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Discovery of substituted benzamides as follicle stimulating hormone receptor allosteric modulators

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ABSTRACT

Follicle-stimulating hormone (FSH), acting on its receptor (FSHR), plays a pivotal role in the stimulation of follicular development and maturation. Multiple injections of protein formulations are used during clinical protocols for ovulation induction and for in vitro fertilization that are followed by a selection of assisted reproductive technologies. In order to increase patient convenience and compliance several research groups have searched for orally bioavailable FSH mimetics for innovative fertility medicines. We report here the discovery of a series of substituted benzamides as positive allosteric modulators (PAM) targeting FSHR. Optimization of this series has led to enhanced activity in primary rat granulosa cells, as well as remarkable selectivity against the closely related luteinizing hormone receptor (LHR) and thyroid stimulating hormone receptor (TSHR). Two modulators, **9j** and **9k**, showed promising in vitro and pharmacokinetic profiles.

Gonadotropins are a family of structurally-related heterodimer glycoprotein hormones that serve important functions in the reproductive process. This family consists of three members: two pituitary and one placenta hormones. Follicle stimulating hormone (FSH) and luteinizing hormone (LH), are released from the anterior pituitary under the control of gonadrotropin-releasing hormone from the hypothalamus, and chorionic gonadotropin (CG) is produced in the placenta. These three gonadotropins are also structurally related to another glycoprotein hormone, thyroid stimulating hormone (TSH).[1] All four glycoprotein hormones are marketed therapeutic drugs. FSH is used clinically in women for the treatment of anovulatory infertility and for controlled ovarian stimulation in in vitro fertilization (IVF) and in men for hypogonadotropic hypogonadism.[2]

FSH exercises its physiological function by acting on its receptor (FSHR) on specific gonadal somatic cells that leads to initiation of ovarian and testicular differentiation and steroidogenesis. FSHR, together with the other glycoprotein hormone receptors (LHR and TSHR), are members of the G-protein coupled receptor (GPCR) superfamily. The superfamily transduces extracellular signals into G protein activation via their seven-helical transmembrane (7TM) domain.[3] Ligand-stimulated FSHR leads to the activation of a cascade of kinases in a cAMP-dependent manner. Glycoprotein hormone receptors are unique among 7TM GPCRs for their large ectodomains at the N-terminus and short cytoplasmic tails at the C-terminus. While the nature of hormone–receptor interaction has been mapped and the structure of FSH binding to the ectodomain of FSHR has been solved,[4–6] the crystal structure of the full-length FSHR, however, has not been available. Consequently, atomic details about how the protein hormone activates the 7TM domain remain unknown.

Although FSH is highly effective and has been heralded as a useful drug for couples who undergo fertility treatment,[7] more than two-thirds of infertile women discontinue infertility treatments before FSH therapy is initiated. The number of patients dropping out of fertility treatment can be as high as 70%.[8] FSH, a protein mol-

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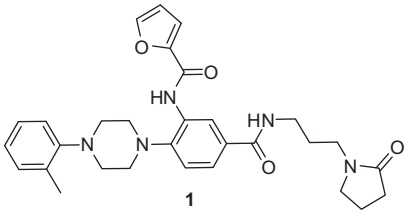


Figure 1. Chemical structure of compound **1**.

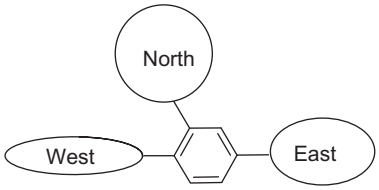


Figure 2. Schematic illustration of the SAR exploration of different regions of the compound series.

