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Small molecule biaryl FSH receptor agonists. Part 1: Lead discovery via encoded combinatorial synthesis

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Abstract—High-throughput screening of two million compounds in 37 distinct encoded combinatorial libraries using FSH receptor transfected cells provided small molecule agonists such as **1** ($EC_{50}=3$ μM) and **2** ($EC_{50}=3.9$ μM), based on which a focused combinatorial library with a total of 31,372 compounds was designed, synthesized, and screened to reveal 72 novel biaryl FSH receptor agonists such as **8a**–**c** as well as a unique combinatorial SAR.

Combinatorial chemistry has emerged over the past decade as a powerful tool for the discovery and optimization of new leads in the pharmaceutical industry.[1] The goal of combinatorial chemistry is to assemble in as short a time as possible a significant number of compounds to meet the ever-increasing need for efficiency in drug discovery. Among the various combinatorial techniques, the encoded combinatorial libraries on polymeric support (ECLiPS™) technology has proved to be an extremely efficient method for assembling a large collection of compounds.[2] In fact, Pharmacopeia has used this technology since 1993 to prepare over 200 combinatorial libraries with over 7.5 million drug-like compounds, from which numerous leads have been identified for a wide variety of biological targets.[2c–g] In this paper, we report the discovery of novel, small molecule biaryl follicle stimulating hormone (FSH) receptor agonists using ECLiPS™ combinatorial libraries.

FSH plays an important role in human reproduction.[3] As a member of the glycoprotein hormone family that also includes luteinizing hormone (LH), thyroid stimulating hormone (TSF), and chorionic gonadotropin (CG), FSH is involved in ovarian follicle maturation in women and spermatogenesis in men. It is a 38 kDa heterodimeric protein composed of two glycosylated subunits—an α-subunit which is conserved across all members of the glycoprotein hormone family and a β-subunit which is specific to the hormone and provides receptor binding specificity. The FSH receptor (FSHR) is a seven transmembrane G-protein coupled receptor expressed on granulosa cells in the female and Sertoli cells in the male. FSH binding to the FSHR activates adenylyl cyclase and causes an increase in the intracellular level of adenosine 3′,5′-cyclic monophosphate (cAMP). In the female, activation of the FSHR ultimately leads to the growth of ovarian follicles and facilitates the selection of a dominant follicle that is able to ovulate upon exposure to the LH surge. Therefore, activation of the FSHR is thought to improve follicle development, ovulation and fertility. Indeed, FSH, purified from urine or prepared through recombinant technology is central to current therapy for low fertility.[4] However, the inconvenience and low patient compliance associated with therapeutic proteins that must be administered via subcutaneous or intra-muscular injection argue for a small molecule drug that is orally or transdermally available. Thus, low molecular weight FSHR

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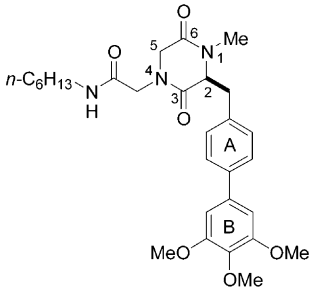
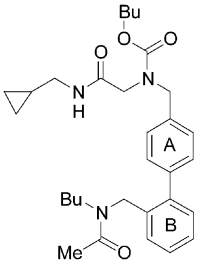
agonists could form the basis for a new therapeutic approach to infertility.

The discovery of small molecule agonists for peptide/protein receptors has historically been a great challenge. However, an increasing number of small molecule agonists for peptide/protein receptors have been identified recently through combinatorial chemistry and high-throughput screening.[5–7] In our laboratories, high-throughput screening of two million compounds in 37 distinct ECLiPS™ combinatorial libraries using a Chinese hamster ovary (CHO) cell line that stably expresses the human FSHR and the cAMP-response-element (CRE)-luciferase-reporter construct[8] revealed small molecule agonists such as **1** ($EC_{50}=3.0$ μM) and **2** ($EC_{50}=3.9$ μM). (EC_{50} values are means of at least two experiments with standard deviations less than 20%.)



