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# Small molecule biaryl FSH receptor agonists. Part 2: Lead optimization via parallel synthesis

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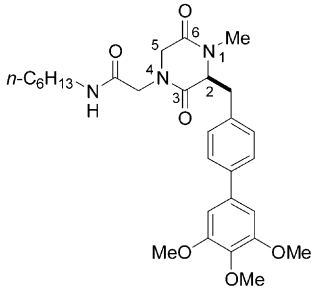
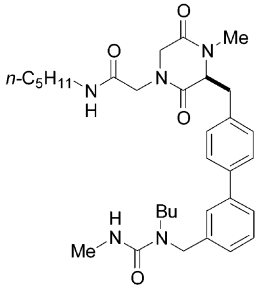
**Abstract**—Potent small molecule biaryl diketopiperazine FSH receptor agonists such as **10c** ($EC_{50}$ = 13 nM) and **11f** ($EC_{50}$ = 1.2 nM) were discovered through the design, synthesis and evaluation of three biaryl diketopiperazine optimization libraries with over 300 compounds. These libraries were prepared via solid-phase parallel synthesis using a cyclization-release method.


As described in the preceding paper, novel biaryl FSH receptor agonists such as **1** ($EC_{50}$ = 3 μM) and **2** ($EC_{50}$ = 1.7 μM) along with a unique combinatorial SAR were discovered through encoded combinatorial synthesis.[1] The results demonstrated that combinatorial synthesis is a powerful tool to aid in the discovery of small molecule agonists for peptide/protein receptors. These biaryl compounds could serve as good starting points in a follow-up optimization program. In this paper, we report the elaboration of **1** and **2** into potent FSH receptor agonists such as **10c** ($EC_{50}$ = 13 nM, MW = 511) and **11f** ($EC_{50}$ = 1.2 nM, MW = 563) via parallel synthesis.

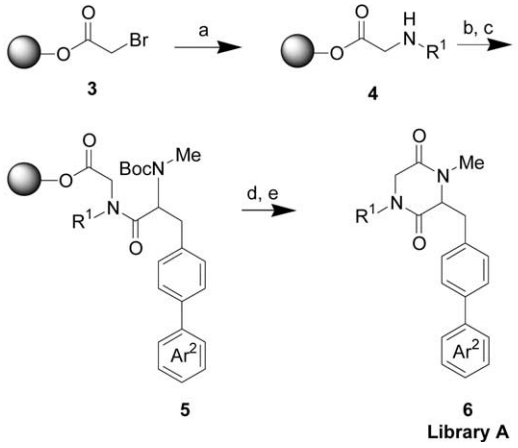



**1**
FSH $EC_{50}$ = 3.0 μM




**2**
FSH $EC_{50}$ = 1.7 μM

Compounds **1** and **2** share a common biaryl diketopiperazine core, thus the optimization efforts were centered on exploring various side chain substituents on the common core. Specifically, three parallel libraries—A (generic structure **6** in Scheme 1), B (generic structure **10** in Scheme 2), and C (generic structure **11** in Scheme 2)—comprising over 300 compounds were designed to investigate the effect of replacing the metabolically vulnerable side chain amide in **1** and **2** with various




**Scheme 1.** Synthesis of parallel library A: (a) $R^1NH_2$, DMSO; (b) Boc-L or D-*N*-Me-4-iodophenylalanine, HATU, DIEA, DMF; (c) $Ar^2B(OH)_2$, $Pd(PPh_3)_4$, $K_2CO_3$, DMF, 65 °C; (d) TFA, $CH_2Cl_2$; (e) $Et_3N$, $CH_2Cl_2$.

