Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 415–418

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Nonsteroidal Progesterone Receptor Antagonists Based on 6-Thiophenehydroquinolines

Lin Zhi,* Christopher M. Tegley,† Barbara Pio,‡ Sarah J. West, Keith B. Marschke, Dale E. Mais and Todd K. Jones‡

Ligand Pharmaceuticals, 10275 Science Center Drive, San Diego, CA 92121, USA

Received 28 October 1999; accepted 13 December 1999

Abstract—Synthesis and biological evaluation of 6-thiophene 1,2-dihydro or 1,2,3,4-tetrahydroquinoline derivatives resulted in a number of potent nonsteroidal antiprogestins.

Introduction

A number of novel classes of nonsteroidal antiprogestins[1–6] have emerged during the past several years aimed at improving the side-effect profile inherent with steroidal antiprogestins and exploring therapeutic opportunities other than as abortifacients, such as the treatment of breast cancer, endometriosis and uterine fibroids or as contraceptive agents.[7,8] Recently, we reported the discovery and preliminary structure–activity relationships (SAR) of 6-phenyl 1,2-dihydroquinoline analogues, the first orally available nonsteroidal antiprogestin pharmacophore, using cotransfection and competitive binding assays as guides.[9,10] The lead compound LG120830 (**1**) demonstrated potent in vivo antiprogestational activity which is equivalent to onapristone (**2**, ZK98,299) in the mouse implantation assay.[9] However, the noted hepatomegaly in the tested animals especially in the high dose groups raised some concerns about the series.[9] To continue the SAR study of the novel pharmacophore and to address the hepatomegaly issue, we explored the pendant 6-aryl group and the dihydroquinoline ring. This report describes our new findings in using 6-thiophene as a bioisostere of the 6-phenyl group as well as preliminary SAR results on quinoline ring modification (Fig. 1).

Chemistry

The preparation of compounds of general structure **3** is depicted in Scheme 1 wherein the bromothiophenes were coupled with the dihydroquinoline boronic acid (**4**)[9] via a palladium catalyzed Suzuki reaction.[11] Removal of the *t*-Boc protection group with TFA afforded compounds **5** and **6** in moderate to high yield. The racemic tetrahydroquinoline analogue **7** was obtained by palladium catalyzed hydrogenation of the corresponding dihydroquinoline analogue **6**. Scheme 2 describes the synthesis of the 3-quinolinone analogues **11** and **12** by a similar palladium catalyzed cross-coupling strategy but using boronic acid **10**. Hydroboration of dihydroquinoline **8** followed by oxidation and methylation provided the 3-quinolinone **9**, which was converted to boronic acid **10** by the standard lithiation/borate-formation/hydrolysis procedures. The preparation of compounds **5c** and **11** are illustrative.[12,13]

Results and Discussion

The biological activity of the new compounds on human progesterone receptor (hPR) were evaluated in a cotransfection assay in CV-1 cells (African green monkey fibroblasts) and in a competitive binding assay.[14] The results are summarized in Table 1. Progesterone, the 6-phenyl analogue LG120830 (**1**) and onapristone (**2**) were used as standards. For the thiophene series **5**, the *meta*-cyano analogue **5c** ($R^2 = CN$, $R^1 = R^3 = H$) is the most active compound, which is consistent with the results of LG120830 series in which a *meta* substituted

*Corresponding author. Tel.: +1-858-550-7663; fax: +1-858-550-7249; e-mail: lzhi@ligand.com

†Current address: Amgen, One Amgen Center Dr., Thousand Oaks, CA 91320, USA.

‡Current address: The R. W. Johnson Pharmaceutical Research Institute, 3535 General Atomics Ct., San Diego, CA 92121, USA.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00011-1

