Allosteric Modulation of 'Reproductive' GPCRs : a case for the GnRH and LH receptors

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CHAPTER 2

G PROTEIN-COUPLED RECEPTORS OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS; A CASE FOR GNRH, LH, FSH AND GPR54 RECEPTOR LIGANDS

The hypothalamic-pituitary-gonadal (HPG) axis, important in reproduction and sex hormone-dependent diseases, is regulated by a number of G protein-coupled receptors. The recently 'deorphanized' GPR54 receptor activated by the peptide metastin is thought to be the key regulator of the axis, mainly by releasing gonadotropin-releasing hormone (GnRH) from the hypothalamus. The latter decapeptide, through the activation of the GnRH receptor in the anterior pituitary, causes the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which subsequently activate their respective receptors on the gonadotrope cells. In this review we will discuss the small molecule agonists and antagonists that are currently being developed to intervene with the action of these four receptors. For GnRH receptors, fourteen different chemical classes of non-peptidic antagonists have been reported, while for the LH receptor three classes of agonists have been described. Both agonists and antagonists have been introduced for the FSH receptor. Recently, the first non-peptidic agonist for GPR54 was reported.

This chapter is an update of a recent publication:

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2.1 INTRODUCTION

The receptors of the hypothalamic-pituitary-gonadal-axis (HPG-axis) that will be discussed in this review all belong to the rhodopsin-like subfamily of G protein-coupled receptors (GPCRs). The human genes of the gonadotropin-releasing hormone (GnRH) [83,84] luteinizing hormone (LH)[85] and follicle-stimulating hormone receptors (FSH)[86] were cloned in the early nineties, whereas human GPR54 cDNA was isolated in 1999.[87] The GnRH receptor is predominantly coupled to the G_q-protein, through which it regulates the biosynthesis and secretion of the gonadotropins, FSH and LH.[10] The FSH and LH receptor belong to the glycoprotein-hormone receptor family together with the thyroid-stimulating hormone (TSH) receptor.[88] These receptors contain a large N-terminus to which the endogenous hormone binds. Activation of the LH and FSH receptor mainly results in the production of intracellular cAMP *via* G_s proteins. These hormones stimulate germ cell development and hormone (estrogen and progesterone) secretion in the ovaries.[89] In addition, LH and FSH to some extent, stimulate the testis to produce testosterone. GnRH secretion in turn is inhibited by estrogen and progesterone, allowing a negative feedback loop in the HPG-axis. Recently, it was shown that a placental peptide, kisspeptin-54 (metastin), activates GPR54, which results in the activation of phospholipase C via G_q.[90] GPR54 has been shown to stimulate the hypothalamic secretion of GnRH.[91]

The endogenous ligands for the GnRH, LH, FSH and GPR54 receptor are either peptide or protein hormones, and can be administered parenterally, also in their recombinant form, if available. However, it would be very desirable to have orally available, non-peptidic, chemical entities as well, which is the focus of intensive research efforts especially in industry. As ligands for the receptors of the HPG-axis have similar clinical applications, this review gives a detailed overview of the search for non-peptidic ligands that have been identified for these receptors. The identification of selective and high affinity ligands for these receptors could be beneficial in the treatment of several sex-hormone dependent diseases, ovarian, prostate, or breast cancer, infertility or as non-steroidal contraceptives.[71,72,92,93] In this review we will first address non-peptidic antagonists for the GnRH receptor, followed by non-peptidic agonists for the LH receptor. Then non-peptidic agonists and antagonists for the FSH receptor will be reviewed, and we conclude by discussing the first non-peptidic agonist for GPR54.

2.2 GNRH RECEPTOR ANTAGONISTS

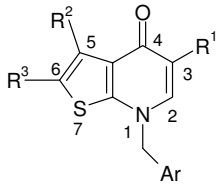
GnRH or its agonist analogs need to be administered in a pulsatile fashion to result in physiologic gonadotropin secretion.[94] A continuous administration of GnRH (agonists) will initially lead to gonadotropin release followed however by antagonism of the HPG axis by subsequent desensitization of GnRH receptors. Initially, analogues of the endogenous ligand GnRH were prepared as agonists and antagonists for this receptor.[95-97] However, peptidic ligands are not preferred as drugs in chronic treatments as they have to be administered by injection due to their susceptibility for biological degradation. Therefore, intensive efforts were undertaken to develop non-peptidic GnRH receptor ligands, which have the potential to be orally bioavailable. To date only non-peptidic antagonists have been identified, which can be classified into fourteen chemical classes. Each of them will be discussed separately, where only the most potent compounds of each class are highlighted. Furthermore, this paragraph includes additional patented compound classes that have not been published (yet). These compounds are classified based on the presence of a mono-, bi- or tricyclic scaffold.