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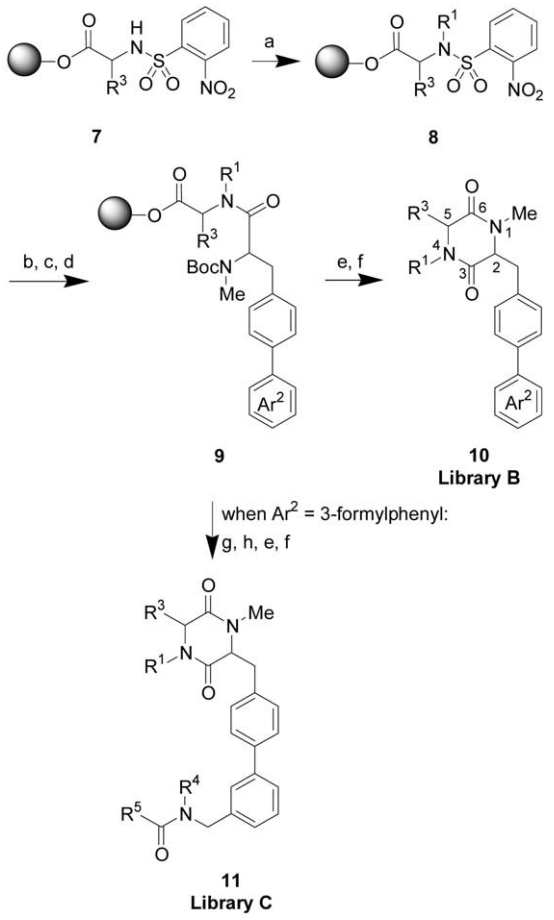
des-amide fragments ($R^1$) in combination with introducing a *C*-5 substituent ($R^3$) on the diketopiperazine ring as well as varying the aryl substituents ($Ar^2$, $R^4$, and $R^5$). The *N*-1-Me group on the diketopiperazine ring, however, was kept unchanged in these libraries because initial analogues of **1** containing other *N*-1-substituents—H, Et, or Pr, for example—all displayed reduced potency ($EC_{50}$ > 20 μM). In order to prepare the three des-amide libraries, given that the side chain amide nitrogen in **1** and **2** was the resin attachment point in the solid-phase synthesis of the original combinatorial library,[1] a new synthetic route was sought. Eventually, a solid-phase cyclization-release method similar to that reported by Chiron[2] and Affymax[3] researchers was developed (Schemes 1 and 2) to generate these des-amide biaryl diketopiperazine libraries.

Thus, as shown in Scheme 1, the synthesis of parallel library A began with bromoacetate **3** that was obtained from esterification of TentaGel™-S-OH resin with bromoacetic acid using 1,3-diisopropylcarbodiimide. Amination of **3** with diverse primary amines $R^1NH_2$ generated **4**. Acylation of **4** with Boc-*N*-methyl-4-iodophenyl alanine (both the L- and D-amino acids were used to explore the stereochemical preference at C-2), followed by Suzuki coupling with various arylboronic acids $Ar^2B(OH)_2$ afforded the biaryl intermediate **5**. Removal of the Boc-protecting group with TFA followed by cyclization-release using triethylamine provided the des-amide biaryl diketopiperazine **6** (library A).



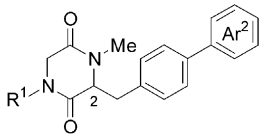

**Scheme 2.** Synthesis of parallel libraries B and C: (a) $R^1OH$, $Ph_3P$, DIAD, NMP, −15 °C to rt; (b) PhSH, DBU, DMF; (c) Boc-L- or D-*N*-Me-4-iodophenylalanine, HATU, DIEA, DMF; (d) $Ar^2B(OH)_2$, $Pd(PPh_3)_4$, $K_2CO_3$, DMF, 65 °C; (e) TFA, $CH_2Cl_2$; (f) $Et_3N$, $CH_2Cl_2$; (g) $R^4NH_2$, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$; (h), $R^5$ derivatization (forming amides, carbamates, ureas).

Next, as shown in Scheme 2, the synthesis of parallel library B (**10**) began with 2-nitrobenzene-sulfonyl-amino acid ester **7** that was prepared from esterification of TentaGel™-S-OH resin with an Fmoc-amino acid (both the L- and D-amino acids were used to explore the stereochemical preference at C-5) using 2,6-dichlorobenzoyl chloride and pyridine followed by Fmoc deprotection using piperidine and subsequent reprotection with 2-nitrobenzene-sulfonyl chloride. Treatment of **7** with various primary alcohols $R^1OH$ under Mitsunobu conditions (based on Liskamp's solid-phase adaptation[4] of the Fukuyama secondary amine synthesis method[5]) provided **8**. Removal of the 2-nitrobenzene-sulfonyl group using PhSH and DBU in DMF followed by acylation with Boc-*N*-methyl-4-iodophenyl alanine (both the L- and D-amino acids were used to explore the stereochemical preference at *C*-2) and subsequent Suzuki coupling with arylboronic acids $Ar^2B(OH)_2$ under standard conditions afforded biaryl intermediate **9**. Deprotection of Boc and cyclization-release afforded the *C*-5 substituted biaryl diketopiperazine **10** (library B).

Finally, as also shown in Scheme 2, the synthesis of parallel library C began with biaryl intermediate **9** when $Ar^2$ = 3-formylphenyl (aldehyde interemediate). Thus, reductive amiation of the aldehyde intermediate with various $R^4NH_2$ followed by acylation with diverse $R^5$ derivatizing agents (acid chlorides, chloroformates, and isocyanates) and subsequent Boc-deprotection and cyclization-release produced the *meta*-substituted biaryl diketopiperazine **11** (library C).

