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Perspective

Non-Peptide Gonadotropin-Releasing Hormone Receptor Antagonists

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Introduction

Gonadotropin-releasing hormone (GnRH[a]) is the principal neuroendocrine regulator of the reproductive system in humans. It is a linear decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, that was first isolated from porcine and ovine hypothalamii.[1,2] This peptide and related isoforms have subsequently been identified in a wide range of species, indicating that core features of the sequence have been conserved over ~600 million years of chordate evolution.[3–6] The GnRH peptide is made by neurons in the hypothalamus and secreted in pulses approximately hourly into the portal blood supplying the pituitary (Figure 1).[7] There, it stimulates secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), into the general circulation. The gonadotropins in turn act at the gonads to support spermatogenesis and synthesis of testosterone in the male and follicular development and production of estrogen and progesterone in the female. Gonadal steroids in turn feed back to regulate the hypothalamus and pituitary.

The actions of GnRH are mediated by the GnRH receptor (GnRH-R).[4] It is a member of the rhodopsin family of seven-transmembrane receptors and was first cloned from the mouse[8] and subsequently from a variety of other species, including human.[3,9–11] It differs from other class A GPCRs by the absence of a C-terminal tail, which is a predominate site of regulation by various kinases in other family members.[12] Binding of GnRH induces a conformational change in the receptor, which in turn activates the GTPases $G_{\alpha q}$ and $G_{\alpha 11}$ resulting in G-protein activation, which stimulates activity of phospholipase C resulting in phosphatidyl inositol turnover,[13] intracellular calcium release, and gonadotropin secretion.[14] Continuous agonist administration causes an initial stimulatory effect, followed by desensitization of the pituitary and eventually down-regulation of gonadotropin secretion over the course of 1−2 weeks.[15–16] This pituitary down-regulation leads to a profound suppression of the reproductive endocrine axis and can be exploited therapeutically to produce medical gonadectomy which reduces circulating sex steroids to levels equivalent to surgical castration.[17] On the basis of this somewhat paradoxical mechanism, several GnRH agonist peptides such as leuprolide, goserelin, and triptorelin are now commercially available in long acting injectable depot formulations for gonadal suppression. They have found widespread utility for a range of steroid hormone dependent diseases[17] and assisted reproductive therapy[18] as outlined in Table 1.

Early studies in the structure–activity relationships of GnRH peptides identified His^2 as critical for receptor activation.[22] Substitutions with D-amino acids at this position and optimization of multiple additional residues in the peptide led to the identification of potent antagonists.[23] These efforts eventually led to multiple peptides that have been evaluated in clinical studies.[20,24]

In contrast to the initial stimulation caused by agonists, antagonists immediately inhibit pituitary gonadotropin secretion.[25] Thus, in patients with advanced prostate cancer, antagonists avoid the initial "flare" in testosterone produced by agonists and result in a more rapid reduction in testosterone.[26,27] Analogously in women, peptide antagonists reduce uterine fibroid volume in 2−4 weeks, more rapidly than is observed with agonist therapy.[28,29] Antagonists also require fewer injec-

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[a] Abbreviations: AUC, area under the curve; b.i.d., twice daily dosing; C_{max}, highest concentration; E_2, estradiol; ECL, extracellular loop; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; GnRH-R, gonadotropin-releasing hormone receptor; GPCR, G-protein-coupled receptor; GTPase, guanosine triphosphatase; HPG, hypothalamic−pituitary–gonadal; im, intramuscular; ip, intraperitoneal; IVF, in vitro fertilization; LH, luteinizing hormone; NTD, N-terminal domain; sc, subcutaneous; TM, transmembrane.

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Table 1. Peptide GnRH-R Modulators Approved for Use in the U.S.

Peptide	Formulations	Approved Indications (US)[a]	Other indications evaluated in clinical trials[c]
Agonists			
leuprolide	s.c. injection s.c. depot i.m. depot	Prostate cancer, endometriosis, uterine fibroids, precocious puberty	Prevention of premature LH surge, fibrocystic breast disease, polycystic ovarian syndrome, premenstrual syndrome; benign prostatic hypertrophy, irritable bowel syndrome, menstrual migraine, mammography improvement, gonadal protection during chemotherapy or radiotherapy, paraphilias, Alzheimer's, breast cancer prevention
goserelin	s.c. injection s.c. depot	Prostate cancer, endometriosis, breast cancer, endometrial thinning	
nafarelin	nasal spray	Endometriosis, precocious puberty	
triptorelin	i.m. depot i.m. injection	Prostate cancer	
histrelin	i.m. depot	Prostate cancer, precocious puberty	
Antagonists			
cetrorelix	s.c. injection	Prevention of premature LH surge	Endometriosis, benign prostatic hyperplasia, uterine fibroids, prostate cancer
ganirelix	s.c. injection	Prevention of premature LH surge	
abarelix[b]	i.m. depot	Prostate cancer	

[a] Based on label information available at the U.S. Food and Drug Administration Web site (www.fda.gov). [b] Marketing discontinued in 2007. [c] For reviews of the clinical uses of peptide GnRH-R agonists and antagonists, see refs 17, 19–21.

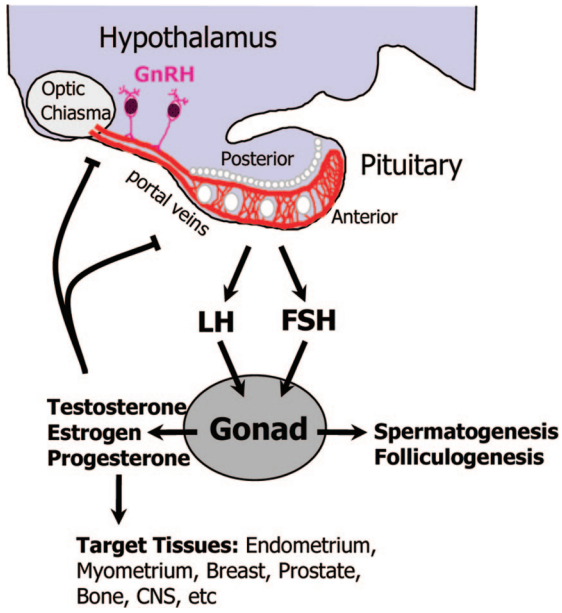

Figure 1. Schematic overview of the hypothalamic−pituitary–gonadal axis.

tions and reduction in the duration of treatment during in vitro fertilization protocols.[20] Several peptide GnRH-R antagonists, including cetrorelix, ganirelix, and abarelix, have subsequently become commercially available for clinical use (although marketing of abarelix has recently been discontinued). Cetrorelix and ganirelix are currently available only in formulations for short acting subcutaneous injection. Abarelix, which was available as a long acting depot for prostate cancer, requires careful patient monitoring because of occurrences of immediate-onset systemic allergic reactions. Depot preparations of the next generation peptide antagonists degarelix[30,31] and ozarelix[32] are currently in late stage clinical development. With the exception of nafarelin, which is formulated as a nasal spray, peptide GnRH-R modulators require parenteral administration. Injection site reactions can sometimes occur. Furthermore, treatment cannot be readily discontinued or modified with the depot preparations.

Since the earliest days of GnRH peptide analogues, there has been significant interest in developing orally active agents. As early as 1982, oral activity of a peptide antagonist to suppress ovulation in female rats was demonstrated, albeit with poor apparent oral bioavailability (<1%).[33] During this period there was considerable effort to design improved peptide analogues,[23] including early application of computational techniques for rational design.[34] Later generations of peptide antagonists contain multiple D-amino acids and highly modified sidechains. Thus, their resemblance to the naturally occurring GnRH peptide is minimal. The bioactive conformation of these peptides is highly compact with various cyclic peptide structures being highly potent.[35,36] This suggested that it could be possible to mimic peptide interactions with GnRH-R using smaller non-peptide molecules. However, these strategies did not lead to what would generally be considered non-peptide, small molecule structures typically used as oral therapeutics.

The first report of a truly non-peptide GnRH-R antagonist was by De and co-workers at Abbott Laboratories who identified the antifungal drug ketoconazole as a weak antagonist of rat GnRH-R.[37] With the cloning of GnRH-R in 1992,[8] screening strategies to identify non-peptide leads became viable. Shortly thereafter, Furuya and co-workers at Takeda Chemical Industries reported a series of potent thieno[2,3-*b*]pyridin-4-one derivatives in the patent literature.[38] As will be discussed below, numerous examples of non-peptide GnRH-R antagonists have appeared in the literature following that initial report, demonstrating that a wide range of chemical structures can satisfy the requirement for high affinity binding to the receptor.[39–42]

Non-peptide antagonists of GnRH-R are an important new class of potential therapeutics for a range of indications, only some of which are currently addressed by peptide GnRH analogues. Oral administration not only avoids injection site

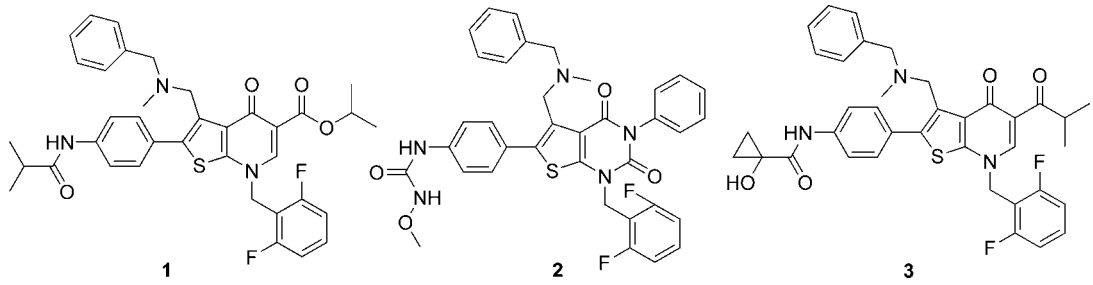

Figure 2. GnRH-R antagonists from thieno[2,3-*b*]pyridin-4-one and thieno[2,3-*d*]pyrimidine-2,4-dione classes.