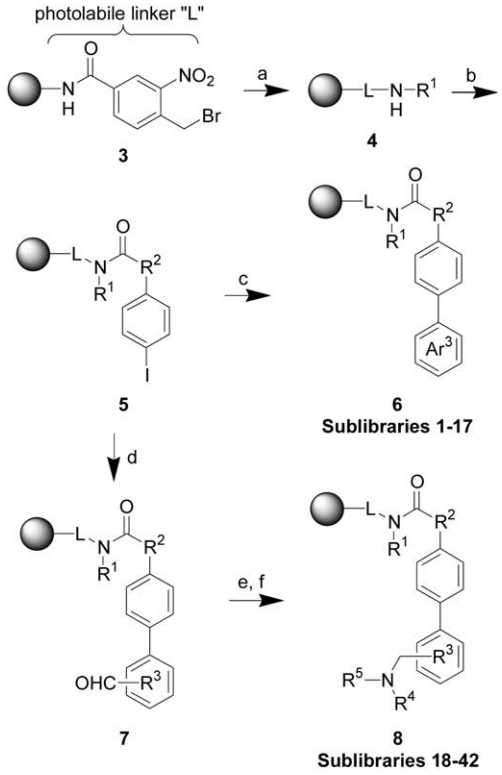
1
FSH EC_{50} = 3.0 μM



2
FSH EC_{50} = 3.9 μM

Compounds **1** and **2** share a common biphenyl subunit but differ in the substituents on each phenyl ring: a heterocyclic diketopiperazine in **1** versus an acyclic carbamate in **2** on the A-ring; and a 3,4,5-trimethoxy substitution in **1** versus an *ortho*-*N*-acetyl-*N*-butyl-aminomethyl fragment in **2** on the B-ring. In addition, compound **1** has a chiral center at the *C*-2 position of the diketopiperazine ring: the *(S)*-enantiomer (as shown) is 6-fold more potent than its antipode—the *(R)*-enantiomer ($EC_{50}=19$ μM, structure not shown). In contrast, compound **2** is achiral.

To expand the limited SAR from **1** and **2**, a focused ECLiPS™ combinatorial library of biaryl compounds (generic structures **6** and **8** in Scheme 1) was designed. The library was to explore a wide variety of side chain substituents in order to identify novel pharmacophoric fragments or novel combinations of known fragments. Specifically, the library was composed of 31 R^1 synthons (including various alkyls such as the hexyl group found in **1** and the cyclopropylmethyl group in **2**); 11 R^2 synthons (including various hetercocylic scaffolds such as the diketopiperazine scaffold in **1** and acyclic scaffolds such as the acyclic carbamate in **2**); 17 Ar^3 synthons (including various aryl groups such as the 3,4,5-trimethoxyphenyl group in **1**) for sublibraries 1–17 (**6**); and 25 R^4/R^5 synthon combinations (various amides, carbamates, ureas, and sulfonamides) appended to the B-ring via *ortho*- (as in **2**), *meta*-, and *para*-substitution for sublibraries 18–42 (**8**).



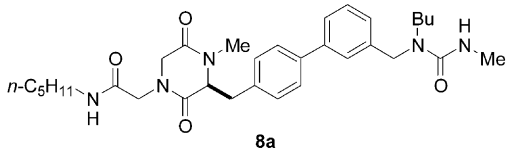
Scheme 1. Solid phase synthesis of a focused 31,372-member ECLiPS™ biaryl combinatorial library: (a) R^1NH_2, DIEA, Bu_4NI, DMF; (b) 4-I-C_6H_4-R^2-CO_2H, HATU, DIEA, DMF; (c) $Ar^3B(OH)_2$, $Pd_2(dba)_3$, $AsPh_3$, CsF, DME/EtOH (4:1 v/v), microwave (50 W); (d) $(OHC)PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, DMF, 55 °C; (e) R^4NH_2, $NaBH(OAc)_3$, TMOF; (f) R^5 derivatization (forming amides, carbamates, ureas, sulfonamides).

As shown in Scheme 1, the library synthesis began with the attachment of 31 primary amines R^1NH_2 to the resin via alkylation of 4-bromomethyl-3-nitro-benzamide derivatized TentaGel™ resin **3** to generate the resin-bound secondary amine **4**. After a pool and split step, **4** was acylated with 11 carboxylic acids 4-I-C_6H_4-R^2-CO_2H to afford amide **5**. After a second pool and split step, one portion of **5** was treated with 17 arylboronic acids $Ar^3B(OH)_2$ under a microwave-assisted Suzuki coupling condition that was developed in our laboratories to afford **6** (sublibraries 1–17). The other portion of **5** was coupled with *ortho*-, *meta*-, and *para*-formyl-phenyl-boronic acids (3 R^3 synthons) under standard Suzuki conditions to afford biaryl aldehyde **7**. After a third pool and split step, aldehyde **7** was reductively aminated with R^4NH_2 followed by R^5 derivatization (25 R^4/R^5 synthon combinations) using acyl chlorides, chloroformates, isocyanates, and sulfonyl chlorides to afford **8** (sublibraries 18–42). The 31 R^1, 11 R^2, and 3 R^3 synthons were encoded, prior to each pool and split step, by haloaromatic alcohol tags that can be detached via oxidative cleavage and analyzed by electron capture gas chromatography.[2] However, the 17 Ar^3 synthons were not encoded and instead the final product **6** was kept as 17 separate sublibraries with each sublibrary containing $31\times11=341$ compounds. Similarly, the 25 R^4/R^5 synthon combinations were also not encoded—the final product **8** was kept as 25 separate sublibraries with each sublibrary containing $31\times11\times3=1023$

compounds. Thus, a focused ECLiPS™ biaryl library with an overall of 31,372 compounds was efficiently prepared.

