Letters

Identification of Substituted 6-Amino-4-phenyltetrahydroquinoline Derivatives: Potent Antagonists for the Follicle-Stimulating Hormone Receptor

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Received May 3, 2004

Abstract: Substituted 6-amino-4-phenyl-tetrahydroquinoline derivatives are described that are antagonists for the G_s-protein-coupled human follicle-stimulating hormone (FSH) receptor. These compounds show high antagonistic efficacy in vitro using a CHO cell line expressing the human FSH receptor. Antagonist **10** also showed a submicromolar IC_{50} in a more physiologically relevant rat granulosa cell assay and was found to significantly inhibit follicle growth and ovulation in an ex vivo mouse model. This compound class may open the way toward a novel, nonsteroidal approach for contraception.

It is well established that gonadotropin hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play an important role in the complex process of human reproduction.[1−3] FSH and LH are glycoproteins with an average weight of ∼30 kDa and act via their specific G_s-protein-coupled FSH and LH receptor, respectively. Upon LH-mediated stimulation of the LH receptor, present on ovarian Theca cells, testosterone is generated (see Figure 1). In a parallel fashion, FSH-mediated activation of the FSH receptor, present on ovarian granulosa cells, leads to the production of the enzyme aromatase.[4] Aromatase converts testosterone into estradiol, required for follicle growth, ovulation, and endometrium proliferation.

Current methods for contraception rely on prevention of follicle maturation, ovulation, and implantation by the action of estrogenic and/or progestagenic steroids.[5] These compounds act via the nuclear estrogen or progesterone receptors, which are present in a variety of tissues. FSH receptors are specifically expressed on granulosa cells present in the ovaries, and therefore, inhibition of the FSH receptor may yield a novel, nonsteroidal approach for contraception. Several reports on low molecular weight (LMW) agonists for the gonadotropin receptors have appeared during the past few years,[6−8] some of which deal with LMW antagonists for the FSH receptor.[9,10] We report here nanomolar, active substituted 6-amino-4-phenyl-tetrahydroquinoline derivatives as a new class of low molecular weight antagonists for the FSH receptor with **10** as a potent representative example.[11] Antagonist **10** showed an IC_{50} of 28 nM (the effective concentration that inhibits 50% of the maximum response) on a cell line expressing the human FSH receptor.

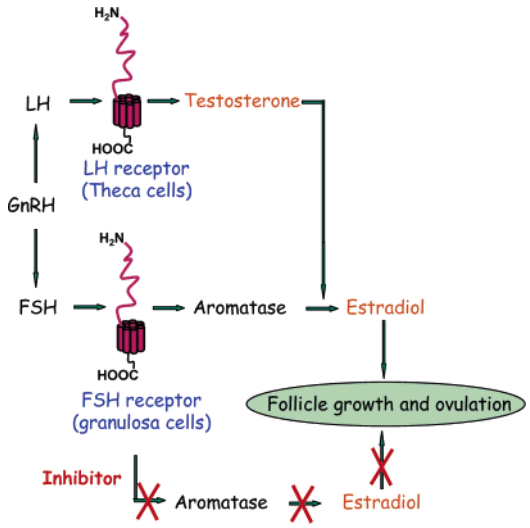

Figure 1. FSH-mediated estradiol production and subsequent follicle growth.

Initially, we aimed at the identification of LMW FSH receptor agonists via a high-throughput screening approach. To this end, we used a Chinese hamster ovary (CHO) cell line stably expressing the human FSH receptor and the cyclic AMP response element (CRE) luciferase reporter construct as a functional readout[12,13] and obtained 6-amino-4-phenyltetrahydroquinoline **4** as a selective FSH-receptor agonistic hit. Agonist **4** showed an EC_{50} of 4.4 μM and had an intrinsic activity (efficacy relative to that of the natural ligand FSH) of 0.85. Preparation of the agonistic hit **4** (Scheme 1) was accomplished using a slightly modified literature procedure. Thus, Skraup reaction[14] of *N*-Boc-1,4-phenylenediamine (**1**) followed by acetylation of the amino group at position 1[15] (**2** → **3**) and subsequent Friedel−Crafts alkylation[16] furnished agonistic hit **4** in reasonable overall yield. Initial structure−activity relationship studies revealed that deletion of the 1-*N*-acetyl, the 4-phenyl, or the 6-amino group all had a detrimental effect on potency.