Taken together, three parallel optimization libraries with over 300 compounds were prepared and each compound (~5 mg) was purified by HPLC prior to the CHO-hFSHR-luciferase assay.[6] The assay results for

**Table 1.** $EC_{50}$ values for select library A compounds in the CHO-hFSHR-luciferase assay

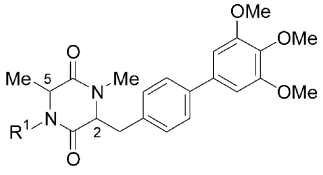


| Compd | $R^1$ | $Ar^2$ | *C*-2 | $EC_{50}$ (nM) |
|---|---|---|---|---|
| **6a** | *n*-$C_9H_{19}$ | 3,4,5-$(MeO)_3C_6H_2$ | *S* | 900 |
| **6b** | *n*-$C_8H_{17}$ | 3,4,5-$(MeO)_3C_6H_2$ | *S* | 400 |
| **6c** | *n*-$C_7H_{15}$ | 3,4,5-$(MeO)_3C_6H_2$ | *S* | 2300 |
| **6d** | *n*-$C_6H_{13}$ | 3,4,5-$(MeO)_3C_6H_2$ | *S* | 3000 |
| **6e** | *n*-$C_5H_{11}$ | 3,4,5-$(MeO)_3C_6H_2$ | *S* | 9000 |
| **6f** | *n*-$C_8H_{17}$ | 3,4-$(MeO)_2C_6H_2$ | *S* | 3300 |
| **6g** | *n*-$C_8H_{17}$ | 3,5-$(MeO)_2C_6H_2$ | *S* | 5000 |
| **6h** | *n*-$C_8H_{17}$ | 3,5-$Me_2$-4-$MeOC_6H_2$ | *S* | 1200 |
| **6i** | *n*-$C_6H_{13}$ | 3,4,5-$(MeO)_3C_6H_2$ | *R* | >50,000 |

select compounds from libraries A, B, and C to highlight the key SAR are presented in Tables 1–3, respectively. ($EC_{50}$ values are means of at least two experiments with standard deviations less than 20%.)

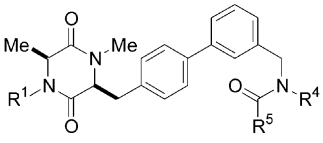
First, as is evident from Table 1, removal of the side chain amide bond is in fact beneficial. The des-amide compound **6a**, while having the same atom length for the *N*-4-substituent as in **1**, shows a 3-fold increase in potency relative to **1**. Shortening the *N*-4 side chain by one carbon atom gives rise to an additional 2-fold increase in potency (**6b**, $EC_{50} = 400$ nM). However, further truncation in chain length causes a gradual decrease in potency (**6c–e**). In addition, branched or cyclic alkyls at *N*-4 all result in inactive compounds ($EC_{50} > 50$ μM, data not shown). Thus, the octyl group appears to be the optimal *N*-4-substituent. Furthermore, replacements of the 3,4,5-trimethoxyphenyl group with other aryls—3,4-dimethoxyphenyl (**6f**), 3,5-dimethylphenyl (**6g**), or 3,5-dimethyl-4-methoxy-phenyl (**6h**), for example—all result in less potent compounds (compare **6f–h** with **6b**). Also, similar to that in the original amide series,[1] the (*S*)-enantiomers in the des-amide series are consistently more potent than the (*R*)-enatiomers (as one example, compare **6d** with **i**).

**Table 2.** $EC_{50}$ values for select library B compounds in the CHO-hFSHR-luciferase assay




| Compd | *C*-5 | *C*-2 | $R^1$ | $EC_{50}$ (nM) |
|---|---|---|---|---|
| **10a** | S | S | *n*-$C_6H_{13}$ | 100 |
| **10b** | *S* | *S* | *n*-$C_7H_{15}$ | 25 |
| **10c** | *S* | *S* | *n*-$C_8H_{17}$ | 13 |
| **10d** | *R* | *S* | *n*-$C_8H_{17}$ | 120 |
| **10e** | *S* | *R* | *n*-$C_8H_{17}$ | 68 |
| **10f** | *R* | *R* | *n*-$C_8H_{17}$ | 1600 |