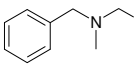
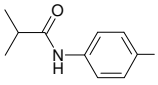
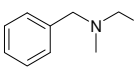
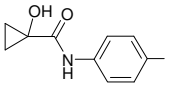
2.2.1 Thieno[2,3-d]pyridin-4-one Derivatives

The first class of non-peptidic antagonists for the human GnRH receptor was described by a research team at Takeda in 1998.[98] Structure-activity relationships (SARs) of peptide agonists and antagonists showed that the type II β-turn involving residues 5-8 (Tyr-Gly-Leu-Arg) of GnRH is important for binding affinity.[99] A compound library was selected that consisted of general GPCR antagonistic structures and screening resulted in a thieno[2,3-d]pyridin-4-one derivative as a lead. Structural similarity was found with the β-turn moiety of GnRH, where the Tyr-, Gly- and Leu-residues were mimicked by the substituents at positions 6, 1 and 3, respectively. Introduction of a basic amino moiety at the 5-position added similarity to the Arg-residue and further optimization resulted in compound **1** (T-98475) (Table 2.1). Compound **1** had a high affinity for the human GnRH receptor (IC_{50} = 0.2 nM) and showed selectivity over other GPCRs interacting with peptide ligands.[98] Although **1** was 20-fold less potent on the monkey GnRH receptor, oral administration in monkeys showed over 70% inhibition of plasma LH-levels *in vivo*. In an extension of this study, Imada and coworkers aimed to further optimize each substituent to improve *in vivo* antagonism.[100] It appeared that all substituents, except on the 6-position, were already optimal. Introduction of the 1-hydroxycyclopropanecarboxamide group yielded compound **2**, which increased the

potency on human receptors by 2-fold, while displaying a 9-fold lower potency on monkey receptors. Oral administration of 60 mg/kg of compound **1** to monkeys resulted in a duration of action of 8 h,[98] whereas 10 mg/kg of **2** suppressed LH-levels for 24 h.[100]

Table 2.1 Binding affinities of thieno[2,3-*b*]pyridin-4-one derivatives (**1**-**2**) at the human GnRH receptor.



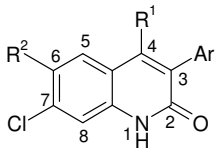
Compound	R^1	R^2	R^3	Ar	IC_{50} (nM)[a]	Ref
1					0.2	98
2					0.1	100

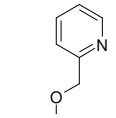
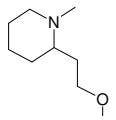
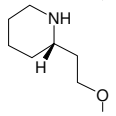
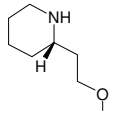
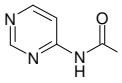
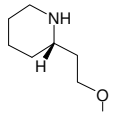
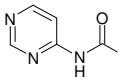
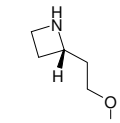
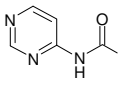
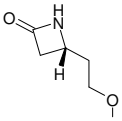
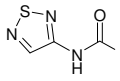
[a] The ability to inhibit binding of ^{125}I-leuprorelin to the cloned human GnRH receptor stably expressed in CHO cells.[98]