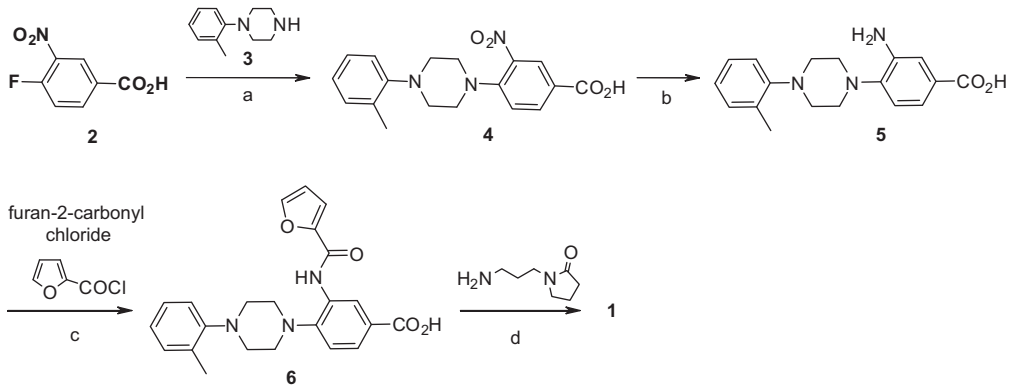
ecule, must be administrated daily or twice daily by injections for two weeks. Although the convenience of FSH injections have been improved with long lasting versions of FSH,[9–12] women report the daily injections are unbearable, and are one of the main reasons that patients who seek fertility treatment decide to drop out.[13] Small molecule drugs, on the other hand, are advantageous, as they can be taken orally.[14] Thus, there is an unmet medical need for bioavailable FSH mimetics that would be more convenient for the patients and potentially improve their treatment compliance. To date, several chemical series of low-molecule-weight (LMW) FSH mimetics have been reported.[15–21] Two have been demonstrated by mutagenesis studies to bind the 7TM domain of FSHR,[19,22] but a defined domain of the receptor where the small molecules binds is unknown and awaits structural studies of the small-molecule/FSHR complex. In addition to being developed for drug candidates, LMW ligands have been useful tools for obtaining GPCR crystals[23] and deciphering the target disulfide pairs.[24] As biased signaling is a general phenomenon in GPCR functions, including FSHR actions,[25] it is advantageous to have several chemical series available for elucidation of the FSHR mechanism and for the 'holy grail' of drug development targeting FSHR.[26] Here we report another chemical series of small molecules targeting FSHR.

The substituted benzamide **1** (Fig. 1) was identified as a promising starting point following screening of a small molecule library. High-throughput screening was performed using a Chinese hamster ovarian (CHO) cell line expressing the recombinant human FSH receptor (CHO-hFSHR) in the presence of an EC_{20} concentration of FSH. This concentration of FSH corresponds to the basal levels of hormone present in the circulation of a great majority of women seeking infertility treatments.[27] Cyclic adenosine monophosphase (cAMP), the second messenger stimulated by FSH, was measured as the readout in this assay.[28] These compounds were also counter-screened in CHO cells expressing human TSHR (hTSHR), human LHR (hLHR) and parental CHO cells. Compounds specific for hFSHR were selected. The EC_{50} of compound **1** in the CHO-hFSHR assay is 164 nM.

Synthesis of **1**, depicted in Scheme 1, is the general synthetic scheme used for derivatives.[29] Displacement of the aryl fluoride of **2** with piperazine **3** in an SNAr reaction followed by hydrogenation of the nitro group of **4** rendered aniline **5** in an overall yield of 35%. Acylation of **5** with furan-2-carbonyl chloride followed by amide coupling of **6** afforded **1** in 50% yield.

In order to investigate the structure-activity relationship (SAR) around the central aromatic ring, analogs were prepared in a fashion similar to Scheme 1. Efficacy of these derivatives to stimulate cAMP in CHO-hFSHR cells was monitored. We first explored the SAR in the northern part (Fig. 2) of the molecule (Table 1). The furan regio-isomer (**7a**) was less potent than compound **1**. Blocking the N–H with a methyl group rendered ~8.8 μM potency in analog **7b**, which indicates that the hydrogen donor is critical for the potency. Sulfonamide replacement of the amide gave little agonist activity (**7c**). 5-Methyl furan analog (**7d**) retained potency, but its isomer **7e** was reduced in potency by about three fold. Replacement of the furan with other five-member rings (**7f**, **7g**, **7h**, **7i**) gave a range of results, while 2-cyclopropyloxazole (**7h)** provided the most promising compound (EC_{50} = 99 nM). Interestingly, the simple cyclopropane analog (**7j)** maintained potency of 199 nM. The phenyl analog (**7k**) showed a reduced potency (554 nM). Replacement of the aryl ring in **7k** with a 2-pyridine (**7i**) retained most of the potency (184 nM). The importance of the N position in the pyridine ring is shown by little agonist activity shown in the two pyridine isomers (**7m** and **7n**).

The SAR in the western region of compound **1**, maintaining either a furan or a 2-cyclopropyloxazole in the northern section, quickly demonstrated a steep SAR around the piperazine phenyl moiety (Table 2). A minor change of the 2-methyl group resulted in a loss of potency. For instance, substitution of the 2-methyl with an electron withdrawing group 2-CF_3 (**8a**) leads to a 6-fold decrease in EC_{50}. The electron donating group 2-OMe (**8b**) analog displayed little agonist activity. Isomers of *o*-tolyl showed reduced potencies as well, with an EC_{50} of 1.4 μM for the *m*-tolyl analog (**8c**). The *p*-tolyl analog (**8d)** loses most of its agonist activity, and replacement of the *o*-tolyl group with 2-pyridine (**8f**) or H



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 36%; (b) Pd/C, H_2, EtOH/MeOH, 94%; (c) Et_3N, DMAP, DCM, 56%; (d) HOBt, EDC, DCM, 84%.