Optimization of Novel Antagonists to the Neurokinin-3 Receptor for the Treatment of Sex-Hormone Disorders (Part II)

Hamid R. Hoveyda,* Graeme L. Fraser, Guillaume Dutheuil, Mohamed El Bousmaqui, Julien Korac, François Lenoir, Alexey Lapin, and Sophie Noël

Euroscreen SA, 47 rue Adrienne Bolland, 6041 Gosselies, Belgium

ABSTRACT: Further lead optimization on N-acyl-triazolopiperazine antagonists to the neurokinin-3 receptor (NK3R) based on the concurrent improvement in bioactivity and ligand lipophilic efficiency (LLE) is reported. Overall, compound 3 (LLE > 6) emerged as the most efficacious in castrated rat and monkey to lower plasma LH, and it displayed the best off-target safety profile that led to its clinical candidate nomination for the treatment of sex-hormone disorders.

KEYWORDS: NK3 antagonist, triazolopiperazine, neurokinin B, LH, FSH, GnRH

The neurokinin-3 receptor (NK3R) is a class A GPCR with neurokinin B (NKB) as its endogenous agonist. We present here the sequel on the lead optimization of N-acyl-triazolopiperazine NK3R antagonists (1 and 2, Figure 1).1 NK3R antagonists were speculated as therapeutically relevant for CNS dysfunctions, e.g., schizophrenia, predicated on the “hyperdopaminergic hypothesis”, which repeatedly met with clinical failures in over a decade of efforts.2 Meanwhile, emerging biology has unambiguously established the role of NK3R/NKB signaling in reproductive neuroendocrinology. Importantly, recent studies have revealed NK3R as a key regulatory component of the hypothalamic–pituitary–gonadal (HPG) axis wherein its tonic activation positively regulates the gonadotropin-releasing hormone (GnRH) pulse frequency.3 In turn, the GnRH pulse frequency is known to differentially control the circulating levels of luteinizing hormone (LH) versus follicle-stimulating hormone (FSH). Thus, high frequency pulses stimulate LH release, whereas low frequency pulses favor FSH induction.4 These gonadotropins ultimately act on the ovary and testis to promote production of gametes and sex-hormone release. In 2011, it was reported that patients with a loss of function mutation in NK3R display a phenotype of normosomic congenital hypogonadotropic hypogonadism, low plasma LH, and attendant low LH/FSH ratios that could be restored through exogenous administration of GnRH.5 We have demonstrated elsewhere6 that the foregoing NK3R antagonists slow the LH pulse and decrease circulating LH levels without affecting FSH, consistent with the literature reports. As such, these antagonists are subtle modulators of gonadotropin secretion unlike GnRH ligands that abrogate both LH and FSH with the consequent decline in plasma estrogen to castration levels thereby triggering menopausal-like adverse events such as bone mineral density loss and incidences of hot flashes.7 Hence, NK3R antagonists offer a potentially safer therapeutic approach due to a decreased rather than abrogated GnRH pulse frequency. Collectively, these findings offer a strong rationale for repositioning NK3R antagonists to address sex-hormones disorders such as polycystic ovary syndrome (PCOS) and uterine fibroids (UF), among others.8

The synthetic approach to the analogues herein was previously described.1 With minor modifications, Scheme 1 was used for the GMP scale-up of 3 in overall 42% yield (2.7 kg) with 99.3% purity and >99.9% enantiomeric excess.

Received: March 17, 2015
Accepted: May 19, 2015
Published: May 19, 2015

Figure 1. Lead progression: iv POC (1), oral POC (2), and clinical candidate (3). (The “magic methyl” groups are shown in red.)
Interestingly, a hydroxyethyl substitution at Ring B (18) afforded an alternative means of reducing lipophilicity (ΔlogD_{2,4} = −0.5 vs 3) with minimal impact on bioactivity, thus resulting in a 0.4-log superior LLE vs 3. Despite this, 18 proved inferior to 3 due to P_{gr} efflux that in turn markedly diminished its brain exposure level (Table 2 and discussion further below).