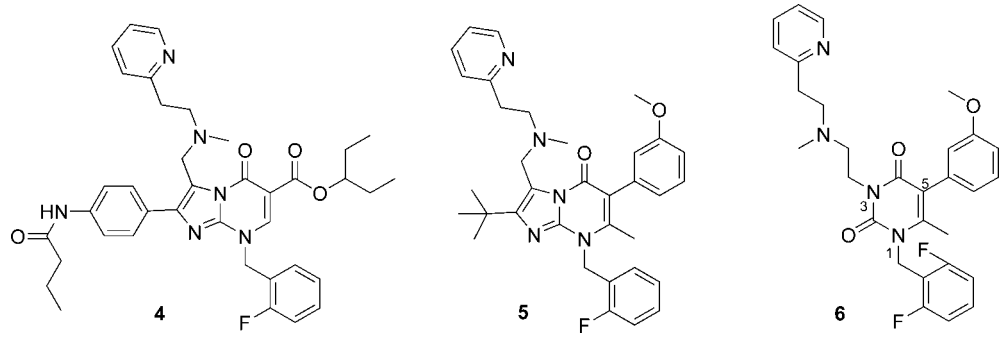

Figure 3. Evolution of SAR from bicylic analogues to monocyclic compounds.

reactions and doctor's office visits for depot insertions (significant improvements from a patient's perspective) but also provides a level of control over the degree of pituitary suppression that is simply not available with a down-regulating agonist or one size-fits-all antagonist depot. As yet, the field is still young with only two compounds reaching phase II clinical trials. Here, we review the developments in the field from the first non-peptide antagonists to the most recent clinical data. We have attempted not only to summarize the available SAR but to put these chemical structures into the context of receptor binding modes and recent advances in the pharmacology of these agents in order to critically assess the ability of typical lead optimization strategies to yield clinically useful agents.

Structure–Activity Relationship of Thieno[2,3-*b*]pyridin-4-one and Related Antagonists

In 1998, Cho and co-workers at Takeda revealed the first series of high affinity non-peptide GnRH-R antagonists in the peer-reviewed literature.[43] SAR studies led to identification of compound **1** (T-98475) (Figure 2), a thieno[2,3-*b*]pyridin-4-one analogue as a highly potent antagonist with an $IC_{50} = 0.2$ nM (binding affinity) versus the cloned human receptor. The compound had reduced binding affinity for both the monkey receptor ($IC_{50} = 4$ nM) and the rat receptor ($IC_{50} = 60$ nM), which were isolated from pituitaries, a common pattern for several classes of non-peptide GnRH-R antagonists. The compound demonstrated in vivo efficacy as measured by LH suppression in castrated male cynomolgus monkeys. Further chemical modification of this lead eventually resulted in a new series of molecules that are based on thieno[2,3-*d*]pyrimidine-2,4-diones.[44] Among them, **2** (TAK-013 or sufugolix, which later reached clinical development and will be discussed below) was a highly potent antagonist against human GnRH-R ($IC_{50} = 0.1$ nM, binding affinity) and monkey receptor ($IC_{50} = 0.6$ nM, binding affinity). Moreover, **2** demonstrated improved in vivo efficacy compared to **1** in castrated monkeys, evidenced by a longer duration of LH suppression even at lower doses. It is also worth noting that the unique methoxyureido group on the left-hand side of **2** improved the oral bioavailability over a urea analogue presumably because of intramolecular hydrogen bonding between the methoxy oxygen atom and the aniline NH which reduces the cost of desolvation during intestinal absorption. Recently, a detailed SAR of the previous series (thieno [2,3-*b*]pyridin-4-ones) was reported by Imada and co-workers.[45] Additional optimization of the initial lead **1** resulted in molecule **3** that is also highly potent both in vitro and in vivo. In castrated male cynomolgus monkeys, **3** suppressed plasma LH for greater than 24 h after oral administration at both 10 and 30 mg/kg, nearly equivalently to **2.** The improved in vivo efficacy compared to **1** could be partially attributed to its improved oral exposure, although direct comparison of oral exposure between **1** and **3** were not available.

SAR and Pharmacophore of Uracil and Related Compounds

Bicyclic imidazolopyrimidinones represented by **4** ($K_i = 7.5$ nM), initially reported by both Takeda and Neurocrine,[46,47] were modified by Gross et al. by replacing the labile ester group on the right-hand side of the molecule with a 3-methoxyphenyl moiety together with introduction of 7-methyl group,[48] resulting in similar potency but with simplified structures (e.g., **5**, $K_i =$ 5.2 nM) (Figure 3).[49] These results enabled Neurocrine scientists to remove part of the imidazole ring to yield the first uracil GnRH-R antagonist (e.g., **6**, $K_i = 34$ nM).[50] Despite the reduced potency of **6** compared to the bicyclic **5**, the uracil core offered a low molecular weight template for further optimization.

Incorporation of an *R*-configured methyl group at the α-position of the 2-aminoethyl side chain of **6** (introduced to restrict the flexible amine-containing side chain) resulted in a 5-fold improvement in potency (**7**, $K_i = 5.2$ nM, Figure 4).[51] In comparison, the *S*-analogue of **7** was much less potent ($K_i =$ 470 nM), suggesting that the steric effect of this methyl group is significant. More importantly, this modification also permitted reducing of the linker between the basic nitrogen and the 2-pyridine group (**8**, $K_i = 5.5$ nM) without compromising potency, simultaneously eliminating the metabolically labile

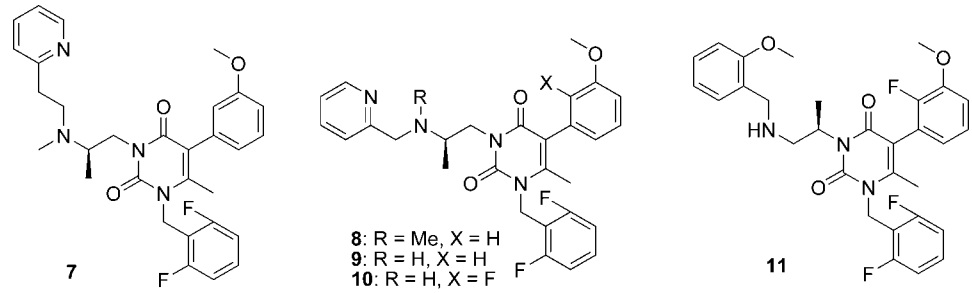

Figure 4. Uracils with methyl group additions designed to reduce flexibility at the 3-position.

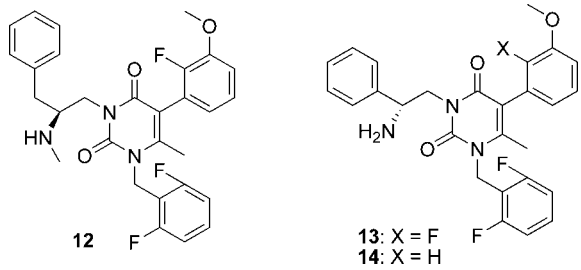

Figure 5. Uracils with further modification at 3-position to enhance potency and metabolic stability.

2-pyridylethylamino group. Moreover, the *N*-methyl group of **8** was determined to be of minimal importance because the secondary amine **9** (K_i = 15 nM) was only slightly less potent. Addition of a fluorine at the 2-position of the 5-(3-methoxyphenyl) group further improved binding affinity over 10-fold (**10**, K_i = 1.1 nM).[52] Similarly, improved binding affinity was also obtained by introducing an *R*-configured methyl group at the β-position of the amino side chain (**11**, K_i = 3.7 nM).[53]

With potent non-peptide antagonists such as **10**, the focus turned to optimizing the pharmacokinetic properties of these compounds. Although the secondary amines (**9**–**11**) were more metabolically stable in vitro than the tertiary amine **7**, their oral bioavailability in rodents was still poor. One of the major products of in vitro liver microsome incubation of **9** or **10** is N-dealkylation. This potential liability was somewhat mitigated by migrating the lipophilic N side chain of **10** to the neighboring methyl group. For example, compound **12** (K_i = 8.1 nM) is a potent GnRH-R antagonist in vitro (Figure 5). This compound also exhibits moderate to good oral bioavailability in mice (42%) and monkeys (22%).[54]

One compound that resulted from continued optimization was **13** (NBI-42902, which also reached clinical development and will be described in detail below), which has a K_i value of 0.56 nM versus human GnRH-R.[55] **13** was also potent at the monkey receptor (K_i = 3.5 nM), though it exhibits negligible affinity at the rat receptor (K_i = 3000 nM). Such species selectivity is very common for this class of compounds, which precludes efficacy experiments in rodents.

One interesting phenomenon observed for 5-(2-fluorophenyl)-6-methyluracils is that the molecules are atropisomeric. For example, **13** exists as a pair of isomers at room temperature. ^{1}H NMR spectra of **13** show two sets of signals in various solvents. In comparison, the des-fluoro analogue **14** (K_i = 2.3 nM) exhibits a single set of resonances under similar conditions. Further experiments using NMR and HPLC demonstrated that the interconversion between the two rotamers (a*R*- and a*S*-**13**) is fast, with a half-life estimated to be about 46 min at 37 °C in aqueous solution.[56] The X-ray crystal structure of **13** shows that the 2-fluoro-3-methoxyphenyl ring is nearly orthogonal to the uracil ring in the solid state (Figure 6), supporting the low-energy conformation and slow rotation of the biaryl bond.[55]

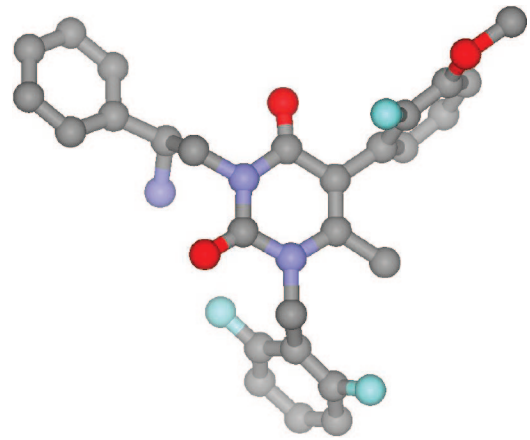

Figure 6. Crystal structure of compound **13**. Reproduced with permission from Tucci, F. C.; et al. *J. Med. Chem.* **2005**, *48*, 1169–1178.[55] Copyright 2005 American Chemical Society.

An attempt to replace the 2-fluoro-3-methoxyphenyl group with various heteroaromatic groups to eliminate atropisomers while retaining potency resulted in some interesting compounds (Figure 7).[57] It was found that a 2-thienyl group was, among other heteroaryl groups, a reasonably good substituent of the 5-phenyl group. For example, the 5-chloro-2-thienyl analogue **15** (K_i = 2 nM) is about 7-fold better in binding affinity than its bioisostere 4-chlorophenyl **16** (K_i = 14 nM). However, there has been no further publication on any follow-up on this class of derivatives.

Since the 1-(2,6-difluorobenzyl) group was inherited from the very early bicyclic compounds, a survey of this group in the uracil class was conducted by Rowbottom et al.[58] A novel synthetic route was developed to efficiently study the SAR at this position, and the results indicated that a benzyl group was important for high potency, since the cyclohexylmethyl possessed low affinity versus human GnRH-R. Replacing one of the two fluorine groups in **17** (K_i = 6 nM) with a chlorine provided an analogue with a greater binding affinity (**18**, K_i = 0.7 nM), which was about 30-fold better than the monofluoro compound **19** (K_i = 19 nM). Highly potent compounds were also obtained from the methylsulfonyl (**21**, K_i = 0.2 nM) and trifluoromethyl (**22**, K_i = 0.5 nM) substitution, which were much higher in affinity than the more polar methylsulfoxide (**20**, K_i = 4.4 nM) (unpublished results).