2.2.2 Quinolin-2-one Derivatives

In 1999 researchers at Merck introduced quinolin-2-one derivatives as a novel class of non-peptidic GnRH receptor antagonists (Table 2.2).[101] The lead compound (**3**), which had micromolar affinity for the rat GnRH receptor (IC_{50} = 10 µM), was identified by screening an in-house compound library. At first the 2-pyridyl substituent at position 4 was replaced by other (nitrogen-containing) ring systems. An alkyl cyclic amine with a 3-carbon spacer between the basic amine and the 4-quinolone oxygen provided the highest binding affinity. The SAR of the 3-aryl group was also described by the same group.[102] As a consequence a 3,5-dimethylgroup was incorporated. Subsequent optimization of the quinolone ring substituents showed that a chlorine atom at the 7-position was important for high affinity. A 10-fold increase in potency was obtained when a 6-nitro group was incorporated resulting in the first nanomolar-affinity compound of this class (**4**; IC_{50} = 32 nM).[101] The chirality and ring size of the alkyl cyclic amine substituent at position 4 was further investigated.[103] It was determinant in binding affinity. Together with the removal of the N-methyl group of this

Table 2.2 Binding affinities of quinolin-2-one derivatives (**3-10**) at the rat or human GnRH receptor.



Compound	R^1	R^2	Ar	IC_{50} (nM)[a]	Ref
3		H		10,000[b]	101
4		NO_2		32[b]	101
5		NO_2		10[b]	103
6				0.9	104
7				0.3	102
8				0.44	105
9				0.1	106

[a] The ability to inhibit the binding of ^{125}I-buserelin to the cloned human GnRH receptor stably expressed in CHO cells.[105]

[b] The ability to inhibit the binding of ^{125}I-buserelin to the rat pituitary GnRH receptor.[101]

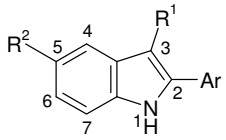
substituent, compound **5** was obtained with an IC_{50}-value of 10 nM at the rat GnRH receptor.[103] At Merck parallel efforts were undertaken to replace the 6-nitro group by different substituted amide groups.[102,104] In both papers, the pyrimidine-carboxamide was the superior substituent (**6**). In addition, a 3,4,5-trimethylphenyl substituent at position 3 further increased the potency by 3-fold (**7**).[102] Due to the availability of a binding assay for the

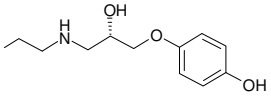
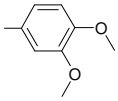
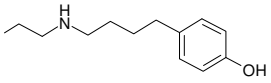
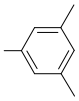
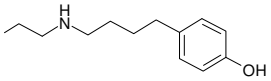
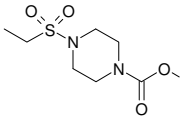
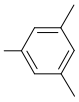
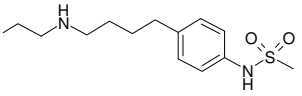
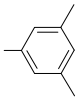
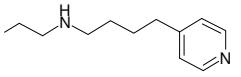
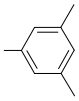
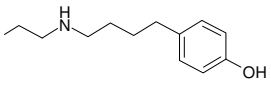
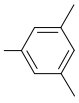
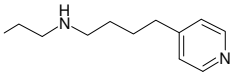
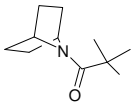
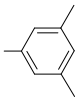
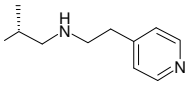
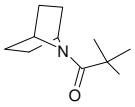
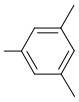
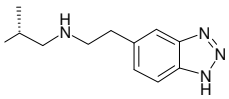
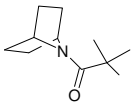
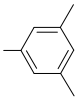
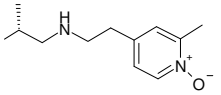
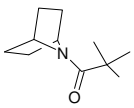
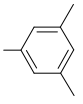
human GnRH receptor, it appeared that these quinolone analogs had a somewhat higher affinity than at the rat GnRH receptor. In addition, for compound **8** it was shown that its affinity at monkey GnRH receptors was equal to the human receptor (IC_{50} = 0.44 nM). However, at the rat GnRH receptor a 10-fold lower affinity was found.[105] Compound **8** was also characterized in *in vivo* studies. Intravenous administration of 3 mg/kg of **8** resulted in 79% suppression of LH and 92% suppression of testosterone blood levels in primates. In 2004, an improved synthetic route was published.[106] In this study, it was shown that also other heterocyclic rings at position 4 yielded a high potency. Replacement of the pyrimidine with a thiadiazole ring only slightly improved the affinity, while changing the cyclic amine for a cyclic amide, such as a γ-lactam moiety (**9**), improved the affinity 4-fold compared to **8**.