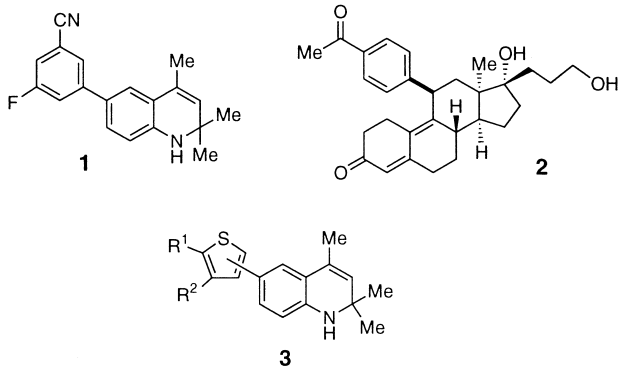
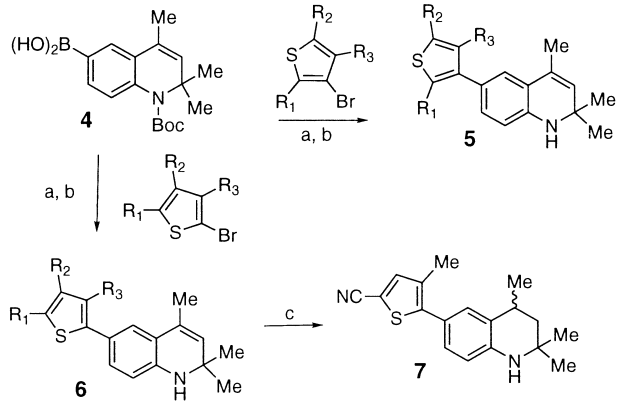
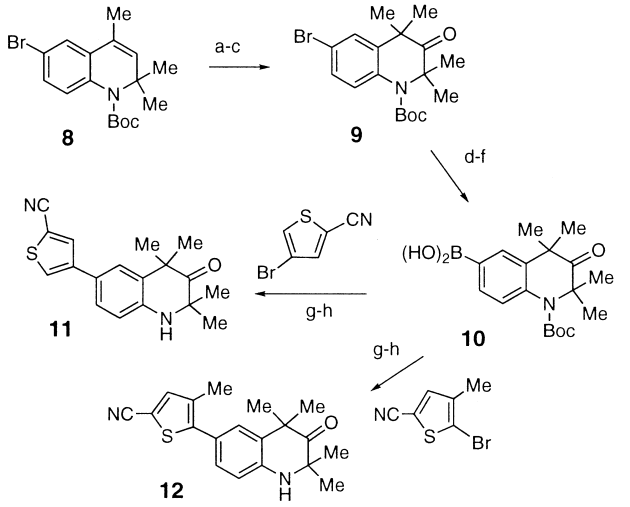


Figure 1. LG120830 (**1**), onapristone (**2**), and general structure of 6-thiophene analogues (**3**).



Scheme 1. (a) $Pd(PPh_3)_4$, K_2CO_3, DME, 80 °C; (b) TFA, CH_2Cl_2, rt; (c) 5% Pd/C, H_2, EtOAc.



Scheme 2. (a) BH_3-THF, H_2O_2, THF, rt; (b) PCC, CH_2Cl_2, rt; (c) NaH, MeI, Toluene, rt; (d) *n*-BuLi, THF, −78 °C; (e) $B(OMe)_3$; (f) H_3O^+; (g) $Pd(PPh_3)_4$, K_2CO_3, DME, 80 °C; (h) TFA, CH_2Cl_2, rt.

electron-withdrawing group on the pendent 6-aryl group enhanced the antiprogestational activity. In the thiophene series **6**, the *meta*-nitro and *meta*-cyano analogues (**6g** and **6h**) are the best compounds. An additional methyl group at the *ortho* position has no effect on biological activity (compare **6h** with **6k**). The SAR around the dihydroquinoline ring was also explored and it was noted that removal of the 3,4-olefin and/or introduction of a 3-ketone group did not have significant impact on the hPR antagonist activity (compounds **7**, **11** and **12**). It is worthy of mention that in a related hPR agonist series the 3,4-olefin or 3-ketone was essential for progestational activity.[15] All of the 6-thiophene 1,2-dihydroquinoline analogues showed good efficacy as hPR antagonists regardless their potency. It is interesting that the analogues without the 3,4-olefin (compounds **7**, **11** and **12**) exhibited excellent hPR antagonist activity at low concentration but behaved as agonists at higher concentration with good efficacy.

The new antiprogestins were also evaluated in the T47D human breast cancer cell line[16,17] and the assay results are summarized in Table 2. Steroidal antiprogestin onapristone showed similar activity in both the cotransfection and T47D assays while the 6-thiophene 1,2-dihydroquinoline analogues behaved quite poorly in the T47D assay. Most of the potent new compounds in CV-1 cells are much less active and some of them behaved as partial agonists in T47D cells.[18]

The cross-reactivity of a number of representative analogues (**5c**, **6g**, **6h**, **6k**, **7**, **11** and **12**) with other steroid receptors was assessed using human androgen (hAR), glucocorticoid (hGR), estrogen (hER), and mineralocortocoid (hMR) cotransfection assays (Table 2). No agonist activity was observed for any of the tested compounds, but antagonist activity was detected, most notably on the AR and GR. Compound **7** is much less selective than compound **6k** in terms of hPR/hAR and hPR/hGR ratios, which suggests that the 3,4-olefin is important for the receptor selectivity. The 3′-methyl of the 2-thiophene series also has a significant impact on the selectivity (compare compounds **6k** and **12** with compounds **6h** and **11**) (Table 3).