The crystal structure of **13** showed that the 1-benzyl group was directed away from the aromatic uracil plane (Figure 6). Pontillo et al. synthesized a series of bicyclic oxazolo- and thiazolopyrimidinones to constrain the 1-benzyl group of **13** (Figure 8).[59] The oxazolopyrimidine **23** (K_i = 230 nM) had a similar binding affinity compared to the thiazolopyrimidine **24**

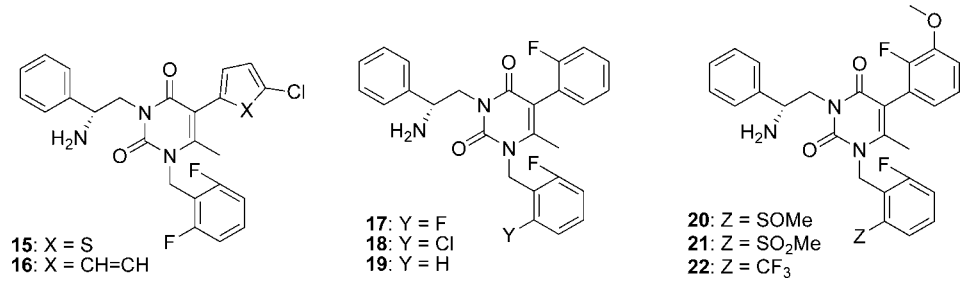

Figure 7. SAR of 1 and 5 positions of uracil class non-peptide GnRH-R antagonists.

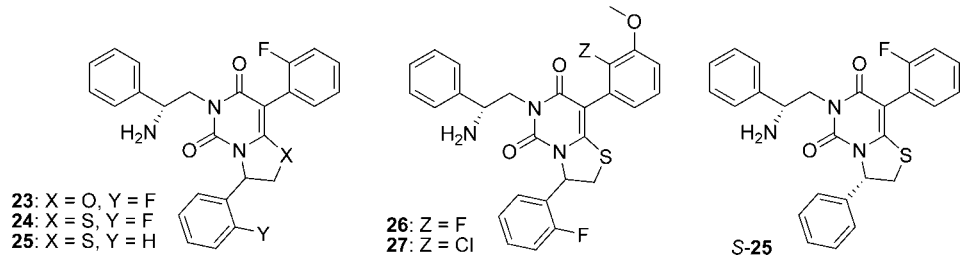

Figure 8. Cyclization of 1,6-substituted uracils to create novel bicyclic structures.

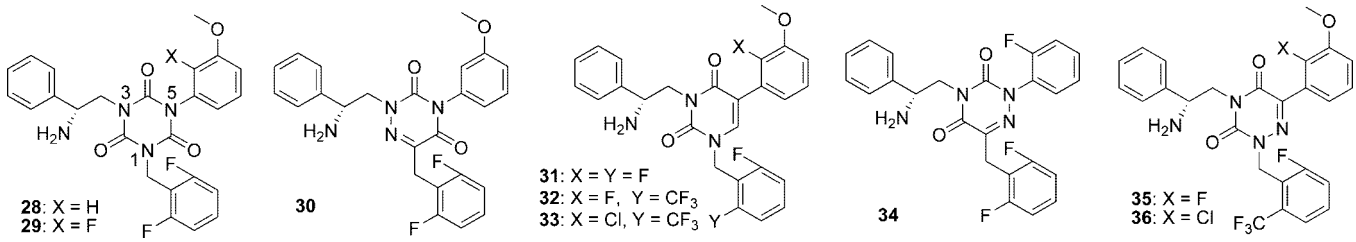

Figure 9. Variety of uracils and uracil mimics that test the requirement for substitution at the 6-position.

(K_i = 220 nM). However, both were considerably less potent than the corresponding uracil analogue **19** (K_i = 20 nM). A fluoro substituent at the bottom phenyl ring had a minimal effect because the unsubstituted analogue **25** (K_i = 230 nM) was equivalent to **24**, suggesting that the SAR in this series diverges from the uracils. The potency of **24** was improved by addition of a 3-methoxy group to the 2-fluorophenyl ring (**26**, K_i = 40 nM), and a potent antagonist **27** (K_i = 4.5 nM) was identified as a pair of diastereoisomers (because of the additional chiral center in the thiazolidine ring). Interestingly, NMR studies did not indicate the presence of atropisomers for either **26** or **27**, suggesting that the five-membered thiazolidine ring allows for faster rotation of the 2-halophenyl group, which most likely contributes to their lower potency compared to the atropisomeric compounds such as **13**. It was determined that the *S*-configured isomer of the dihydrothiazolidine was preferred because *S*-**25** (K_i = 110 nM) had approximately 2-fold improved affinity over the mixture **25**. However, the corresponding *R*-isomer was not isolated to confirm that preference. Identification of the bioactive isomer in one of the more potent compounds in this series such as **27** would help to define the receptor bound conformation of the substituted 1-benzyl group typically found in the uracil series.

A series of triazine-*s*-triones was synthesized and studied as GnRH-R antagonists by Guo and co-workers (Figure 9).[60] Compound **28**, which was a direct analogue of the uracil **14**, was only moderately potent (K_i = 37 nM).[61] Similarly, the 2-fluoro-3-methoxyphenyltriazine-*s*-trione **29** (K_i = 9 nM) was about 15-fold less potent than the uracil analogue **13**. It is worth noting that **29** existed as a pair of atropisomers at room temperature based on ^{1}H NMR. In comparison, the 4-(3-methoxyphenyl-1,2,4-triazine-3,5-dione **30** had a K_i value of 110 nM, about 4-fold less potent than **28**.[62] Whether this apparently reduced potency of these series, compared to the 6-methyl-uracils, is due to the 6-carbonyl group or due to more subtle geometric differences around the N−C bond connecting the 5-substituent to the core is unclear.

Uracils without the 6-methyl group were also studied.[63] These compounds were generally about 10-fold less potent than their 6-methyluracil counterparts. For example, **31** has a K_i of 5.3 nM. However, SAR showed that the potency of this class of compounds could be improved. Thus, the 1-(2-fluoro-6-trifluoromethyl)benzyl analogue of **31** had a 9-fold increase in binding affinity (**32**, K_i = 0.64 nM). Its 5-(2-chloro-3-methoxy)phenyl derivative **33** (K_i = 0.45 nM) also exhibits high affinity. As expected, none of these compounds were atropisomeric at room temperature. By comparison, the 2-(2-fluorophenyl)-1,2,4-triazine-3,5-dione **34** (K_i = 580 nM) was only weakly active.[62]

6-Azauracils were also synthesized and studied by Pontillo and co-workers as GnRH-R antagonists.[62] The SAR of this series of compounds was quite similar to that of uracils, with the azauracils consistently less potent. For example, the 2-chloro-3-methoxyphenyl **36** (K_i = 2.3 nM), while exhibiting over 5-fold higher affinity compared with the corresponding fluoro analogue **35** (K_i = 13 nM), was about 5-fold less potent than the corresponding uracil **33**.

As is clear, the pharmacophore of uracils and related compounds as GnRH-R antagonists has been studied in great detail, and the key features are summarized in Figure 10. While the uracil core holds the 1-benzyl, 3-aminoethyl, and 5-phenyl groups in position for interaction with the receptor, the 2-, 4-, and 6-moieties play different roles. The 2-carbonyl group has

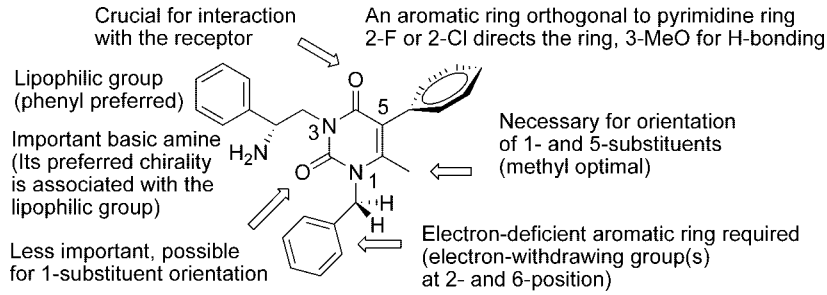

Figure 10. Summary of uracil pharmacophore as human GnRH-R antagonists.

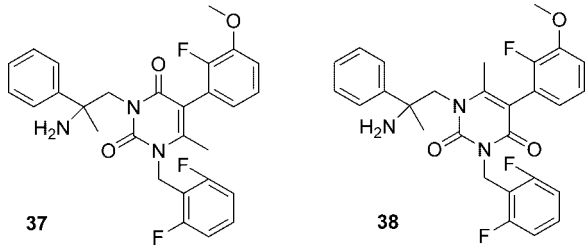

Figure 11. Isomeric uracil analogues illustrate the importance of the "top" carbonyl group on the uracil core.

a minimal effect based on the comparison between **28** and **30**. By contrast, the 4-carbonyl moiety appears to be critical. For example, while the uracil **37** (K_i = 23 nM) possessed good binding affinity, its regioisomer **38** was inactive in the same equilibrium binding assay, demonstrating the importance of the carbonyl group being in the 4-position with that arrangement of side chain interactions of **37** (unpublished observations) (Figure 11).

The 6-methyl group of the uracil template also significantly contributes to the high potency of this series of compounds. While the des-methyl analogue **31** was approximately 10-fold less potent than **13**, an ethyl derivative also exhibited reduced binding affinity. The main role of this methyl is most likely to orient the 5-phenyl ring, as well as the 1-benzyl group. The 1-benzyl group is steered away from the uracil plane and requires electron-withdrawing groups for high potency. The 5-phenyl group needs to be orthogonal to the uracil ring, and chlorine is better than fluorine, which is superior to a proton at the 2-position to achieve this orientation. The biologically preferred atropisomer has not been determined. The 3 side chain is relatively flexible, and the requirements are a basic amine and a lipophilic group, with a phenyl moiety preferred.

Lastly, a series of isoguvacines were synthesized and studied as analogues of the uracil GnRH-R antagonists,[64] and relatively potent analogues such as **39** (K_i = 15 nM) were identified (Figure 12). This compound possessed a similar structure on the left side of early uracil compounds such as **40** (K_i = 30 nM).[50] The SAR on the right sides of these molecules was substantially different, however, and did not fit the general uracil pharmacophore described above, suggesting an as yet to be determined alternative binding mode.