2.2.3 Indole Derivatives

Another class of non-peptidic GnRH antagonists was described by Chu and coworkers (Table 2.3).[107] An indole-based lead (**10**) was identified after in-house screening, having micromolar binding affinity at the rat GnRH receptor (IC_{50} = 3 μM). In a first attempt to increase the affinity, the substituents at positions 2 and 3 were optimized. It appeared that neither the stereochemistry nor the ether linkage in lead compound **10** were important for GnRH receptor affinity. In addition, replacement of the aryl group at position 2 for a 3,5-dimethylphenyl resulted in a 60-fold increase in receptor affinity (**11**; IC_{50} = 50 nM).[107] In an extension of this study, the effect of substituents at position 5 on receptor affinity was studied.[108] It was shown that a functionalized piperazinyl group, especially when it was sulfonylated, increased the binding affinity over 10-fold (**12**; IC_{50} = 4 nM). Since compounds **10**-**12** were phenol derivatives, and therefore metabolically unstable, the Merck group continued to study phenol ring surrogates.[109,110] It appeared that a hydrogen bond donating group in combination with a four-carbon spacer resulted in the most active compounds. The methanesulfonamide group in compound **13** resulted in a ligand with a similar affinity.[109] Notably, the affinity of compound **13** was almost 25-fold lower on the human GnRH receptor (IC_{50} = 170 nM). It was shown that the introduction of a heterocyclic 4-pyridyl substituent also resulted in a potent compound (**14**) with an affinity between that of **11** and **13**.[110] At the same time, the substituents at the 5-position of the indole were further explored.[111] Carboxamide groups, particularly those derived from secondary amines, increased receptor affinity. Interestingly, the affinity of these compounds for the human receptor increased (**15**;

Table 2.3 Binding affinities of indole derivatives (**10-19**) at the rat or human GnRH receptor.



Compound	R^1	R^2	Ar	IC_{50} (nM)[a]	Ref
10		H		3,000[b]	107
11		H		50[b]	107
12				4[b]	108
13		H		7[b]	109
14		H		16[b]	110
15				5.7	111
16				1.4	112
17				0.6	113
18				0.6	114
19				0.3	115

[a] The ability to inhibit the binding of ^{125}I-buserelin to the cloned human GnRH receptor stably expressed in CHO cells.[105]

[b] The ability to inhibit the binding of ^{125}I-buserelin to the rat pituitary GnRH receptor.[98]

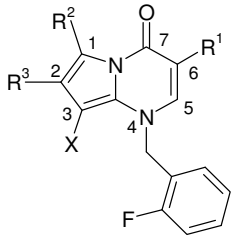
IC_{50} = 5.7 nM). Furthermore, these compounds were also most effective in antagonizing LH release from pituitary cells. Combining the optimal substituents of compounds **14** and **15** resulted in compound **16**, which had the highest affinity for the human GnRH receptor so far (IC_{50} = 1.4 nM).[112] In addition, the 5-substituent was *gem*-dimethylated, which was favorable to reduce metabolic cleavage. Several attempts were made to improve the pharmacodynamic and pharmacokinetic properties of this class of compounds. Introduction of a chiral β-methyl group at the 3-substituent and reducing the four-carbon to a two-carbon spacer, resulted in higher potency and oral bioavailability (**17**).[113] Oral administration of 10 mg/kg of compound **17** in castrated male rats completely suppressed plasma LH levels for 13 h. Notably, the affinity of **17** was almost 3-fold lower for the rat GnRH receptor than the human receptor. The pyridine portion of **17** was modified in two separate studies.[114,115] Firstly, the introduction of a benzotriazole group (**18**) resulted in a two-fold increase in the potency (IC_{50} = 0.6 nM), and maintained oral bioavailability and low plasma clearance.[114] In addition, the cytochrome P450 3A4 inhibition that was found for some analogues of **18** was substantially decreased. Secondly, the introduction of an *ortho* methyl to the pyridine portion of **17** and oxidation of the pyridine nitrogen resulted in compound **19**.[115] Compound **19** had a lower oral bioavailability in dogs (25% compared to 37%), but a longer terminal half-life (5 h compared to 2.7 h) and a 2-fold higher affinity than compound **18**.