**Table 3.** $EC_{50}$ values for select library C compounds in the CHO-hFSHR-luciferase assay




| Compd | $R^1$ | $R^4$ | $R^5$ | $EC_{50}$ (nM) |
|---|---|---|---|---|
| **11a** | *n*-$C_6H_{13}$ | Bu | Me | 150 |
| **11b** | *n*-$C_8H_{17}$ | Bu | Me | 14 |
| **11c** | *n*-$C_6H_{13}$ | Bu | OMe | 8.9 |
| **11d** | *n*-$C_8H_{17}$ | Bu | OMe | 2.7 |
| **11e** | *n*-$C_6H_{13}$ | Bu | NHMe | 7.2 |
| **11f** | *n*-$C_8H_{17}$ | Bu | NHMe | 1.2 |
| **11g** | *n*-$C_8H_{17}$ | $MeOC_2H_5$ | Me | 9.7 |
| **11h** | *n*-$C_8H_{17}$ | $MeOC_2H_5$ | OMe | 1.8 |
| **11i** | *n*-$C_8H_{17}$ | $MeOC_2H_5$ | NHMe | 3.6 |

Second, as can be seen in Table 2, incorporation of a single (*S*)-methyl group at the C-5 position of the diketopiperazine ring results in a remarkable 30–100-fold increase in potency (compare **10a–c**[7] with **6d–b**). However, compounds bearing bigger C-5-substituents—(*S*)-Et, Pr, or Bu—are all less potent than **10c** (data not shown), suggesting that the (5*S*)-Me is the optimal substituent. In addition, the (2*S*,5*S*)-*cis*-stereochemistry affords the most active compound (**10c**, $EC_{50} = 13$ nM), relative to which, the corresponding (2*S*,5*R*)-*trans*-isomer (**10d**) has a 9-fold potency drop ($EC_{50} = 120$ nM); the (2*R*,5*S*)-*trans*-isomer (**10e**) a 5-fold potency drop ($EC_{50} = 68$ nM); and the (2*R*,5*R*)-*cis*-isomer (**10f**) a greater than 100-fold potency drop ($EC_{50} = 1600$ nM).

Finally, as shown in Table 3, similar to the trimethoxy series, the *meta*-aminomethyl series also shows a substantial potency enhancement with the incorporation of a single (5*S*)-Me on the diketopiperazine ring (**11a–i**, $EC_{50} < 150$ nM) relative to the *C*-5 unsubstituted compounds ($EC_{50} > 1000$ nM, data not shown). In addition, the *N*-4-octyl compounds (**11b**,**d**,**f**) all exhibit higher potency levels (by 3–10-fold) than the corresponding *N*-4-hexyl compounds (**11a**,**c**,**e**). Furthermore, the $R^4$ butyl side chain can be replaced with other groups such as a methoxyethyl to provide equipotent compounds (**11g–i** versus **11b**, **11d**, **11f**). Finally, ureas and carbamates are generally more potent than their corresponding amides. For example, **11e** (urea) and **11c** (carbamate) are both >10-fold more potent than **11a** (amide).

Some of the most potent compounds were further evaluated in a CHO-hFSHR cell-based cAMP accumulation assay[6] and the data is presented in Table 4. As can be seen, both the trimethoxy series (**10b–c**) and the *meta*-substituted series (**11b–f**) display agonistic activity in the cAMP accumulation assay, generating efficacies between 47% (**10b**) and 85% (**11e**) relative to FSH (100%).

In summary, we have described the optimization of low micromolar compounds **1** and **2** into potent low nanomolar FSH receptor agonists such as **10c** and **11f** through the design, synthesis, and evaluation of three parallel libraries with over 300 compounds. This work has demonstrated that parallel synthesis is a valuable tool for lead optimization in drug discovery. Further study of these compounds will be reported in due course.

**Table 4.** $EC_{50}$ and efficacy values for compounds **10b–c** and **11b–f** in the CHO-hFSHR cell-based cAMP accumulation assay

| Compd | $EC_{50}$ (nM) | Efficacy (%) |
|---|---|---|
| **10b** | 32 | 54 |
| **10c** | 32 | 47 |
| **11b** | 35 | 57 |
| **11c** | 48 | 59 |
| **11d** | 31 | 49 |
| **11e** | 9.5 | 85 |
| **11f** | 7.9 | 72 |

## References and notes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7. Characteristic analytical data for **10c**: $^1$H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, 2H, $J=8.3$ Hz), 7.14 (d, 2H, $J=8.3$ Hz), 6.97 (s, 2H), 4.20 (t, 1H, $J=4.4$ Hz), 3.89 (s, 6H), 3.86 (s, 3H), 3.75 (q, 1H, $J=7.0$ Hz), 3.56 (m, 1H), 3.34 (dd, 1H, $J=4.2$ Hz, 13.8 Hz), 3.16 (dd, 1H, $J=4.2$ Hz, 13.8 Hz), 3.02 (s, 3H), 2.85 (m, 1H), 1.57 (m, 1H), 1.41 (m, 1H), 1.23 (m, 10H), 0.84 (t, 3H, $J=6.6$ Hz), 0.57 (d, 3H, $J=7.0$ Hz); MS (ESI): $m/z$ 511.3 ($MH^+$).