Other Classes of Non-Peptide GnRH Antagonists

Several other classes of GnRH-R antagonists exemplified by the macrolide A-198401 (**41**),[65] indole **42**,[66] quinolone **43**,[67,68] and furan **44**[69,70] have been reported and extensively reviewed previously (Figure 13).[40–42]

Most recently, benzimidazole derivatives have become a new family member of non-peptide GnRH-R antagonists. Hashimoto and co-worker at Bayer Japan[71] disclosed a lead optimization effort based on their initial screening hit **45** (IC_{50} = 3.4 μM, human Ca^{2+}mobilization assay), which was optimized to **46** (IC_{50} = 0.12 μM) (Figure 14). A further improvement was achieved by Li and co-workers[72] yielding the potent compound **47** that has an IC_{50} below 10 nM in the same assay. In this family, the urea moiety, which functions as both hydrogen bond donor and acceptor, was identified as the critical interaction site with the receptor to bolster activity. Generally speaking, the series has no species selectivity issues as judged by its equal potency for the human and rat receptors. However, there has not yet been any further report on in vivo activity of compounds from this class.

In addition, Tatsuta and co-workers[73] reported the SAR based on another benzimidazole "hit" **48** (IC_{50} = 10 μM, human Ca^{2+} mobilization assay) that was obtained from HTS. However, no single-digit (or sub-) nanomolar activity compounds have been described. Compound **49** is one example that was relatively potent (IC_{50} = 41 nM) for human receptor.

Independently, another benzimidazole class of non-peptide human GnRH-R antagonists was revealed recently by Pelletier and co-workers at Wyeth.[74] SAR studies based on the HTS screening "hit" **50** (IC_{50} = 0.8 μM, competition binding) led to a series of potent GnRH-R antagonists exemplified by **51** (IC_{50} = 6.6 nM)[75] and **52** (IC_{50} = 5.2 nM) (Figure 15).[76] These compounds, however, do not have optimal druglike characteristics because of CYP3A4 inhibition, low permeability, and poor aqueous solubility. Compound **52** was also reported to lack in vivo activity in rats (30 mg/kg, po), which the authors attribute to its poor oral bioavailablity. Eventually, further optimization of the "top" heterocycle led to **53**, which not only has a very high affinity for human GnRH-R (IC_{50} = 1.6 nM, competition

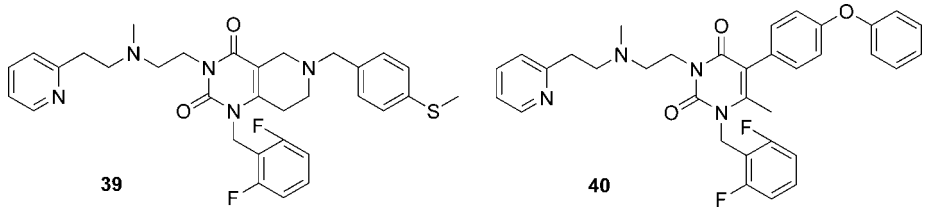

Figure 12. Isoguvacine GnRH-R antagonists.

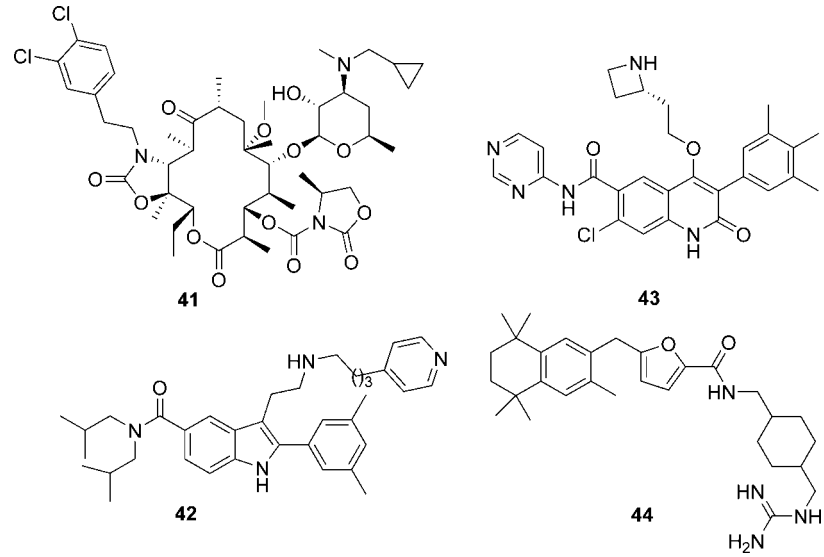

Figure 13. Additional classes of GnRH-R antagonists published before 2003.

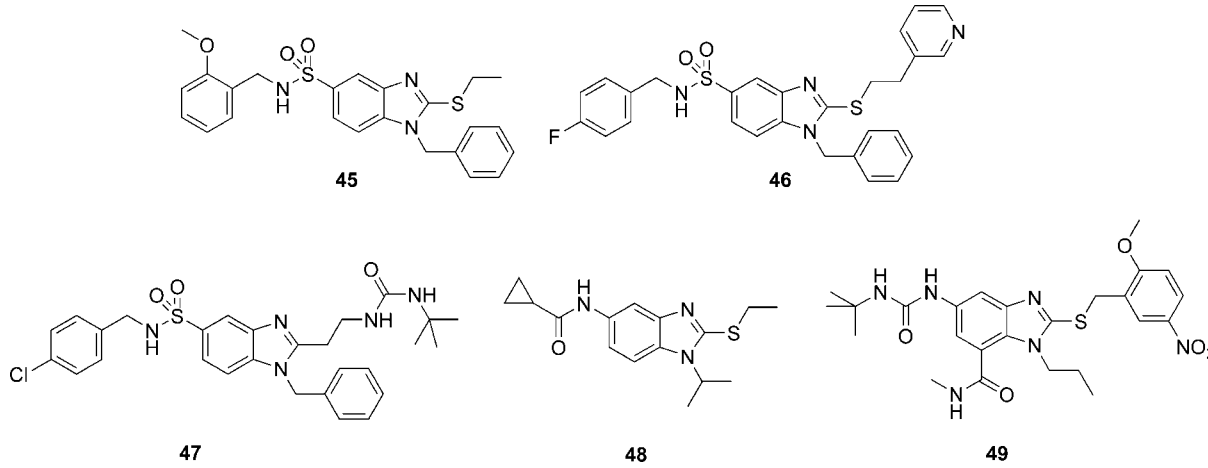

Figure 14. Representative benzimidazole GnRH-R antagonists.

binding) but also is highly bioavailable ($F = 72\%$) in rats and demonstrates impressive LH suppression in castrate rats with oral dosing at 30 mg/kg, despite being approximately 10-fold less potent versus the rat receptor ($IC_{50} = 18$ nM).[74] The improved oral bioavailablity of **53** compared to similar analogues was attributed to a reduction of the polar surface area and the number of the hydrogen bond donors.[77] However, it is unclear whether **53** still possesses the CYP3A4 inhibition liability of earlier compounds that could hinder its progress toward further development.

In 2006, Li and co-workers[78] at Pfizer reported a furan analogue **54** (Figure 16) that exhibited picomolar affinity to both human and rat GnRH-Rs. The compound is likely the product of a lead optimization effort on an earlier series (e.g., **55**).[69,70,79] Compound **54** was also reported to be highly selective versus 54 other G-protein-coupled receptors, enzymes, and ion channels. Furthermore, it also demonstrated dose-dependent suppression of testosterone to castrate-levels in gonad-intact male rats by oral administration. There is no report on further development of this series into preclinical or clinical studies.

Most recently, Chen and co-workers[80] disclosed another novel series of non-peptide GnRH-R antagonists based on the tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine template. The most potent compound **56** is a highly active antagonist versus both the human and rat receptors ($IC_{50} = 1.5$ and 0.2 nM, respectively). It also demonstrates significant LH suppression in castrate male rats via ip administration. Interestingly, SAR of this class of molecules seems to overlap partially with an earlier indole series of antagonists reported by Merck (e.g., **57**)[81] on the right-hand side of the molecule, although the SAR does not correlate as well on the left-hand side.

Structural Pharmacology of Non-Peptide Ligands

The variety of chemical series that can be developed into potent GnRH-R antagonists suggests that a wide range of different receptor interactions are utilized. However, the receptor binding site has evolved to recognize a relatively small peptide. So how can this be accomplished? To answer these questions, relatively extensive investigations into the structural basis of receptor pharmacology have been conducted.

As a basis for this analysis, a model to translate the sequence and topology of the receptor into three-dimensional structural hypotheses is required (Figure 17). The crystal structure of rhodopsin has been used extensively to create three-dimensional homology models of class A GPCRs.[82] Such models have been recently developed for human GnRH-R[4,83,84] and have been used to examine the relationship between GnRH peptide sequence conformations, receptor selectivity, and activation,[4,85,86] as

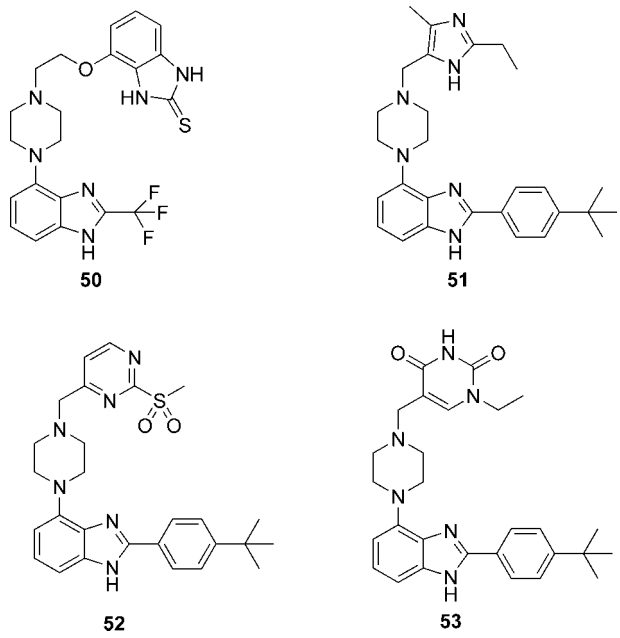

Figure 15. Representative piperazine-benzimidazole GnRH-R antagonists.

well as the interactions of several classes of non-peptide antagonists.[84,87,88] A comparison of the inactive states of each model suggests that they are quite similar and are robust enough to model regiospecific interactions and occasionally well-defined enough to permit atomic-level hypotheses. Considerable work has been published on deciphering the interactions between the peptide and the receptor.[4,83,85,86] These models predict a relatively large pocket formed by the helical bundle that effectively binds the hairpin structure of GnRH agonist and antagonist peptides.[4] Figure 17 displays the proposed overall tertiary structure of the GnRH receptor with several key residues demonstrated to interact with non-peptide antagonists highlighted.