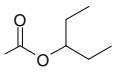
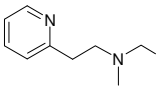
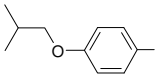
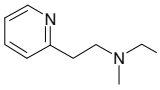
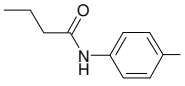
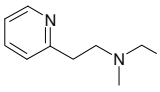
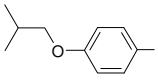
2.2.4 Pyrrolo[1,2-a]pyrimidin-7-one Derivatives

Pyrrolo[1,2-*a*]pyrimidones, as a novel class of heterocyclic non-peptidic antagonists for the human GnRH receptor were introduced by Neurocrine Biosciences (Table 2.4).[116] All non-basic compounds were inactive. At position 2 a hydrophobic aromatic ring with an extra hydrogen bond acceptor was preferred and at position 4 a 2-fluorobenzyl group was most potent. The potency was increased when the *para*-substituent at the 2-aromatic ring was replaced with the more lipophilic isobutoxy group, yielding nanomolar affinity. Introduction of a medium-sized lipophilic ester group at position 6 resulted in high binding affinity at the human GnRH receptor (**20**; K_i = 25 nM).[116] Compound **20** was highly selective for the human receptor, as the affinity at the rat GnRH receptor was almost 300-fold lower. Further optimization by Zhu *et al.* proved that removal of the cyano-group at the 3-position resulted in more potent compounds.[117] At position 2, a hydrogen bond acceptor together with a lipophilic group and a linear, rather than branched, alkyl group provided a drastically

increased affinity (**21**; K_i = 1.2 nM). For other compounds in this class functional antagonism was shown by their inhibition of GnRH-stimulated calcium flux. The removal of the cyano group in compound **20** resulted in a compound that was relatively unstable under acidic conditions, but more potent. Therefore, Tucci *et al.* introduced a fluoro substituent as a smaller electron-withdrawing group at position 3.[118] As a result the core was stabilized, while maintaining a high affinity for the human GnRH receptor (**22**; K_i = 9 nM). It appeared that introduction of the 3-fluoro group in compound **22** resulted in an electron-poor density of the 4-(2-fluorobenzyl) moiety, while the cyano group of **20** yielded an electron-rich moiety. In this study, compound **22** was docked into a 3D-model of the human GnRH receptor. It was believed that the 2-fluorobenzyl group interacts with one of two tyrosine residues in transmembrane domain (TM) VI (Y283 and Y284) of the receptor. A ligand with an electron-poor aromatic ring, like **22**, could therefore interact with the electron-rich aromatic ring of the tyrosine residues by π stacking, resulting in a higher binding affinity.

Table 2.4 Binding affinities of pyrrolo[1,2-a]pyrimidin-7-one derivatives (**20-22**) at the human GnRH receptor.



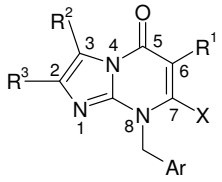
Compound	R^1	R^2	R^3	X	K_i (nM)[a]	Ref
20				CN	25	[116]
21				H	1.2	[117]
22				F	9	[118]

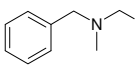
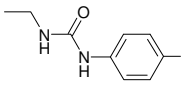
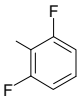
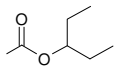
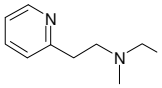
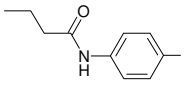
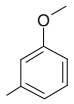
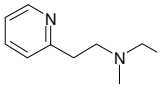
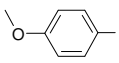
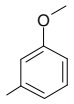
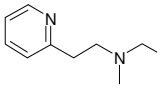
[a] The ability to inhibit the binding of des-Gly^{10}[^{125}I-Tyr^5,DLeu^6,NMeLeu^7,Pro^9-NEt]-GnRH to the cloned human GnRH receptor stably expressed in HEK293 cells.[119]