The lead compound **5c** (LG121046) demonstrated potent oral antiprogestational activity in rodent models such as the mouse implantation[9] and decidualization assays. No hepatomegaly was observed at the pharmacological doses.[19]

Conclusion

The SAR study around the pendent 6-phenyl group of LG120830 series generated 6-thiophene analogue series (**3**), which served as bioisostere of 6-phenyl analogues but without having the hepatomegaly effect in rodents. The preliminary SAR at the 1,2-dihydroquinoline moiety revealed the sensitivity of the new structure towards the steroid hormone receptor selectivity. The unique biological activity and the chemistry simplicity of the orally available pharmacophore offer great opportunities for developing clinically attractive selective non-steroidal antiprogestins.

Table 1. Cotransfection and binding data for the new compounds[a,b]

				hPR Cotransfection assay in CV-1 cells				hPR
				Agonist		Antagonist		Binding
No.	R^1	R^2	R^3	Efficacy (%)	EC_{50} (nM)	Efficacy (%)	IC_{50} (nM)	K_i (nM)
		Progesterone		100	2.9 ± 0.9	—	—	3.5 ± 0.2
1		LG120830		—	—	82 ± 3	30 ± 4	10 ± 1
2		Onapristone		—	—	95 ± 1	2.2 ± 0.4	18 ± 3
5a	CN	H	H	—	—	88 ± 3	318 ± 169	>100
5b	H	CHO	H	—	—	83 ± 11	245 ± 22	37 ± 16
5c	H	CN	H	34 ± 10	1800 ± 700	84 ± 8	24 ± 9	3.5 ± 0.6
5d	Me	CN	H	—	—	78 ± 7	109 ± 32	31 ± 10
5e	Me	CN	Me	—	—	74 ± 6	124 ± 31	36 ± 5
6a	H	H	H	—	—	85 ± 11	416 ± 98	237 ± 80
6b	H	H	Me	—	—	76 ± 10	232 ± 48	25 ± 5
6c	H	Br	H	29	2700	91 ± 4	105 ± 31	32 ± 12
6d	Cl	H	H	—	—	75 ± 5	43 ± 18	12 ± 3
6e	Br	H	H	—	—	78 ± 12	65 ± 31	31 ± 7
6f	CHO	H	H	—	—	71 ± 10	729 ± 204	80 ± 8
6g	NO_2	H	H	—	—	77 ± 3	31 ± 20	2.6 ± 1.0
6h	CN	H	H	35	2900	77 ± 11	27 ± 9	55 ± 16
6i	CN	H	Br	—	—	86 ± 4	52 ± 15	433 ± 93
6j	CN	Br	H	—	—	95 ± 4	242 ± 80	249 ± 58
6k	CN	H	Me	—	—	89 ± 6	33 ± 13	26 ± 2
7				88 ± 2	2290 ± 290	80 ± 7	31 ± 14	6.2 ± 2.2
11				73 ± 5	1190 ± 440	79 ± 1	14 ± 5	12.4 ± 5.7
12				95 ± 13	350 ± 200	73 ± 6	10 ± 1	3.9 ± 1.87

[a]Efficacy for agonist assays is defined in % versus progesterone = 100. Efficacy for antagonist assays is % inhibition of transscriptional 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 a single determination. "—" = not active (< 20% efficacy and/or >10 μM potency).

Table 2. T47D Assay data for reference compounds and the new analogues[a]

				hPR T47D Assay			
				Agonist		Antagonist	
No.	R^1	R^2	R^3	Efficacy (%)	EC_{50} (nM)	Efficacy (%)	IC_{50} (nM)
		Progesterone		100	1.8 ± 0.3	—	—
1		LG120830		40	2200	59 ± 5	37 ± 22
2		Onapristone		—	—	83 ± 8	3.3 ± 2.2
5a	CN	H	H	—	—	90	278
5b	H	CHO	H	—	—	95	102
5c	H	CN	H	37 ± 2	245 ± 47	63 ± 3	106 ± 11
5d	Me	CN	H	43	1400	67 ± 3	120 ± 26
5e	Me	CN	Me	58	580	39 ± 2	150 ± 21
6a	H	H	H	—	—	95	700
6b	H	H	Me	—	—	75 ± 5	105 ± 35
6c	H	Br	H	35	3500	84 ± 4	190 ± 20
6d	Cl	H	H	33	2650	69	151
6e	Br	H	H	—	—	80	71
6f	CHO	H	H	—	—	95	78
6g	NO_2	H	H	35	1024	60	70
6h	CN	H	H	41	1800	55	80
6i	CN	H	Br	60	700	40	120
6j	CN	Br	H	—	—	100	250
6k	CN	H	Me	45	500	56 ± 2	123 ± 22

[a]See Table 1 for legend.