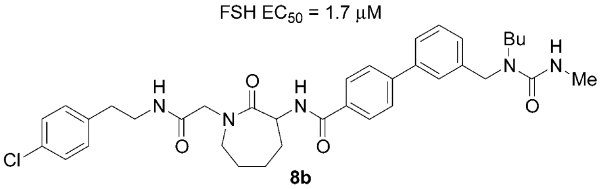
Screening of the 31,372-member biaryl library in the high-throughput CHO-hFSHR-luciferase assay was carried out in two stages. First, a survey screen was performed in which one copy of each sublibrary was arrayed in a 96-well plate as a mixture of ~10 compounds per well (~5 μM/compound, photocleaved from the resin beads) with the goal of identifying active sublibraries. Next, the active sublibraries were selected for a follow-up screen in which 3 copies of each active sublibrary was arrayed in 96-well plates in a single compound per well format (~5 μM/compound, photocleaved from the resin beads) with the goal of identifying the active compounds. The structures of the active compounds were determined via analyzing the haloaromatic alcohol tags from the source beads.[2]

Overall, 72 distinct structures with EC_{50} <10 μM were identified from several sublibraries including, for example, 9 structures from sublibrary 1 (**6**: Ar^3 = 3,4,5-trimethoxyphenyl) and 25 structures from sublibrary 30 (**8**: R^4 = Bu, R^5 = MeHNCO). These structures revealed novel pharmacophoric fragments as well as novel combinations of known fragments. Three representative structures, **8a–c**, from sublibrary 30 are shown below.



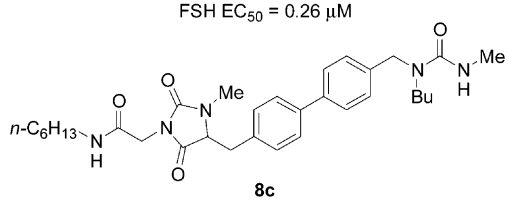
8a

FSH EC_{50} = 1.7 μM



8b

FSH EC_{50} = 0.26 μM



8c

FSH EC_{50} = 2.5 μM

An interesting *combinatorial SAR* emerged when plotting the frequency of synthons that appeared for all the active compounds. As an illustration, Figure 1 depicts the synthon frequency plots for the 25 structures identified from sublibrary 30:(A) R^3–R^2 combinations and (B) R^3–R^1 combinations.

As is evident in Fig. 1(A), the required combinations of R^3–R^2 for activity are extremely stringent. In particular, the R^3-*ortho*-compounds prefer the acyclic carbamate scaffold (R^2#3). For example, this particular relationship was found in **2**. The R^3-*meta*-compounds, however,

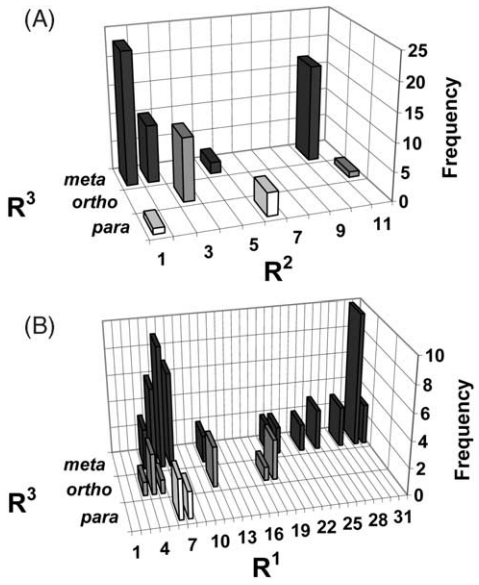


Figure 1. The distribution frequency of synthons for the 25 active compounds discovered from sublibrary 30: (A) R^3–R^2 combinations and (B) R^3–R^1 combinations.

favor the (*S*)-diketopiperazine scaffold (R^2#1, as in **8a**), the (*R*)-diketopiperazine scaffold (R^2#2), or the azepinone scaffold (R^2#10, as in **8b**)—all of which are novel combinations. And finally, the R^3-*para*-compounds select the hydantoin scaffold (R^2#6, as in **8c**)—which is also a novel combination. Such a striking combinatorial SAR readily obtained through encoded combinatorial synthesis would have taken much longer to acquire if using the traditional one-variation-at-a-time approach.

Similarly, the required combinations of R^3–R^1 for activity, as shown in Figure 1(B), are also stringent—though to a lesser extent. As can been seen, the R^3-*ortho*-compounds favor the butyl (R^1#4), cyclopropylmethyl (R^1#11), or tetrahydrofuran-2-methyl (R^1#18) substituents; the R^3-*meta*-compounds prefer the pentyl (R^1#5, as in **8a**), hexyl (R^1#6, as in **8c**), heptyl (R^1#7), or 4-chlorophenethyl (R^1#30, as in **8b**) susbsitutents; and the R^3-*para*-compounds select the hexyl (R^1#6) or heptyl (R^1#7) susbstituents.

In summary, we have described the discovery of novel small molecule biaryl FSH receptor agonists along with a unique combinatorial SAR through ECLiPS™ combinatorial synthesis. Further elaboration of these compounds is described in the following paper.[9]

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