s, 3H). Anal. calcd for $C_{23}H_{22}FNOS_2$: C, 67.12; H, 5.39; N, 3.40. Found: C, 67.17; H, 5.33; N, 3.29.

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9. This series of compounds are relative stable in solid state or under yellow light even in solution.

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11. A detailed description of the assay will be published elsewhere.