2.2.5 Imidazolo[1,2-a]pyrimidin-5-one Derivatives

In 2002 Takeda[120] and Neurocrine Biosciences[121] both introduced imidazolo[1,2-a]pyrimidin-5-one derivatives. The thiophene ring of the thieno[2,3-b]pyridin-4-one derivatives (**1**-**2**) and the pyrrole ring of the pyrrolo[1,2-a]pyrimidin-7-one derivatives (**20**-**22**) were replaced by an imidazole ring, respectively (Table 2.5). These replacements resulted in potent GnRH receptor antagonists with improved pharmacokinetic profiles and increased stability under acidic conditions. Sasaki *et al.* showed that similar substituents as at compound **1** resulted in a functional antagonist at the human GnRH receptor with comparable binding affinities (**23**; IC_{50} = 0.3 nM).[120] Wilcoxen *et al.* confirmed the importance of the basic nitrogen and the attached benzyl group at position 3.[121] The substitution pattern of their most potent compound (**24**) was similar as **23** with a K_i value of 7.5 nM. Modeling of this compound showed that the basic tertiary amine possibly interacts with an aspartic acid in TM

Table 2.5 Binding affinities of imidazolo[1,2-a]pyrimidin-5-one derivatives (**23**-**26**) at the human GnRH receptor.



Compound	R^1	R^2	R^3	Ar	X	K_i (nM)[a]	Ref
23					H	0.3[b]	[120]
24					H	7.5	[121]
25					CH_3	4.6	[122]
26					CH_3	1.2	[123]

[a] The ability to inhibit the binding of des-Gly[10][^{125}I-Tyr[5],DLeu[6],NMeLeu[7],Pro[9]-NEt]-GnRH to the cloned human GnRH receptor stably expressed in HEK293 cells.[119]

[b] IC_{50} value, determined by the ability to inhibit binding of ^{125}I-leuprorelin to the cloned human GnRH receptor stably expressed in CHO cells.[98]

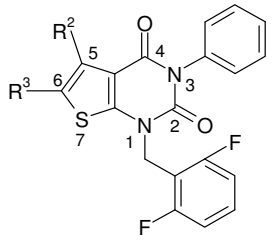
VII (Asp302) and the pyridine ring could provide π-π interaction with an aromatic residue in the receptor.[123] Although this class of compounds was more stable, Neurocrine Biosciences focused on replacing the ester group at position 6, as it is hydrolyzed *in vivo*.[122] This group may function as both a lipophilic group and a hydrogen bond acceptor, and was therefore replaced by a phenyl group bearing a hydrogen-bond accepting group. Replacement of the *n*-butyroylamidophenyl (**24**) by a 4-methoxyphenyl group at position 2 resulted in a 30-fold decreased binding affinity (data not shown). However, in combination with the 3-methoxyphenyl and a methyl group at position 6 and 7, respectively, compound **25** emerged with a K_i-value of 4.6 nM.[122] The 7-methyl was speculated to force the 5-phenyl ring into a perpendicular conformation, which may be preferred for π-π interaction with an aromatic residue in the GnRH receptor. Furthermore, it was postulated that the *para*-substituted phenyl group at position 2 might play a less important role in binding. Introduction of a *tert*-butyl moiety indeed resulted in similar binding affinity as compound **25**. Some potency was gained upon the introduction of a 1-methyl-1-methoxycarbonylethyl group at position 2 (**26**; K_i = 1.2 nM). This modification led to high-affinity functional GnRH receptor antagonists with a reduced molecular weight.[123] However, the affinity of this novel class of antagonists for the rat GnRH receptor was almost 100-fold lower,[121,122] in agreement with earlier reports by Takeda and Merck on their low molecular weight antagonists.[113,116]

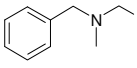
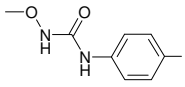
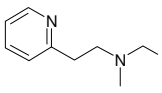
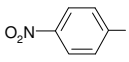
2.2.6 Thieno[2,3-d]pyrimidin-2,4-dione Derivatives