Similar to what has been done with GnRH peptide variants,[4] the SARs of several non-peptide classes have been examined using mutant receptors to determine the molecular interactions that define binding of each class to the receptor. Initially, these studies focused on understanding the determinants of species selectivity, which has been observed in several non-peptide antagonist classes though not in peptides.[67,90] The wide array of diverse pharmacophores that give rise to GnRH-R non-peptide antagonists, however, causes a conundrum when considering the location of binding interactions between them and the receptor. Nearly all non-peptide GnRH-R antagonists identified appear competitive with the radiolabeled agonist peptides, consistent with being orthosteric ligands (i.e., a ligand that binds at the site of the endogenous agonist that initiates receptor signaling).[91] However, it seems unlikely that the many different ligands could interact with the receptor in the same way or in a manner that completely mimics the binding of peptides. Recently, a large-scale receptor mutagenesis effort was undertaken to compare the binding modes of different classes of non-peptide antagonists.[84] This analysis revealed that each class possesses a distinct set of interactions with the receptor and that the individual sets partially overlap with one another as well as with the set of residues defined by peptide agonists.

One of the earliest successful classes of non-peptide antagonists was a series of thienopyrimidinediones developed by Takeda. Exemplified by **2**, these molecules possess high receptor affinity and were efficacious in in vivo nonhuman primate models of gonadal axis suppression.[44]

Several key sites of interactions for the thienopyrimidinedione series were determined such that a ligand docking model of **2** bound to the receptor could be developed (Figure 18).[87] Several distinct interactions between the non-peptide and the receptor were found, notably on the extracellular sides of TM6, TM7, and ECL3. Alanine mutations at D302$^{(7.32)}$ and H306$^{(7.36)}$ indicate that those residues are part of a hydrogen-bond network important for anchoring the most potent compounds in this series, those with a trans urea substituent on the 6-(4-aminophenyl) group.[87] A separate regiospecific interaction was determined between the *N*-benzyl-*N*-methylamino substituent and L300$^{(6.68)}$ in ECL3 and Y290$^{(6.58)}$ in TM6. The deciphering of both of these interactions was critical in dispelling a long-standing assumption that the positively charged amino group in this series (and observed to be important in the SAR of several other non-peptide classes) mimicked the interaction of Arg^8 in GnRH peptides,[43,92] which forms a charge-based interaction with D302$^{(7.32)}$.[93,94] The final model predicts that the molecule makes contact with several other areas of the receptor as well, including the N-terminal domain and TM3. It would be interesting to determine if the recently developed potent urea-containing benzimidazoles (e.g., **47**) mimic the urea−TM7 interactions proposed for **2**.

As described above, considerable SAR has been described by Neurocrine for a series of uracil-based non-peptide GnRH-R

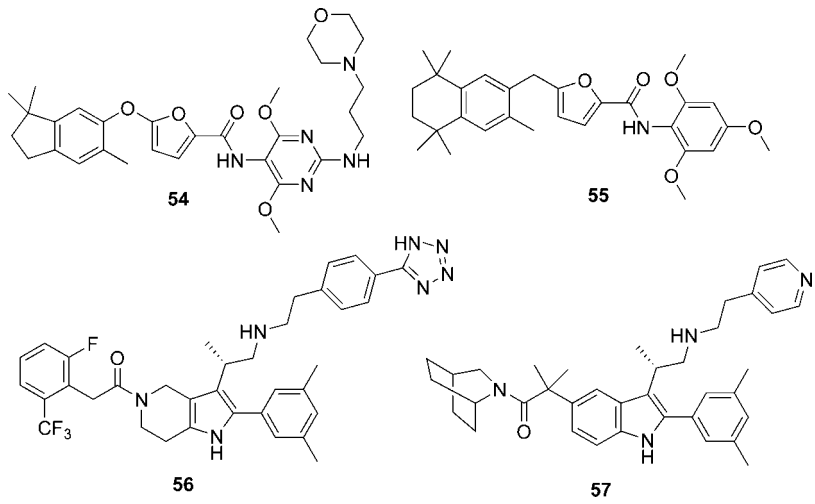

Figure 16. Furan and tetrahydropyrrolopyridine GnRH-R antagonists.

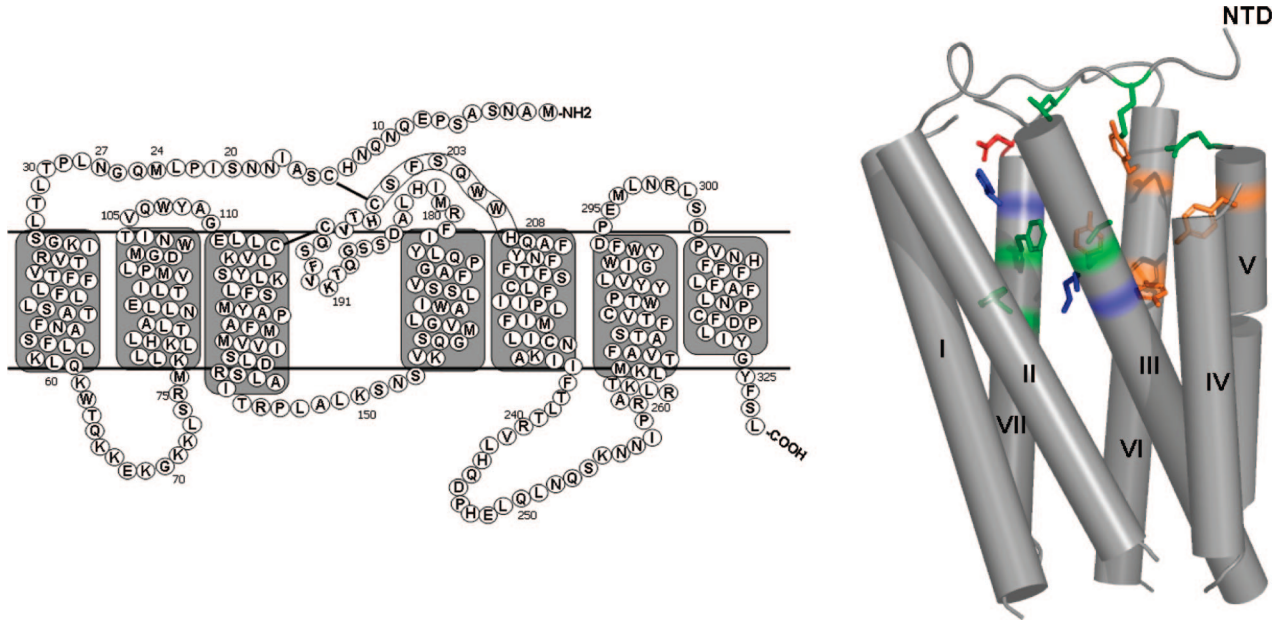

Figure 17. Structure and three-dimensional model of human GnRH-R. Left: Primary structure and predicted secondary structure of human GnRH-R. Transmembrane helical regions are indicated by gray cylinders. Right: three-dimensional model of hGnRH-R. Transmembrane helical regions are depicted by gray cylinders and indicated with Roman numerals. Several residues important for the binding of various non-peptide GnRH-R antagonists are highlighted: M24(green) , S118$^{(3.29)}$(green), K121$^{(3.32)}$ (blue), Q208$^{(5.35)}$ (green), Y211$^{(5.38)}$(orange), Y283$^{(6.51)}$ (orange), Y284$^{(6.52)}$ (orange), Y290$^{(6.58)}$ (orange), L300$^{(6.68)}$ (green), D302$^{(7.32)}$ (red), H306$^{(7.36)}$ (blue), F309$^{(7.39)}$ (green), F313$^{(7.43)}$ (green). (Residues from GnRH-R are referred to by their standard one-letter code, their position in the human primary sequence, and their reference position in the GPCR nomenclature of Ballesteros and Weinstein,[89] superscripted (e.g., D302$^{(7.32)}$). Residues in the N-terminal domain are only referred to by their primary sequence position (e.g., M24).) Reproduced with permission from Betz, S. F.; et al. *J. Med. Chem.* **2006**, *49*, 637–647.[84] Copyright 2006 American Chemical Society.

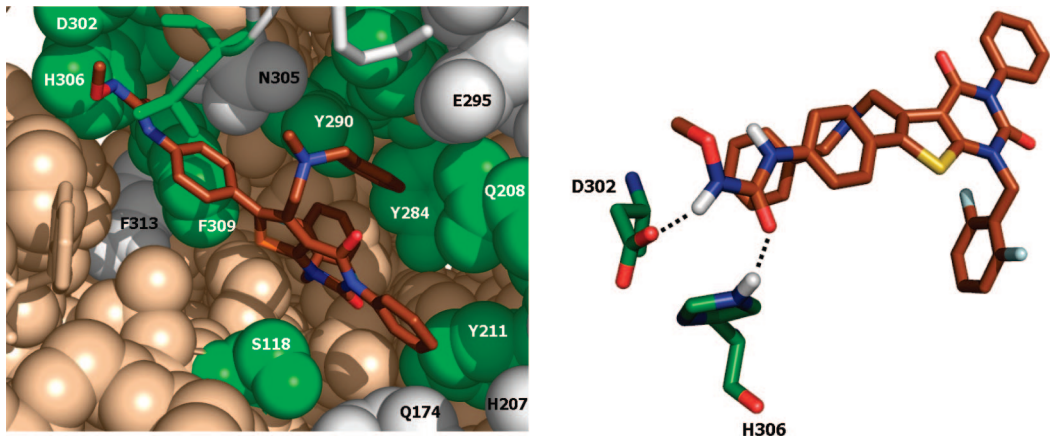

Figure 18. Molecular interaction of **2** with human GnRH-R. Left: Docking model of **2** (shown in brown sticks) bound to a space-filling model of human GnRH-R. Residues found to be important for the binding of **2** to the receptor are shown in green. Residues that were mutated and did not affect the binding of **2** to the receptor are shown in white. Certain residues in ECLs are shown as sticks for clarity. Right: Hypothesized hydrogen-bonding interactions between residues in TM7 and the urea moiety of **2**. Reproduced with permission from Betz, S. F.; et al. *J. Med. Chem.* **2006**, *49*, 6170–6175.[87] Copyright 2006 American Chemical Society.

antagonists, including the clinical compound **13**. This compound has been the basis for understanding the SAR of this series and its interactions with the receptor. Early determinations indicated that **13** (perhaps unsurprisingly given the evolution of its SAR) possessed a set of binding interactions similar to that of **2**,[84] spanning the NTD, TM3, TM6, and ECL3. Substitutions on the difluorobenzyl group were most sensitive to mutations in TM6 deep within the binding pocket, notably Y283$^{(6.51)}$ and Y284$^{(6.52)}$, suggesting aromatic dipolar interactions between these electron rich side chains in the receptor and the electron deficient ring in the ligands.[95] The phenylglycine moiety is in proximity to Y290$^{(6.58)}$ based on SAR of compounds with different substituents at the 3-position of the uracil and mutant receptors at this residue (unpublished observations). With this information, a docked structure of **13** bound to the receptor was generated (Figure 19).[88] In the model, the amine is pointing toward hydrogen bonding residues in TM3 (e.g., S118$^{(3.29)}$), though pseudosymmetric rotation of the phenylglycine by 180° would point the amine toward the extracellular side of the receptor, creating potential interactions with ECL2 or ECL3.