The hERG SAR herein (Table 1) was governed by the interplay between lipophilicity and the hydrogen-bond acceptor (HBA) count in the heteroaryl Ring D, as previously reported. For instance, in progressing from 8 to 3 (ΔlogD_{2,4} = 1.5), the hERG IC_{50} was improved by over 12-fold. However, the poor hERG IC_{50} = 1.6 μM in 11 (ΔlogD_{2,4} = 1.7) was in keeping with the increased HBA count in the Ring D oxadiazole (N + N + O) in stark contrast to the thiadiazole (N + N) Ring D variations (3 and 15–18), all of which displayed a superior hERG IC_{50} ≥ 39 μM (ΔlogD_{2,4} = 1.3–2.4). Interestingly, the Ring B hydroxyl group in 18 did not adversely impact hERG (IC_{50} = 50 μM) suggesting that the HBA effect on hERG SAR is primarily a Ring D related effect. Finally, the Ring B magic methyl also reduced hERG efficacy, i.e., 3 (IC_{50} > 100 μM) vs 12 (IC_{50} = 50 μM). Compound 3 was the best overall in the hERG and CYP safety profile evaluation.

Based on the free drug hypothesis, the unbound fraction rather than total drug is relevant for PKPD analysis. The NK-R is mainly expressed on KNDy neurons in the ARC region of the hypothalamus that is part of the circumventricular organs lacking blood–brain barrier and are therefore exposed to blood solutes. As such, both the unbound plasma (f_{u}) and the unbound brain levels (b_{f,u}) must be considered here (Table 2). While lipophilicity alone does not correlate well to albumin binding, this trend is often apparent in a congenic series. Hence, a compound with balanced lipophilicity such as 3 (ΔlogD_{2,4} = 1.5) displayed high f_{u} and b_{f,u} levels (>50%) in contrast to the more lipophilic congeners, e.g., 16 (Table 2). It is noteworthy that despite an increase in unbound plasma concentration, the systemic clearance levels (CL_{f}) remained low (e.g., 3, Table 2). The comparatively lower CL_{f} in para substituted phenyl Ring A (3, 15) against the unsubstituted congener 17 is likely due to the metabolic blocking effect. All analogues except 18 displayed high Caco-2 permeability with no evidence of appreciable P_{gr} efflux (ER = 0.6–1.2), consistent with the high oral availability (%F) and brain-to-plasma ratios observed. The so-called P_{gr} rule-of-4 suggests that increasing the number of HBA atoms to (N + O) ≥ 8 tends to confer an increasing likelihood of P_{gr} efflux. This is in keeping with the P_{gr} efflux in 18 (ER = 3.8) given its HBA atom count (N + O = 8). As with 2, a complete oral absorption (%F > 100) was also observed in rat with compound 12 and in monkey with 3. This phenomenon is well-known and various underlying causes have been reported. No drug accumulation was observed in 5-day once-daily oral dosing studies in rats (3 and 12) or monkeys (3), despite administration of elevated doses (e.g., up to 1 g/kg in rats with 12), in step with the relatively short half-life values and the previous related observations with 2. Moreover, no adverse hepatotoxicity (AST, ALT, and bilirubin levels normal) was detected in these subchronic studies. Furthermore, 3 displayed the highest b_{f,u} = 0.525 and brain unbound concentration (C_{brain,u} = 343 nM) herein (Table 2). In contrast, 18 although nearly completely unbound in the brain displayed a comparatively low C_{brain,u} = 45.6 nM consistent with its elevated P_{gr} efflux ratio.
The key PKPD parameters for interpreting the LH inhibition data are the unbound plasma and brain levels normalized with respect to the bioactivity, i.e., $C_{\text{plasma,u}}/K_i$ and $C_{\text{brain,u}}/K_i$ (Table 3). The plasma and brain levels were determined at the $T_{\text{max}}$ for the minimum effective dose (MED). As noted before for 2 and 8, a statistically significant effect was attained at $C_{\text{plasma,u}}/K_i \geq 7.6$ and $C_{\text{brain,u}}/K_i > 1$ in rat oral LH inhibition studies. This was also the case here, i.e., for analogues 3, 12, and 16−18, with MED values ranging from 3 mg/kg (3) to 30 mg/kg (12 and 17). For example, in rats, 3 was 20-fold more efficacious in vivo against the initial POC lead 2 despite being 3-fold right-shifted in $K_i$. This ameliorated efficacy is reflected in their respective MED-normalized plasma and brain PKPD parameters (Table 3, the last two columns). Otherwise stated, the >1-log LLE superiority of 3 vs 2 underscores the greater unbound exposure levels and consequently the greater in vivo efficacy of 3. Likewise, the monkey LH data (Figure 2) mirrored these trends with 3 4-fold more efficacious (MED levels) although nearly equipotent to 2 in monkey $K_i$ values (Table 3) in keeping with the significantly better MED-normalized plasma PKPD parameter for 3 vs 2.