To explore the requirements for FSH receptor agonism of the tetrahydroquinoline (THQ) compound class, several analogues were prepared. Surprisingly, introduction of a 4-chlorophenylcarbonyl group at position 6 of the THQ scaffold (e.g., **5**, Table 1) induced a switch from micromolar full agonistic activity to nanomolar full antagonistic activity in the CHO-hFSHR(luc) assay. This observation was further exploited in the prepara-

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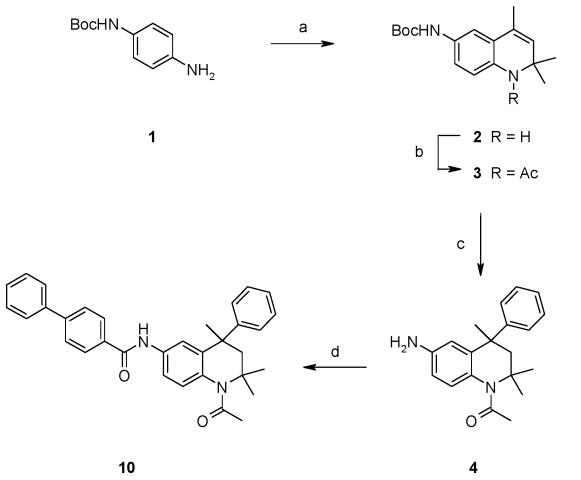
10.1021/jm049676l CCC: $30.25
Published on Web 07/16/2004

Scheme 1. Representative Synthesis of Substituted 6-Amino-4-phenyltetrahydroquinoline Derivatives[a]

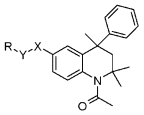


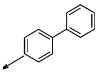
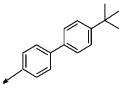
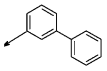
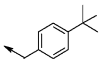
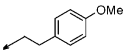
[a] Reagents and conditions: (a) mesityl oxide, I_2, 100 °C, 2 h, 42%; (b) AcCl/Ac_2O, pyridine/CH_2Cl_2, room temp, 18 h, 70%; (c) benzene, $AlCl_3$, 70 °C, 1 h, 71%; (d) 4-phenylbenzoyl chloride, *N,N*-diisopropylethylamine, THF, 88%.

tion of THQs **6**−**19** (Table 1; synthesis, step d in Scheme 1). All tetrahydroquinoline derivatives **6**−**19** were tested in the CHO-hFSHR(luc) assay in an antagonistic setup using the natural ligand FSH for receptor stimulation and addition of the presumed antagonist. The resulting IC_{50} values are listed in Table 1. As can be seen from this table, aromatic substituents at position 6 of the THQ scaffold are preferred for FSH receptor antagonism, although aliphatic derivatives are tolerated (analogues **5**, **8**, **9** vs **6**). Heteroaromatic tetrahydroquinolines show potencies in the low 10^{-8} M range, as exemplified by **7**. Analogues containing phenyl substituents with electron-withdrawing groups and analogues with electron-donating groups show potencies in the single-digit nanomolar range (see **8** and **9**, IC_{50} of 7 and 5 nM, respectively). It was established that the FSH receptor can accommodate biphenyl substituents (analogues **10**, **12**, and **13**) yielding nanomolar inhibitors, indicating the presence of a large lipophilic pocket. However, space is limited because introduction of an extra *tert*-butyl group on the biphenyl substituent (analogue **11**) led to a dramatic drop in potency. It is of interest to mention that apart from the nature of the substituent on position 6, the mode of connection of that substituent to the THQ scaffold is also of importance for potency. For example, replacement of the amide linkage for a sulfonamide linkage had a detrimental effect on potency (analogue **14**). This also accounts, albeit to a lesser extent, for the replacement of the amide by a urea linkage (**15**). Alkylated 6-aminotetrahydroquinolines or 6-hydroxytetrahydroquinolines (exemplified by analogues **16** and **17**, respectively) were a factor 100−1000 less potent than comparable amides, as also counts for the "reversed" amides **18** and **19**.