The thieno[2,3-d]pyridin-4-one derivative **1** showed high binding affinity *in vitro*. However, *in vivo* antagonism was not as effective, due to its low oral bioavailability. Therefore, another research program to identify non-peptidic GnRH receptor antagonists was conducted.[124] A novel class of ligands was introduced with a thieno[2,3-d]pyrimidin-2,4-dione core (Table 2.6). Previous studies showed that the *N*-benzyl-*N*-methylaminomethyl and 2,6-difluorobenzyl substituents were important for receptor binding. Only the 3- and 6-substituents were therefore investigated. A phenyl group at position 3 resulted in the highest affinity when a 4-methoxyphenyl group was present at position 6 (data not shown). Since the methoxyphenyl group is a metabolic target, different *para*-acylaminophenyl substituents were investigated. These compounds showed high receptor affinity and it was therefore hypothesized that the 6-substituent should contain a hydrogen-bond donor and a small alkyl group. Compound **27** (TAK-013), with a 6-(4-methoxyurea-phenyl group had an IC_{50}-value

of 0.1 nM and similar binding affinity at the monkey GnRH receptor (IC_{50} = 0.6 nM). Compound **27** showed effective *in vitro* functional antagonism and *in vivo* efficacy after oral administration. Oral administration of 10 mg/kg of compound **27** completely suppressed plasma LH levels for more than 24 h in monkeys.[124] An urea moiety is widely acknowledged to cause low oral adsorption due to its strong hydrogen bonding. However, molecular modeling studies suggested that through the introduction of the methoxy oxygen, an intramolecular hydrogen bond is formed. This would result in an increased apparent lipophilicity and therefore a higher oral bioavailability. Further effort was put into making more water-soluble analogues of compound **27**. As was already shown by Wilcoxen *et al.*, replacement of the benzyl by a pyridine ring at position 5 did not affect the binding affinity.[121] The advantage of incorporation of a pyridine ring is that it is slightly basic, which reduces the lipophilicity and increases the water solubility. Although introduction of a nitro group at position 6 resulted in a lower affinity for the *N*-benzyl analogue, in combination with a 2-pyridylethyl group an affinity comparable to **27** was obtained (**28**; K_i = 0.6 nM).[125] This

Table 2.6 Binding affinities of thieno[2,3-b]pyrimidin-2,4-dione derivatives (**27-28**) at the human GnRH receptor.



Compound	R^2	R^3	K_i (nM)[a]	Ref
27			0.1[b]	124
28			0.6	125

[a] The ability to inhibit binding of des-Gly^{10}[^{125}I-Tyr^5,DLeu^6,NMeLeu^7,Pro^9-NEt]-GnRH to the cloned human GnRH receptor expressed in HEK293 cells.[119]

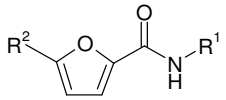
[b] IC_{50} value, determined by the ability to inhibit binding of ^{125}I-leuprorelin to the cloned human GnRH receptor stably expressed in CHO cells.[98]

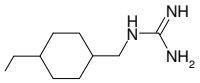
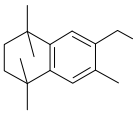
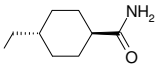
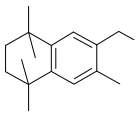
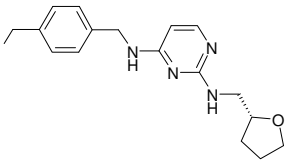
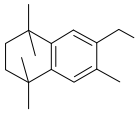
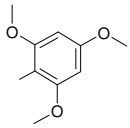
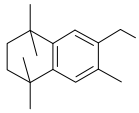
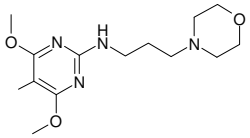
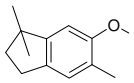
finding is in line with the previously mentioned receptor model suggested by Takeda (section 2.2.5), where the pyridyl side chain is in close proximity to Asp302 in TM VII.[123]

2.2.7 Furamide Derivatives

In 2002 Pfizer identified the first class of non-peptidic GnRH receptor antagonists without a 5-6 membered heterocyclic core (Table 2.7).[126] Compound **29** was identified through screening of the in-house libraries. Although **29** was a potent and functional GnRH receptor antagonist (K_i = 40 nM), the guanidine moiety was suspected to cause potential absorption problems.

Table 2.7 Binding affinities of furamide derivatives (**29**-**33**) at the human GnRH receptor.