In summary, 3 proved a superior lead candidate based on bioactivity, LLE, LE, and Fsp$^3$ (Table 1) criteria. Apart from its excellent hERG and CYP safety profile, 3 was highly efficacious in LH inhibition, showed >2.5-log selectivity against Nk3R and Nk2R subtypes, proved >300-fold selective against related HPG axis receptors (KOR, GnRH, GnIH-R, GPR54), and was highly selective in the broad CEREP off-target screen (<25% specificity).

### Table 1. Human Nk3R In Vitro Bioactivity, LogD7.4, Ligand Efficiency Metrics$^{10−12}$ and Off-Target Safety SAR

| Cpd | Structure | pK$\alpha$, pIC$\alpha$ | logD$\alpha$ | LLE | LE | Fsp$^3$ | CYP panel IC$\alpha$ (µM)$^b$ | hERG (µM)$^b$ | IC$\alpha$ |
|-----|-----------|------------------------|-------------|------|-----|--------|-----------------------------|--------------|---------|
| 1   | See Figure 1 | 8.7, 7.7 | 5.0 | 3.7 | 0.33 | 0.15 | 19, 7, 3, 4, 21 | 1.4 |
| 2   | See Figure 1 | 7.9, 7.8 | 3.1 | 4.8 | 0.38 | 0.24 | 50, >100, 21, 34, 26 | 24 |
| 3   | See Figure 1 | 7.6, 7.7 | 1.5 | 6.1 | 0.43 | 0.31 | 90, >100, 42, 48, >100 | >100 |
| 8   | ![Structure](image1) | 8.5, 8.4 | 3.0 | 5.5 | 0.41 | 0.24 | 79, 39, 13, 19, 67 | 8 |
| 9   | ![Structure](image2) | 5.0, 4.8 | 0.8 | 4.2 | 0.28 | 0.31 | -- | -- |
| 10  | ![Structure](image3) | 6.9, 6.8 | 0.7 | 6.2 | 0.39 | 0.31 | >100, >100, 42, 34, 86 | -- |
| 11  | ![Structure](image4) | 7.6, 7.4 | 1.7 | 5.9 | 0.39 | 0.39 | >100, >100, 64, 56, 100 | 1.6 |
| 12  | ![Structure](image5) | 7.0, 6.8 | 1.2 | 5.8 | 0.41 | 0.27 | >100, >100, 82, 56, >100 | 50 |
| 13  | ![Structure](image6) | 5.9, 5.9 | 1.1 | 4.8 | 0.34 | 0.27 | -- | -- |
| 14  | ![Structure](image7) | 6.7, 6.2 | 2.0 | 4.7 | 0.36 | 0.35 | >100, >100, 12, 4, 51 | -- |
| 15  | ![Structure](image8) | 7.7, 7.5 | 2.0 | 5.7 | 0.43 | 0.31 | >100, 88, 7, 50, 99 | 66 |
| 16  | ![Structure](image9) | 8.1, 8.2 | 2.4 | 5.7 | 0.40 | 0.31 | >100, >100, 45, 57, 54 | 39 |
| 17  | ![Structure](image10) | 7.3, 7.2 | 1.3 | 6.0 | 0.42 | 0.31 | 31, >100, 12, 17, 63 | 50 |
| 18  | ![Structure](image11) | 7.5, 7.1 | 1.0 | 6.5 | 0.39 | 0.35 | >100, >100, 77, 54, >100 | 50 |

$^a$N = 3, %RSD ≤ 5. $^b$CYP 3A4, 2D6, 2C9, 2C19, and 1A2, respectively (N = 2, <10% variability). $^c$N = 3, coefficient of variation < 6%.
Table 2. Permeability, Plasma and Brain Fraction Unbound, Brain Exposure, and PK Data

| Cpd | species | plasma \(K_i\) (nM) | LLE | MED (mg/kg) | \(T_{\text{max}}\) (min) | \(C_{\text{plasma,u}}/K_i\) | \(C_{\text{brain,u}}/K_i\) | \(C_{\text{plasma,u}}/K_i\)/MED | \(C_{\text{brain,u}}/K_i\)/MED |
|-----|---------|-------------------|-----|------------|------------------|----------------|----------------|----------------|----------------|
| 2a  | rat     | 76                | 4.0 | 60         | 150              | 16.4           | 2.32           | 0.273          | 0.039          |
| 2c  | monkey  | 20                | 4.6 | 20         | 60               | 13.3           | 5.03           | 7.33           | 1.68           |
| 3c  | rat     | 219               | 5.2 | 3          | 150              | 22.0           | 192            | 38.4           | 1.24           |
| 8a  | rat     | 22                | 4.6 | 10         | 150              | 15.0           | 12.4           | 1.5            | 1.24           |
| 12a | rat     | 2033              | 4.5 | 30         | 150              | 22.5           | 7.77           | 0.75           | 0.09           |
| 16a | rat     | 85                | 4.7 | 10         | 150              | 23.8           | 5.87           | 2.38           | 0.59           |
| 17a | rat     | 573               | 4.9 | 30         | 45               | 47.3           | 7.71           | 1.58           | 0.26           |
| 18a | rat     | 244               | 5.6 | 10         | 45               | 38.9           | 1.82           | 3.89           | 0.18           |