During the analysis of several different classes of GnRH-R antagonists, a comparison of the binding "footprints" of **13** and a series of hydrophobic furan-based antagonists[69,70,79] indicated that the two classes of molecules had nearly mutually exclusive sets of residues that impacted binding affinity.[88] It was determined that the furan antagonist **55** interacted primarily with

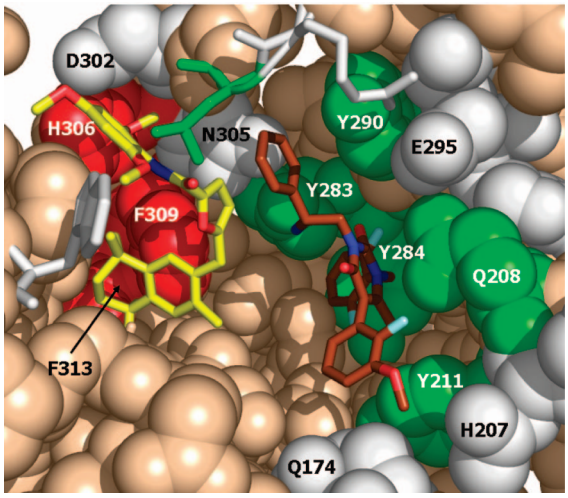

Figure 19. Docking model of **13** and **55** simultaneously bound to human GnRH-R. Residues found to be important for the binding of **13** (shown in brown sticks) to the receptor are shown in green. Residues found to be important for the binding of **55** (shown in yellow sticks) to the receptor are shown in red. Residues that were mutated and did not affect the binding of either compound are shown in white. Residues in ECLs are shown as sticks for clarity. Reproduced with permission from Sullivan, S. K.; et al. *Biochemistry* **2006**, *45*, 15327–15337.[88] Copyright 2006 American Chemical Society.

TM7. Compound **55** was shown to increase the dissociation rate of [^{3}H]-**13**, indicating that the two compounds are allosteric.[91] Furthermore, analysis of the effect of **55** on the dissociation rate of radiolabeled [^{125}I-His^5,D-Tyr^6]GnRH showed that it was also an allosteric antagonist to the peptide as well as to **13**. However, because of the extremely high degree of cooperativity, **55** appears competitive in binding assays against the radiolabeled agonist peptide.[88] A comparison of the binding of **13** and **55** is shown in Figure 19.blockq

Potent non-peptide antagonists with an indole-based core have been described by Merck.[66,81,96] This class of non-peptides (exemplified by the "molecular chaperone" IN3, **57**; see discussion below) has achieved subnanomolar affinity. Initial work on a comparison of different non-peptide classes[84] suggested that a similar indole antagonist bound primarily in the area of the pocket was derived from the interactions of TM6 and TM7. Interestingly, that region is highly conserved among class A GPCRs,[97] which could potentially lead to selectivity issues. For example, in addition to its high affinity at human and rat GnRH-R, **57** has moderate affinity at both the $5HT_{2B}$ and α_1 receptors (165 and 114 nM, respectively; unpublished observations).

Investigation with different molecules in this class showed that the aliphatic amide moiety in the series interacted with F313$^{(7.43)}$L. Further SAR determined that the 4-pyridyl moiety interacted with the aromatic ring of Y290$^{(6.58)}$. Finally, sensitivity of the methyl-substituted aromatic ring to mutations at L300$^{(6.68)}$ provided adequate anchor points for the docking of **57** to the receptor (unpublished observations). The resulting docked model is shown in Figure 20.

A series of quinolone GnRH-R antagonists (e.g., **43**) of similar structure were examined with respect to one mutation (F313$^{(7.43)}$L) in TM7 that was largely responsible for the selectivity differences between the human and dog receptors.[67] Cui et al. model the interaction of the quinolone core near F313$^{(7.43)}$L, but corroborating SAR to support the modeled orientation was not presented. Although the quinolone series has yet to be tested versus a large collection of GnRH-R mutant

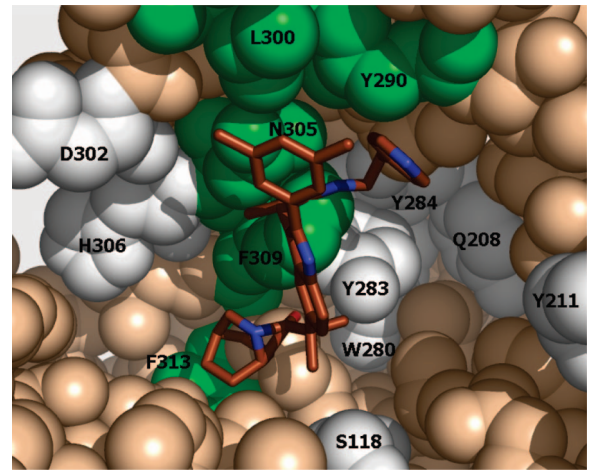

Figure 20. Docked model of **57** bound to space-filling model of human GnRH-R. Residues found to be important for the binding of **57** (shown in brown sticks) to the receptor are shown in green. Residues that were mutated and did not affect the binding of either compound are shown in white.

receptors, it seems likely that they interact with the receptor in a similar manner to the indole antagonists.

An essential conclusion to these reciprocal SAR-mutagenesis experiments is that the human GnRH-R binding pocket is large enough to support several different non-peptide antagonist binding modes, each capable of achieving high affinity. A critical need has been to translate this knowledge toward which features are required for superior functional efficacy and receptor selectivity.

GPCR antagonists can be classified as either surmountable or insurmountable, depending on whether an excess of competing agonist in the presence of the antagonist does (surmountable) or does not (insurmountable) restore the maximum response in a dose–response curve.[98] The basis for insurmountable antagonism can be thought of as removing functional receptors from the assay system. This can occur if the antagonist causes the internalization of the receptor but also can occur if the antagonist dissociates slowly from the receptor. To date, this effect has been described for several receptor systems in varying degrees of detail[99–104] and has been amenable to assay development for high-throughput analysis of non-peptide interactions with GnRH-R.[105]

The analysis of compounds not under equilibrium conditions can confound interpretation of SAR because in competition binding or functional assays, affinities (or potencies) can be underestimated. For example, in a more detailed competition-based receptor kinetics study of uracil analogues, Sullivan et al. showed that the binding affinities of **21** and **22** were 64 and 44 pM, respectively, significantly greater than those determined by standard "equilibrium" competition binding experiments (K_i = 0.2 and 0.5 nM for **21** and **22**, respectively). By use of the kinetics-based method, the K_i for **13** is 90 pM.[106] The scale of these differences underscores the need for careful investigation of the putative potency of high affinity and/or insurmountable GnRH-R antagonists.

Currently, two different classes of non-peptide GnRH-R antagonists that present insurmountable antagonism in functional assays using the human receptor have been examined in detail: uracils and thienopyrimidinediones.[106,107] In each case, clear compound SAR dictates changes in dissociation rate, which in turn gives rise to insurmountable functional antagonism in vitro. For the uracil series, an important component is a replacement

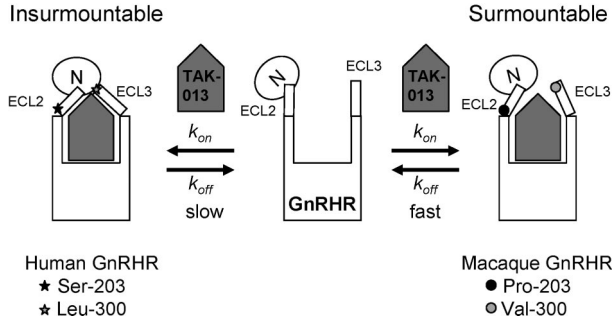

Figure 21. "Trap door" model for insurmountable antagonism of human GnRH-R. Reproduced with permission from Kohout, T. A.; et al. *Mol. Pharmacol*. **2007**, *72*, 238–247.[107] Copyright 2007 American Society for Pharmacology and Experimental Therapeutics.

of one of the fluorines on the difluorobenzyl ring of **13** with a more electron-withdrawing substituent (**21**, **22**).[106] That ring has been predicted to interact with tyrosines in TM6 within the receptor binding pocket.[84,88] For compounds similar to **2**, insurmountability can be achieved with the inclusion of the trans-urea moiety that was identified as having a specific hydrogen-bonding interaction with D302$^{(7.32)}$ and H306$^{(7.36)}$.[87]

The two series do, however, share a number of common features, including sensitivity to mutations on the extracellular side of the receptor, specifically, the NTD (M24), ECL2 (S203$^{(5.30)}$), and ECL3 (L300$^{(6.68)}$). Mutations at these three residues have been shown to be responsible for the majority of human−rat selectivity in the uracil series and similar compounds.[90] Similarly, ECL2/3 changes can recapitulate the selectivity that both series display between the monkey and human receptors.[107]

Extending this observation, Kohout et al. undertook a thorough examination of the impact of changes in these extracellular loop residues on the dissociation kinetics and functional insurmountability of a series of thienopyrimidinediones.[107] In contrast to its human GnRH-R activity, **2** is fully surmountable versus the monkey receptor, but the replacement of the monkey receptor residues with the corresponding human residues at positions 203 and 300 manifested in a "human-receptor-like" slow dissociation rate and functional insurmountability. Kohout et al. hypothesize that species-dependent functional insurmountability is dependent on a "trap door" mechanism in which ECL2, ECL3, and the N-terminal domain (both the human and monkey GnRH-Rs have methionine at position 24) form a closed conformation that slows egress of the compound from the receptor/non-peptide complex (Figure 21). The human GnRH-R/non-peptide complex is slower to dissociate than the analogous complex with the monkey receptor. Accordingly, the corresponding rat receptor complex is predicted to dissociate the most quickly of the three.

What emerges is a unified picture of non-peptide binding to human GnRH-R (Figure 22). The most potent insurmountable compounds seem to possess not only specific "anchoring" interactions within the transmembrane region but also the capacity to stabilize the "closed" version of the ECL−NTD interaction. Other non-peptide classes (e.g., indoles) appear to interact primarily with a subpocket formed by TM6 and TM7 but do not stabilize the ECL−NTD interaction. These compounds are less sensitive to changes in the composition of the ECL−NTD complex and are correspondingly less selective versus other species' GnRH-Rs. In addition, because the region where these compounds have many important interactions is highly conserved among class A GPCRs,[97] they are likely to be less specific in general toward other receptors, which could have important toxicological and developability impacts.