Table 3. Cross-reactivity data in cotransfection assays for selected analogues[a,b]

No.	hAR Efficacy (%)	hAR IC_{50} (nM)	hGR Efficacy (%)	hGR IC_{50} (nM)	hER Efficacy (%)	hER IC_{50} (nM)	hMR Efficacy (%)	hMR IC_{50} (nM)
1	88	210	—	—	—	—	80 ± 2	>1000
2	93 ± 4	269 ± 57	100 ± 0	27 ± 4	27 ± 4	>1000	34 ± 9	>1000
5c	91 ± 7	494 ± 145	83 ± 10	2600 ± 200	—	—	88 ± 2	2050 ± 800
6g	89	1460	—	—	—	—	—	—
6h	79	1110	61	3200	—	—	—	—
6k	99	200	99 ± 1	780 ± 230	—	—	97 ± 2	2000 ± 600
7	87 ± 3	45 ± 10	98 ± 1	113 ± 18	—	—	89 ± 12	1000 ± 500
11	90 ± 1	1100 ± 490	98 ± 1	900 ± 170	—	—	56 ± 15	2700 ± 300
12	91 ± 5	150 ± 70	99 ± 2	35 ± 8	—	—	83 ± 3	2100 ± 800

[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.

References and Notes

1. Pathirana, C.; Stein, R. B.; Berger, T. S.; Fenical, W.; Ianiro, T.; Mais, D. E.; Torres, A.; Goldman, M. E. *Mol. Pharm*. **1995**, *47*, 630.
2. Hamann, L. G.; Farmer, L. J.; Johnson, M. G.; Wang, X.-N.; Marschke, K. B.; Jones, T. K. *J. Med. Chem*. **1998**, *41*, 623.
3. Combs, D. W.; Reese, K.; Phillips, A. *J. Med. Chem*. **1995**, *38*, 4878.
4. Combs, D. W.; Reese, K.; Cornelius, L. A.; Gunnet, J. W.; Cryan, E. V.; Granger, K. S.; Jordan, J. J.; Demarest, K. T. *J. Med. Chem*. **1995**, *38*, 4880.
5. Kurihara, K.; Tanabe, K.; Shinei, R.; Okonogi, T.; Ohtsuka, Y.; Omoto, S. *J. Antibiot*. **1997**, *50*, 360.
6. Kurihara, K.; Tanabe, K.; Yamamoto, Y.; Shinei, R.; Ajito, K.; Okonogi, T. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1837.
7. Kettel, L. M. *Clin. Obstetr. Gynecol*. **1995**, *38*, 921.
8. Baulieu, E. E. *Ann. NY Acad. Sci*. **1997**, *828*, 47.
9. Pooley, C. L. F.; Edwards, J. P.; Goldman, M. E.; Wang, M.-W.; Marschke, K. B.; Crombie, D. L.; Jones, T. K. *J. Med. Chem*. **1998**, *41*, 3461.
10. Hamann, L. G.; Winn, D. T.; Pooley, C. L. F.; Tegley, C. M.; West, S. J.; Farmer, L. J.; Zhi, L.; Edwards, J. P.; Marschke, K. B.; Mais, D. E.; Goldman, M. E.; Jones, T. K. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2731.
11. Suzuki, A. *Pure Appl. Chem*. **1991**, *63*, 419.
12. Preparation of **5c**: A solution of 4-bromothiophene 2-carboxaldehyde (1.0 g, 5.2 mmol) and hydroxylamine-*O*-sulfonic acid (2.4 g, 21 mmol) in CH_3CN/H_2O (995%, 20 mL) was stired at 65 °C for 8 h and quenched with aqueous NaOH (10%, 10 mL). The mixture was extracted with EtOAc and concentrated. Chromotography afforded 4-bromo-2-cyanthiophene as a white solid (0.50 g, 51%); ^{1}H NMR (400 MHz, $CDCl_3$ 7.54 (d, J = 1.3 Hz, 1H), 7.50 (d, J = 1.3 Hz, 1H). A mixture of the bromothiophene (0.50 g, 2.7 mmol), $Pd(PPh_3)_4$ (22 mg, 0.02 mmol), compound **4** (0.30 g, 0.63 mmol) and aqueous K_2CO_3 (1 M, 1 mL) in toluene (50 mL) and EtOH (10 mL) was heated at 80 °C for 2 h and was diluted with EtOAc. The mixture was washed with brine, concentrated and then treated with TFA (2 mL) in CH_2Cl_2 (20 mL) for 2 h at rt. Chromatography of the crude mixture afforded compound **5c** as a white solid (0.16 g, 91%); mp 66–67 °C; ^{1}H NMR (400 NMz), $CDCl_3$) 7.79 (d, J = 1.3 Hz, 1H), 7.46 (d, J = 1.3 Hz, 1H), 7.20 (d, J = 1.9 Hz, 1H), 7.16 (dd, J = 8.0 and 1.9 Hz, 1H), 6.46 (d, J = 8.0 Hz, 1H), 5.37 (s, 1H), 3.84 (bs, 1H), 2.03 (s, 3H) and 1.30 (s, 6H).