* PK doses: iv, 1 mg/kg (rat), 10 mg/kg (monkey); oral, 3 mg/kg (rat), 5 mg/kg (monkey). Brain exposure dose: 1 mg/kg. Mean values for \(N = 3 - 4\) rats, or 4 monkeys, per group. All rat data at 60 min: (B/P)u = \(C_{\text{brain,u}}/C_{\text{plasma,u}}\). In the AMES genotoxicity test (up to 100 \(\mu\)M), Compound 3 (ESN364) is currently in phase 2 clinical trials for the treatment of PCOS and UF.

**Table 3. PKPD Analysis of the Oral LH Inhibition Studies**

| Cpd | species | \(K_i\) (nM) | LLE | MED (mg/kg) | \(T_{\text{max}}\) (min) | \(C_{\text{plasma,u}}/K_i\) | \(C_{\text{brain,u}}/K_i\) | \(C_{\text{plasma,u}}/K_i\)\)/MED | \(C_{\text{brain,u}}/K_i\)/MED |
|-----|---------|----------------|-----|------------|------------------|----------------|----------------|----------------|----------------|
| 2   | rat     | 76             | 4.0 | 60         | 150              | 16.4           | 2.32           | 0.273          | 0.039          |
| 3   | monkey  | 20             | 4.6 | 20         | 60               | 13.3           | 5.03           | 7.33           | 1.68           |
| 8   | rat     | 22             | 4.6 | 10         | 150              | 15.0           | 12.4           | 1.5            | 1.24           |
| 12  | rat     | 2033           | 4.5 | 30         | 150              | 22.5           | 7.77           | 0.75           | 0.09           |
| 16  | rat     | 85             | 4.7 | 10         | 150              | 23.8           | 5.87           | 2.38           | 0.59           |
| 17  | rat     | 573            | 4.9 | 30         | 45               | 47.3           | 7.71           | 1.58           | 0.26           |
| 18  | rat     | 244            | 5.6 | 10         | 45               | 38.9           | 1.82           | 3.89           | 0.18           |

* Plasma concentrations coincident with LH measurements. MED determined by a significant decrease (\(p < 0.05\)) in LH vs baseline with a lower non-significant dose established in all cases.

**Figure 2.** Oral LH inhibition with 3 (0.5% MC/water) in castrated cynomolgus monkey (2-way ANOVA and Dunnet’s comparison to the vehicle; ***p < 0.001, **p < 0.01).**

inhib at 10 \(\mu\)M). Finally, 3 showed no effect either in Langendorff cardiac safety in rabbits (up to 30 \(\mu\)M) or in AMES genotoxicity test (up to 100 \(\mu\)M). Compound 3 (ESN364) is currently in phase 2 clinical trials for the treatment of PCOS and UF.

**ASSOCIATED CONTENT**

*Supporting Information*

Experimental details for the synthesis and characterization of compounds, X-ray structure of 3 (accession code: CCDC 1052911), and pharmacology and profiling assays. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00117.

**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: hhoveyda@euroscreen.com. Tel: +32-71.348.502.*

**Funding**

This work was supported by the Ministry of Sustainable Development and Public Works, Walloon Region, Belgium.

**Notes**

The authors declare no competing financial interest.

**Biography**

Hamid Hoveyda obtained his Ph.D. from the University of British Columbia (Vancouver, Canada) followed by postdoctoral stints at Harvard University (NSERC fellow) and University of Alberta (Edmonton, Canada). He began his industrial career at the AstraZeneca Research Institute (CA, USA) working on the applications of diversity-oriented synthesis in drug discovery. Later, at Tranzyme Pharma (Canada), he led the discovery of two ghrelin agonists that advanced into clinical development for the treatment of GI disorders. Since 2007, he has led medicinal chemistry efforts on various GPCR targets at Euroscreen (Belgium). His scientific contributions have been captured in over fifty publications and patents.
ACKNOWLEDGMENTS

We thank Prof. Tony Plant and Dr. Suresh Ramaswamy (University of Pittsburgh) for the monkey LH assays.