Receptor Regulation by Non-Peptide Antagonists

Down-regulation of the pituitary gonadotroph by chronic agonist treatment is now well established.[15] The effect of antagonist treatment on pituitary regulation is less clear. In part, this has been hindered by lack of high quality antibodies to the naturally expressed receptor and the influence of epitope tags on the underlying regulation being studied.[108] It has been reported that chronic administration of the peptide antagonist cetrorelix to rats results in decreased cell surface pituitary GnRH-R binding and mRNA expression but increased receptor concentration in nuclei.[109] In contrast, non-peptide GnRH-R antagonists have been shown to *increase* expression (in transfected cell lines) of wild-type human GnRH-R as well as hypogonadotropic hypogonadism-causing mutant GnRH-Rs.[110,111] This ability for pharmacologic rescue appears to be a property of several different chemical classes of non-peptide GnRH-R antagonists.[112] The observation in heterologous expression systems that the relatively low expression levels of the human receptor at the plasma membrane can be increased through treatment with a non-peptide (and its subsequent removal) suggests that similar mechanisms may work against non-peptide antagonist efficacy in vivo. However, this is difficult to test experimentally because rat and mouse receptors are more efficiently trafficked to the plasma membrane than their human counterpart and the responsiveness of cells expressing these receptors is not increased by non-peptides.[113]

In Vivo Pharmacology

Qualitatively, non-peptide GnRH-R antagonists behave much as one would expect in in vivo experiments based on extensive experience with peptide antagonists. Oral administration of the orthosteric indole antagonist **57** (rat GnRH-R $IC_{50} = 1.7$ nM, whole cell binding) to a castrate rat can rapidly eliminate LH pulsatility and suppress circulating LH to very low levels.[81] The macrolide **41** also shows very high affinity for the rat receptor ($pK_i = 9.2$) and is a potent inhibitor of LH release from cultured rat pituitary cells ($pA2 = 8.8$).[65] This compound also shows rapid suppression of circulating LH (to ~20% of vehicle-treated baseline) in castrate male rats following oral administration with an approximate minimum effective concentration in vivo of 110 ng/mL. Intravenous administration of this compound resulted in castrate levels of testosterone in intact rats 6−9 h after administration. The allosteric furan antagonist **55** (100 mg/kg) can also completely suppress circulating LH in castrate rats for 8 h.[79] Castrate levels of testosterone could also be achieved in intact rats but required plasma concentrations of the compound greater than 2 μM. The K_i determined by competition with a radiolabeled peptide agonist of this compound is 3.8 ± 0.8 nM[79] but plasma protein binding in the rat was estimated to be >99.9%, providing a potential explanation for the high concentrations required to achieve maximum testosterone suppression in vivo. Thus, both orthosteric and allosteric antagonists can achieve maximum testosterone suppression in the rat given sufficient exposure of the antagonist at the pituitary.

Because of the weak activity of **2** and **13** at rat GnRH-R, cynamolgus macaques were used preclinically to characterize the in vivo activity of these compounds. Compound **2** has a very high binding affinity for the macaque GnRH-R ($IC_{50} =$ 0.6 nM) when expressed in CHO cells and is also potent in functional inhibition of arachadonic acid release ($IC_{50} = 10$ nM)

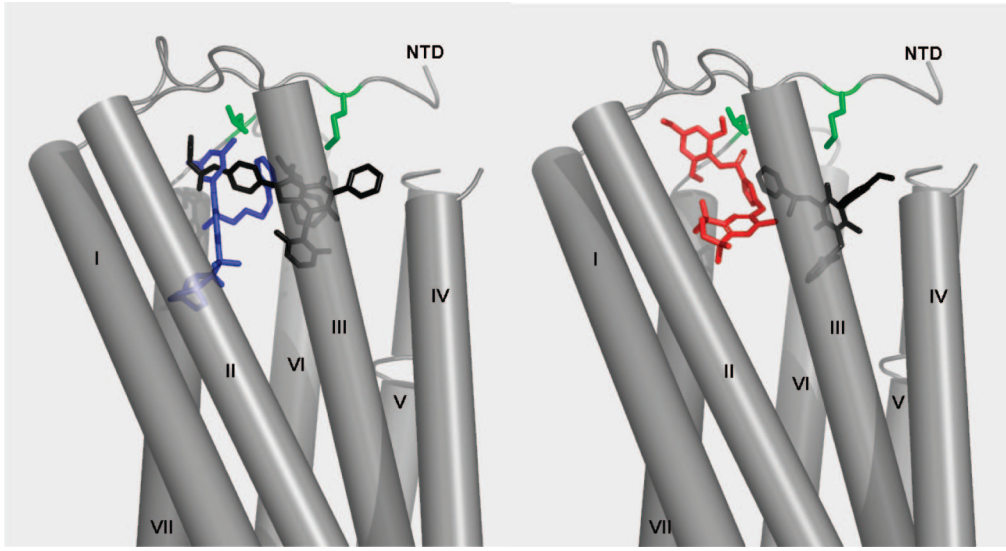

Figure 22. Comparison of different binding motifs to human GnRH-R. Left: **57** binds primarily to TM6 and TM7, an area of low GPCR specificity. Compound **2** possesses interactions with multiple TMs 3, 5, 6, and 7 as well as interactions with the "trap door" residues L300$^{(6.68)}$ and M24 (each shown in green). Right: Similarly, **55** binds primarily to TM6 and TM7, while **13** has interactions with multiple TMs 3, 5, and 6 as well as interactions with the "trap door" residues. M24 position is shown for hypothesis purposes only.

in these cells.[44] Potency for inhibition of LH release from cultured macaque pituitary cells was 36 nM.[114] In castrate male macaques, **2** (10 mg/kg, po) suppresses LH to 18% of predose baseline by 8 h after administration.[44] Higher doses (30 mg/kg) suppresses LH to 11% of baseline versus vehicle. Exposure in cynomolgus macaques following oral administration of 10 mg/kg is high compared to its receptor affinity (C_{max} = 0.21 μM, AUC_{0-6} = 0.85 μM·h),[44] suggesting that the receptor should be fully occupied. However, while chronic treatment of macaques with very high doses of **2** (30 mg/kg three times per day for 80 days) showed strong suppression of circulating LH, it did not suppress FSH.[114] This regimen abolished menstrual cyclicity during treatment, and the monkeys returned to normal cyclicity and steroid profiles after discontinuation. Estrogen levels, however, although reduced, did not remain at castrate levels as has been reported in similar experiments for monkeys treated chronically with peptide agonists.[115] Whether this lack of complete gonadal suppression in the macaque is due to lack of FSH suppression and/or residual levels of LH is unclear.

In comparison, the orthosteric surmountable antagonist **13** suppresses LH in castrate macaques to 32% of pretreatment doses. Antagonist plasma concentrations of 10−50 ng/mL were required to maintain a maximal level of pituitary suppression.

Data for intact male monkeys (rhesus macaque) are only available for the quinolone, **43**.[68] While its binding affinities for human and rhesus GnRH-Rs are similar (0.4 and 0.5 nM, respectively), it is somewhat less potent at the monkey receptor in an inositol phosphate functional assay (IC_{50} = 1.0 and 7.0 nM, respectively). Following iv dosing of this compound, LH pulsatility was prevented and overall LH exposure reduced an average of 79% (based on LH AUC). Testosterone was reduced to near castrate levels, but residual LH and testosterone levels suggest that the HPG axis was not completely suppressed.

Overall, as with peptide antagonists, castrate levels of testosterone can be achieved (at least in the rat) with sufficient exposure. While the data are not complete, the overall picture for three different classes of first generation non-peptide antagonists in monkeys suggests that these compounds may not suppress the HPG axis as completely as has previously been demonstrated for peptide agonists. However, differences in animals, experimental conditions, and hormone assay methods make these types of historical comparisons problematic.

One area where non-peptides may diverge from the peptide antagonists is the lack of injection site reactions and systemic allergic reactions that hindered development of early peptide GnRH antagonists.[116] These side effects are thought to associated with the direct stimulation of histamine release by mast cells and do not appear to be mediated by GnRH-R.[117,118] This was mitigated in third-generation peptide antagonists such as cetrorelix and ganirelix, which have been widely used in patients undergoing IVF treatment. However, abarelix (which was approved for use in prostate cancer but subsequently withdrawn from the market) showed cases of immediate-onset systemic allergic reactions, some resulting in hypotension and syncope. While this may be a direct effect of the peptide, it has also been suggested that carboxymethylcellulose in the depot formulation of abarelix may be the cause of this hypersensitivity.[119] Because of these considerations, direct stimulation of histamine release was thought to be an unlikely action of the non-peptide antagonists, and this was subsequently confirmed for two different chemical classes.[65,120]

Clinical Development

The development of non-peptide GnRH-R antagonists is still in its early stages, and as yet no compound has achieved regulatory approval for clinical use. Several compounds have entered clinical development, but detailed reports of results are sparse. Given the early leadership of the Takeda group, it was not surprising that their compound **2** was the first non-peptide GnRH-R antagonist to be evaluated in humans. Results of several studies have been described at scientific meetings[121–125] and provide the first demonstrations of oral non-peptide GnRH-R antagonist activity in humans. In normal healthy male volunteers,[123] this compound showed suppression of serum testosterone at doses as low as 10 mg, despite relatively low exposure (C_{max} = 15 ± 3 ng/mL; AUC(0,8) = 173 ± 31 (ng·h)/mL; mean ± SD). Doses up to 200 mg (C_{max} = 274 ± 178 ng/mL; AUC(0,8) = 2563 ± 1595 (ng·h)/mL) were well tolerated, and increasing dose showed increasing gonadal suppression. A combined single and multiple dose study in