In a second optimization cycle, the linkage of the biphenyl group of **10** to the core was further examined, as well as the presence of substituents at the 4-phenyl substituent (R1, Table 2) or at the scaffold (R2, Table 2). IC_{50} values for analogues **20**−**28** are depicted in Table 2. Introduction of small substituents on the 4-phenyl moiety was tolerated, yielding antagonists with potencies 2−10 times less than the corresponding

Table 1. IC_{50} Values for Analogues **5**−**19** on CHO-hFSHR(luc) Assay



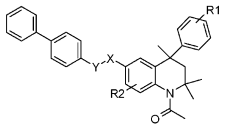
cmpd	X	Y	R	IC_{50} (nM)[a]
5	NH	C(O)		31
6	NH	C(O)		76
7	NH	C(O)		27
8	NH	C(O)		7
9	NH	C(O)		5
10	NH	C(O)		28
11	NH	C(O)		74% @E-05M
12	NH	C(O)		9
13	NH	C(O)		25
14	NH	$S(O)_2$		2800
15	NH	C(O)NH		580
16	NH	bond		930
17	O	bond		80% @E-05 M
18	C(O)	NH		25% @E-05M
19	C(O)	NH		1880

[a] Data are obtained from duplicate measurements and are representative of at least two independent experiments. SD of pIC_{50} is typically lower than 0.2.

unsubstituted 4-phenyl (**20**−**22**). Small substituents could be introduced on the THQ scaffold while maintaining potency (analogues **23** and **24**). Again, changing the nature of the connection between the 6-substituent and the scaffold greatly influenced potency. The nature of the linkage follows the same trends in SAR that were observed for the first series of compounds. Thus, alkylated/elongated derivatives (**25** and **27**) and "reversed" amides (cf. analogue **28**) were shown to be far less potent or even inactive compared to the corresponding amide **10**.

To examine whether potency is located in one single enantiomer, 6-amino-4-phenyltetrahydroquinolinyl hit **4** was chirally purified using a normal phase chiral column. Subsequently, the 6-amino groups in the individual isomers were reacted with 4-phenylbenzoyl chloride to afford the two chirally pure derivatives of **10**. It

Table 2. In Vitro Potencies of 6-Biphenyl-Containing Tetrahydroquinolines



cmpd	X	Y	R^1	R^2	IC_{50} (nM)[a]
20	NH	C(O)	4-Me	H	50
21	NH	C(O)	2-OMe	H	170
22	NH	C(O)	4-OH	H	240
23	NH	C(O)	H	7-Me	5
24	NH	C(O)	H	8-OMe	30
25	CH_2	NH	H	H	n.a.
26	O	C(O)	H	H	54
27	O	CH_2	H	H	166
28	C(O)	NH	H	H	44% @E-05 M
10 ***(isomer I)***	NH	C(O)	H	H	n.a.
10 ***(isomer II)***	NH	C(O)	H	H	4

[a] Data are obtained from duplicate measurements and are representatives of at least two independent experiments. SD of pIC_{50} is typically lower than 0.2. n.a. = not active.