ABBREVIATIONS

ARC, arcuate nucleus; AST, aspartate transaminase; ALT, alanine aminotransferase; bfα, brain fraction unbound; (B/P)α, unbound brain-to-plasma; CYP, cytochrome P-450; FSH, follicle-stimulating hormone; Fp, fraction sp carbon content; fα, plasma fraction unbound; GnRH, gonadotropin-releasing hormone; HBA, hydrogen-bond acceptor; hERG, human ether-à-go-go related gene; HP/ICD, 9% hydroxypropyl-β-cyclo-dextrin; HPG, hypothalamic–pituitary–gonadal; KNDy, kispeptin-neurokinin B-dynorphin A neuron; LH, luteinizing hormone; LE, ligand efficiency; LLE, ligand lipophilicity efficiency; MC, methyl cellulose; MED, minimum effective dose; NBK, neurokinin B; NKR, neurokinin-3 receptor; Pglycoprotein; PKPD, pharmacokinetic–pharmacodynamic; POC, proof-of-concept; T1/2, elimination half-life; Vα, steady-state volume of distribution.

REFERENCES

(1) Hoveyda, H. R.; Fraser, G. L.; Roy, M.-O.; Dutheuil, G.; Batt, F.; El Bousmaqui, K. J.; Lenoir, F.; Lapin, A.; Noël, S.; Blanc, S. Discovery and optimization of novel antagonists to the human neurokinin-3 receptor for the treatment of sex-hormone disorders (Part 1). J. Med. Chem. 2015, 58, 3060−3082 (Cpd1, 2, and 8 were reported in ref 1 as 3, 31, and 39, respectively). (2) Dawson, L. A.; Porter, R. A. Progress in the development of neurokinin 3 modulators for the treatment of schizophrenia: molecule development and clinical progress. Future Med. Chem. 2013, 5, 1525−1546 and references therein. (3) For a recent review, see: Skorupski, K.; George, J. T.; Anderson, R. A. The kispeptin-GnRH pathway in human reproductive health and disease. Hum. Reprod. Update 2014, 0, 1−16. (4) Marshall, J. C.; Griffin, M. L. The role of changing pulse frequency in the regulation of ovulation. Hum. Reprod. 1993, 8, S7−61. (5) Francou, B.; Bouligand, J.; Voican, A.; Amazit, L.; Trabado, S.; Fagart, J.; Meduri, G.; Brailly-Tabard, S.; Chanson, P.; Lecomte, P.; Guiochon-Mantel, A.; Young, J. Normosomic congenital hypogonadotropic hypogonadism due to TAC3/TACR3 mutations: characterization of neuroendocrine phenotypes and novel mutations. PLoS One 2011, 6, e25614. (6) Fraser, G. L.; Hoveyda, H. R.; Clarke, I. J.; Ramaswamy, S.; Plant, T. M.; Rose, C.; Millar, R. P. The NK3 receptor antagonist ESN364 interrupts pulsatile LH secretion and moderates levels of ovarian hormones throughout the menstrual cycle. Endocrinology, submitted for publication. (7) Rigs, M. M.; Bennets, M.; van der Graaf, P. H.; Martin, S. W. Integrated pharmacometrics and systems pharmacology model-based analysis to guide GnRH modulator development for management of endometriosis. CPT: Pharmacometrics Syst. Pharmacol. 2012, 1, e11. (8) Millar, R. P.; Newton, C. L. Current and future applications of GnRH, kispeptin and neurokinin B analogues. Nat. Rev. Endocrinol. 2013, 9, 451−466 and references therein. (9) Hopkins, A. L.; Keserü, G. M.; Lesson, P. D.; Rees, D. C.; Reynolds, C. H. The role of ligand efficiency metrics in drug discovery. Nat. Rev. Drug Discovery 2014, 13, 105−121 and references therein. (10) Leeson, P. D.; Empfield, J. R. Reducing the risk of drug attrition associated with physicochemical properties. Annu. Rep. Med. Chem. 2010, 45, 381−391 and references therein. (11) LE = (1.37/HAC) × pKc. HAC = number of non-hydrogen atoms (ref 9). (12) Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: increasing saturation as an approach to improving clinical success. J. Med. Chem. 2009, 52, 6752−6756.