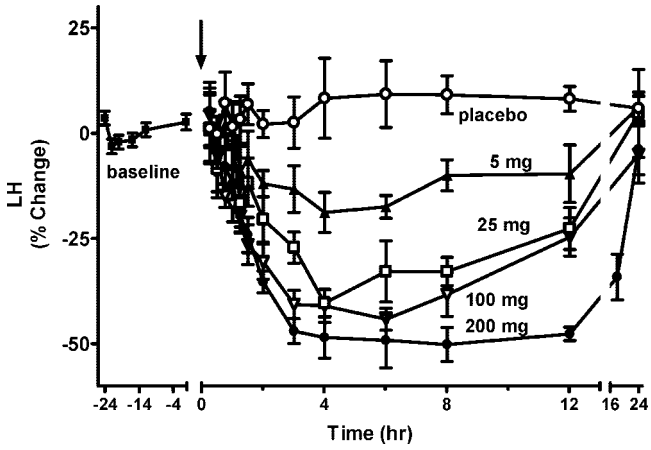

Figure 23. Suppression of serum LH in postmenopausal women following oral administration of **13**. Subjects were administered 5 mg (▲, $N = 6$), 25 mg (□, $N = 6$), 100 mg (▽, $N = 6$), or 200 mg (●, $N = 6$) of **13** or vehicle (○, $N = 8$) at $t = 0$. Values shown are mean (±SEM) percentage changes from the average gonadotropin concentrations for each individual baseline during the 24 h prior to administration of antagonist. A predose mean baseline LH curve for all subjects ($N = 56$) is shown (■). An arrow indicates the time ($t = 0$) at which antagonist was administered. Reproduced with permission from Struthers, R. S.; et al. *J. Clin. Endocrinol. Metab*. **2006**, *91*, 3903–3907.[126] Copyright 2006 The Endocrine Society.

postmenopausal women[121] showed rapid suppression of LH and FSH following **2** administration that persisted for at least 36 h after the last dose. Following administration of 100 mg b.i.d. for 14 days, nadir levels of circulating LH became essentially undetectable (compared to 16 IU/L in placebo treated subjects) and FSH was reduced substantially as well (3 IU/L vs 55 IU/L in placebo). However, this was associated with a significant reduction of exposure and $t_{1/2}$ and a dose-dependent increase in the ratio of urinary 6-hydroxycortisol to cortisol consistent with induction of CYP3A4. In a 14-day study of **2** with 5–100 mg administration to healthy premenopausal women, LH and E_2 were suppressed at all dose levels, though no significant effect on FSH was observed.[122] At the highest dose (100 mg) estradiol was suppressed to very low levels (median E_2 concentration of 6 pmol/L), although low levels, with greater variability, were seen even at the lowest dose (5 mg). Exposure and $t_{1/2}$ were reduced on day 14, and a dose dependent increase in the urinary 6-hydroxycortisol/cortisol ratio was observed, consistent with the observations in postmenopausal women. Although the compound was evaluated in a phase II study in patients with endometriosis, it did not meet criteria for advancement into phase III studies according to information on Takeda's Web site.

Recently, a study of the uracil compound **13** on pituitary suppression in 56 postmenopausal women was published.[126] Doses between 5 and 200 mg were evaluated. The compound was rapidly absorbed following oral administration (t_{max} = 0.4–1.1 h) and a dose dependent suppression of LH was observed (Figure 23). Because of the relatively short pharmacokinetic half-life (2.7 ± 0.3 to 4.8 ± 0.8 h), the duration of LH suppression is dependent upon the dose, but maximum suppression is maintained for 12 h or more with 200 mg. Analysis of the pharmacokinetic and pharmacodynamic data suggested that concentrations above 20 ng/mL are required to maintain maximum LH suppression. Thus, the range of pituitary suppression that can be achieved illustrates a key difference between oral GnRH antagonists and the down-regulating peptide agonists that essentially act as an on/off switch. In addition, compared to various peptide antagonist depots, the degree and duration of LH suppression during the day can be varied. Suppression of FSH was less pronounced, as has been seen in some previous studies with peptide GnRH-R antagonists.[25,127,128] This compound did not advance into phase II studies, but a second compound from Neurocrine, NBI-56418 (also known as elagolix), was advanced to clinical development[129] and has recently completed two 3-month phase IIa studies in patients with endometriosis.[130] This compound is currently being evaluated in additional phase IIb trials.

One of the most difficult problems in drug discovery is the prediction of clinical pharmacokinetics and pharmacodynamics from preclinical information. As discussed above, species-dependent differences in receptor activity of many non-peptide GnRH-R antagonists make this challenge even more difficult. With the data available on **2** and **13** some interesting observations can be made. For example, **2** suppresses LH and estrogen, but not FSH, in female cynomolgus macaques and premenopausal women. Although menstrual cyclicity in macaques is abolished, estradiol levels are not maximally suppressed in contrast to premenopausal women, who achieve profoundly suppressed levels. Although the compound has ~6- to 170-fold lower affinity for the monkey receptor than the human receptor,[44] depending on the assay, high exposure in the macaques due to three times a day dosing should have resulted in nearly complete receptor occupancy. However, we have also recently shown that in addition to differences in affinity of **2** for human and macaque GnRH-Rs, the resulting functional pharmacology is also different. At the human receptor, **2** is an insurmountable antagonist as measured by inositol phosphate production, while under similar conditions it is a fully surmountable antagonist at the macaque receptor.[107] Thus, in the physiologic context of pulsatile GnRH secretion, the insurmountable antagonism at the human receptor may result in more complete prevention of receptor signaling than the surmountable antagonism at the monkey pituitary.

On the other hand, **13** is a surmountable antagonist of both human and macaque receptors.[120] Yet despite similar receptor pharmacology between the species, relative potency and efficacy remain difficult to predict a priori. In castrate macaques, concentrations of the antagonist of ~10−50 ng/mL (20−100 nM) were required to maintain maximum pituitary suppression. Surprisingly, this is comparable to the plasma concentrations required to suppress LH in postmenopausal women[126] even though its potency as assessed by in vitro assays is reduced 10- to 17-fold between the two species. Thus, comparison of the preclinical and clinical results for **2** and **13** illustrates the inherent difficulties in quantitatively predicting pharmacologic effects in humans from in vitro and nonhuman in vivo data.

Conclusions and Future Directions

The diverse and critical roles of biologically active peptides acting at GPCRs together with the example of the archetypical non-peptide opiates have inspired efforts by many groups toward the discovery of non-peptide drugs. Initial peptidomimetics emerged from the field of peptide chemistry and were based on defining the bioactive peptide conformation, with attempts to replicate that pharmacophore on a non-peptide scaffold.[131–133] While often elegant, successes were relatively scarce. Subsequently, improvements in screening technologies were employed to identify lead structures that were then optimized using medicinal chemistry. Non-peptide ligands for many different peptide receptors have now been identified using this approach,[134,135] and approved drugs against several of these have emerged.

These include angiotensin antagonists (losartan, candesartan, valsartan, irbesartan, olmesartan, telmisartan, eprosartan), CCR5 antagonists (maraviroc), neurokinin antagonists (aprepitant), endothelin antagonists (bosentan), and vasopressin antagonists (conivaptan).

What is remarkable about the field of non-peptide GnRH-R antagonists is that although the native peptide ligand is relatively small, a wide variety of chemical classes can be recognized by the receptor binding pocket with high affinity. Although some residues in the receptor interact with both peptide and non-peptide ligands, there is no clear correspondence between functional groups to indicate a peptidomimetic relationship. Moreover, different classes of non-peptide ligands bind to different subregions of the receptor active site. In some cases these are partially overlapping and in other cases these are nonoverlapping, resulting in non-peptide pairs that bind with allosteric pharmacology. Despite the broad chemical diversity available for high affinity ligands to human GnRH-R, very few have led to drug candidates thus far. In part, we speculate that this may be due to the inherent "drugability" of the specific subsite of the receptor recognized by each chemical class. For example, ligands that bind deeply in the transmembrane region of the receptor utilize interactions with residues that are broadly conserved across class A GPCRs, and thus lead optimization efforts are continuously forced to swim upstream against selectivity issues while trying to solve all the other typical pharmaceutical optimization challenges. In contrast, successful subsites, such as that recognized by uracils and thienopyrimidinediones, utilize nonconserved regions in the extracellular domains and can result in very high affinity binding, which may not be possible at other subsites. Thus, studies with GnRH-R begin to provide a structural explanation for what has long been recognized by medicinal chemists working on GPCRs: some chemical series are simply intractable dead ends, while others lead to rich veins of good drug candidates.

Orally available, non-peptide GnRH-R antagonists may offer more than simply a more convenient and acceptable route of administration compared to GnRH peptide drugs. The ability to easily modify dosage to vary the degree and duration of pituitary suppression is a fundamental change in paradigm from the peptide depots. How this will be utilized in the human population remains to be determined through clinical studies, some of which are already underway. In the area of women's health, this may enable suppression of the menstrual cycle and maintence of low, but not menopausal, estrogen levels in order to treat benign gynecological conditions such as endometriosis or uterine fibroids, without incurring hypoestrogenic side effects such as hot flashes and bone loss. Analogous approaches may be suitable for benign prostate hyperplasia. With the wide range of indications that can be treated by reduction in gonadal steroids, many potential opportunities exist for non-peptide GnRH-R antagonists to make a significant impact on the practice of medicine. Results from clinical trials in the coming years will tell us how well various individual compounds live up to this potential.

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Biographies

Stephen F. Betz earned his Ph.D. in Chemistry from the University of North Carolina at Chapel Hill where he studied protein structure and stability. He pursued postdoctoral studies in the laboratory of William DeGrado at the DuPont Merck Pharmaceutical Company where he focused on protein engineering and de novo design. He moved to pharmaceutical discovery in the Research NMR Group at Abbott Laboratories, working on protein structure, structure-guided drug design, assay development, and compound screening in several different therapeutic areas. Subsequently, he led laboratory efforts at GeneFormatics, a biotechnology company founded on protein function annotation and characterization. Currently, he is Director of Endocrinology and Metabolism at Neurocrine Biosciences, and works on the discovery and development of GnRH-R antagonists and non-peptide modulators of other targets.

Yun-Fei Zhu received his Ph.D. in Organic Chemistry from Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences in China, and pursued his postdoctoral studies in the laboratory of Professor Murray Goodman in Department of Chemistry, University of California, San Diego. He joined Neurocrine Biosciences in 1997 and is currently Director of Chemistry in the Endocrinology and Metabolism group. His research has involved discovery of small molecule IGF-BP inhibitors and CRF-R2 and MCH receptor antagonists, and he is currently focused on the discovery and development of novel non-peptide GnRH-R antagonists for treatment of hormone-dependent diseases.

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R. Scott Struthers received his Ph.D. in Physiology and Pharmacology from the University of California, San Diego, under the supervision of Professor Wylie Vale at the Salk Institute for Biological Studies. Following his degree, Dr. Struthers joined Biosym Technologies where he led their contract research efforts developing and applying computational tools for drug discovery. He subsequently cofounded ScienceMedia Inc. to develop science education software. In 1998, he joined Neurocrine Biosciences and initiated the company's efforts to discover non-peptide GnRH-R antagonists. His research interests include reproductive and metabolic endocrinology, GPCR ligand recognition and signaling, and drug discovery. Dr. Struthers is currently Senior Director and Head, Endocrinology and Metabolism at Neurocrine Biosciences.

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