Table 3. Followup Pharmacology for FSHR Antagonist **10**

assay (readout)	result
CHO-hFSHR (cAMP)	IC_{50} = 10 nM
rat GFSHR-17 granulosa cell line (cAMP)	IC_{50} = 540 nM
mouse ex vivo follicle culture (follicle ∅, % ovulated follicles)	significant inhibition of follicle growth at 10 μM, 78% inhibition of ovulation[a]

[a] Ovulation was seen in 3 out of 14 follicles. In a control experiment ovulation was seen in 12 out of 14 follicles.

was established that FSH receptor antagonistic activity resides in one enantiomer (isomers I and II of **10** in Table 2).[17]

Additional pharmacological evaluation of antagonist **10** is summarized in Table 3. No differences were found using cAMP instead of luciferase as a functional readout in the CHO-hFSHR assay (IC_{50} = 10 nM) with the natural ligand FSH for receptor stimulation. In a physiologically more relevant assay using the granulosa cell line GFSHR-17 with stable expression of the rat FSH receptor, rat granulosa cells were incubated with FSH and treated with **10**.[18] It was established that antagonist **10** was able to fully inhibit FSH-induced cAMP production, albeit with a 20-fold lower IC_{50} than found for the human receptor. The latter effect may be explained by a species difference, but also differences in assay and cell conditions may account for this observation.

Finally, **10** was tested in an ex vivo mouse follicle culture assay.[19] Addition of **10** to immature mouse follicles in the presence of FSH led to a significant inhibition of follicle growth compared to mouse follicles that were stimulated with FSH in the absence of **10**. Furthermore, ovulation of the mouse follicles that were treated with **10** was greatly impaired (78% inhibition of ovulation).

It is well documented that the natural gonadotropin ligands interact with the large extracellular domain (ECD) of their receptors.[20−22] It is assumed that binding of FSH or LH to the ECD induces a conformational change in the hinge region followed by relaxation of the transmembrane domain and subsequent receptor and G_s protein activation. In contrast to an earlier reported FSH receptor antagonistic compound class,[10] our tetrahydroquinolines were not able to displace ^{125}I-labeled FSH in a binding assay (see Figure 2). Therefore, we postulate that our lipophilic LMW compounds find their interaction within the seven-transmembrane (7-TM) part of the G-protein-coupled FSH receptor rather than in the large hydrophilic extracellular domain.

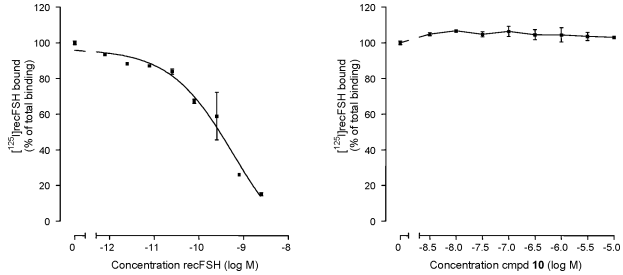


Figure 2. Displacement of [^{125}I]FSH binding to membranes prepared from CHO-K1 cells expressing the human FSH receptor by various concentrations of unlabeled FSH en LMW antagonist **10**. Results are shown as the mean ± SE of triplicate observations from a single representative experiment.

As a result of this 7-TM binding, the FSH−ECD complex may no longer be able to induce the conformational change within the 7-TM domain which is necessary to activate the receptor and the G_s protein. Allosteric interactions of synthetic antagonists have been described in the literature for peptide-binding G-protein-coupled receptors such as the angiotensin receptors.[23−26]

In conclusion, we have discovered a new class of low molecular weight antagonists for the G_s-protein-coupled FSH receptor. Substituted 6-amino-4-phenyltetrahydroquinoline derivatives such as **10** may serve as starting points for further optimization to evaluate the feasibility of FSH receptor antagonists as a novel method for contraception. Further progress in this area will be reported in due course.

Acknowledgment. The authors are grateful to Professor Abraham Amsterdam from The Weizmann Institute of Science, Rehovot, Israel, for providing the GFSHR-17 cell line.

Supporting Information Available: Representative experimental procedures, spectral data for the characterization of substituted 6-amino-4-phenyltetrahydroquinoline derivatives, and detailed assay experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM049676L