13. Preparation of **11**: To a solution of compound **8** (6.5 g, 18 mmol) in THF (20 mL) at 0 °C was added BH_3-THF (1 M, 29 mL) and the reaction mixture was warmed to room temperature and stirred for 2 h till no starting material was left. The reaction was quenched with aqueous NaOH (3 M, 40 mL) and treated with 30% H_2O_2. Extraction of the reaction mixture with EtOAc (3×) followed by removal of solvent and flash chromatography afforded the crude 3-hydroxy tetrahydroquinoine as a white solid (5.3 g, 78%). To a solution of the hydroxy intermediate in CH_2Cl_2 was added PCC (4.8 g, 22 mmol) portion-wise and stirred at room temperature for 2 h. The mixture was filtered through a plug of Florisil to remove chromium and was concentrated to afford the crude 3-ketone compound (4.3 g, 81%). To a solution of the 3-ketone (3.0 g, 8.1 mmol) in toluene (70 mL) at −78 °C was added lithium bis(trimethylsilyl)amide (1 M in THF, 24 mL) slowly and warmed to 0 °C. Excess iodomethane (2.5 mL) was added to the reaction mixture and was allowed to stir at rt till the starting material was consumed. Standard work up followed by chromatography provided compound **9** as yellow oil (1.8 g, 58%). To a solution of compound **9** (0.10 g, 0.26 mmol) in ether (3 mL) at −78 °C was treated with *n*BuLi (1.6 M in hexane, 0.19 mL) and stirred for 5 min before the addition of $B(OMe)_3$ (0.09 mL, 0.78 mmol). The reaction was warmed to room temperature and quenched with aqueous HCl (0.5 M) to adjust the PH < 2. Extraction with EtOAc followed by chromatography afforded compound **10** in 60% yield. The cross-coupling reaction between compound **10** and 4-bromo-2-cyanothiophene was performed by a similar procedure as described above to give compound **11** as colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) 7.83 (d, J = 1.4 Hz, 1H), 7.54 (d, J = 1.4 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.30 (dd, J = 8.0 and 2.0 Hz, 1H), 6.73 (d, J = 8.0 Hz, 1H), 3.78 (bs, 1H), 1.49 (s, 6H) and 1.36 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 214.5, 143.6, 143.0, 136.3, 130.3, 126.2, 126.1, 125.2, 123.8, 116.5, 114.5, 110.6, 60.1, 47.0, 27.6 and 24.5.
14. Rosen, J.; Day, A.; Jones, T. K.; Jones, E. T. T.; Nadzan, A. M.; Stein, R. B. *J. Med. Chem*. **1995**, *38*, 4855.
15. Zhi, L.; Tegley, C. M.; Marschke, K. B.; Mais, D. E.; Jones, T. K. *J. Med. Chem*. **1999**, *42*, 1466.
16. Lorenzo, D. D.; Albertini, A.; Zava, D. *Cancer Res*. **1991**, *51*, 4470.
17. Beck, C. A.; Weigel, N. L.; Moyer, M. L.; Nordeen, S. K.; Edwards, D. P. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 4441.
18. See activity differences of related nonsteroidal progestin series in CV-1 and T47D cell lines (ref 15).
19. Results of these experiments will be reported in due course.