Compound	R^1	R^2	K_i (nM)[a]	Ref
29			40 ± 5	126
30			13 ± 4	126
31			9.3 ± 0.9	127
32			6.0 ± 0.8	128
33			0.4 ± 0.1	129

[a] The ability to inhibit [^{125}I]GnRH-A to the cloned human GnRH receptor stably expressed in HEK293 cells.[128]

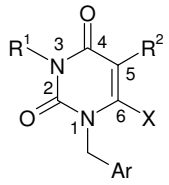
Therefore, a variety of other functional groups were examined. The SAR study revealed that the guanidine moiety on the cyclohexyl ring could be replaced by different substituents, resulting in species as well as potency differences. Introduction of a carboxylic acid amide resulted in the most potent compound (**30**) with a 3-fold increase in receptor affinity.[126] Both compound **29** and **30** preferred the human over the rat GnRH receptor by approximately 20-fold. The guanidine moiety was also modified to a 'caged' form with mono- or diaminopyrimidine substituents, and the cyclohexyl ring was replaced for a benzyl ring (e.g. **31**).[127] Compound **31**, a diaminopyrimidine derivative, was a potent and functionally active antagonist *in vitro* and *in vivo*. Intravenous administration of 20 mg/kg of **31** suppressed LH levels for at least 6 h in castrated rats, whereas intramuscular administration significantly lowered testosterone blood levels for up to 24 h in intact rats.[127] In addition, compound **31** was profiled against other drug targets and showed 50- to > 100-fold selectivity for human GnRH receptors, except for dopamine D_2 receptors and sodium channels. Based on the previous SAR studies,[126,127] compound **32** was developed, which contains a 2,4,6-trimethoxyphenyl group at the amide bond.[128] Anderes and coworkers evaluated **32** for its bioavailability and *in vivo* activity. Compound **32** had a high affinity for the human GnRH receptor (K_i = 6.0 nM) and showed functional antagonism *in vitro*. In addition, **31** had a similar affinity at the rat GnRH receptor and showed over a 1000-fold selectivity against other drug targets, except for dopamine D_2, 5-HT_{2a} serotonin receptors and calcium channels. In contrast to compound **31**, this compound was orally active. Although at a relatively high concentration (100 mg/kg), oral administration of **32** completely suppressed LH and testosterone blood levels for up to 8 h in castrated rats and 24 h in intact rats, respectively.[128] Notably, Neurocrine Biosciences reported that **32** acts as a negative allosteric modulator rather than as an orthosteric ligand for both a peptide GnRH agonist and a non-peptidic antagonist.[53] Hence, since *in vivo* activity was previously shown,[128] the allosteric mechanism is proven to be effective. Interestingly, a close analog of **33** was shown to be a negative allosteric modulator as well (*Chapter 3*). Moreover, in the same study it was shown that the GnRH receptor contains another allosteric site to which amiloride analogues bind. These compounds have been shown to modulate several GPCRs. Some additional structural changes (e.g. addition of a morpholino group) resulted in **33**, which was highly potent with improved oral activity.[129] Compound **33** had identical affinity values at rat and human GnRH receptors (K_i = 0.4 nM). Oral administration of 50 mg/kg of **33** completely suppressed LH and testosterone blood levels for up to 24 h in castrated rats and 12 h in intact rats, respectively.

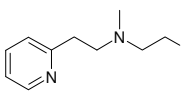
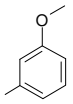
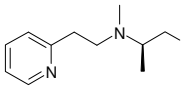
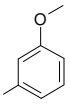
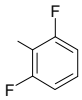
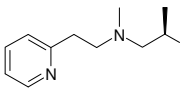
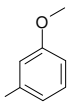
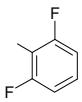
Importantly, **33** was highly selective over other drug targets and had low potential for interaction with various cytochrome P450s.

2.2.8 Pyrimidin-2,4-dione Derivatives

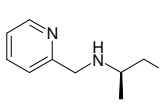
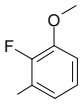
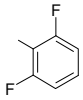
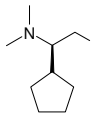
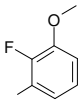
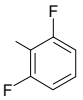
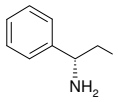
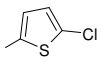
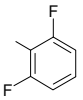
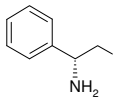
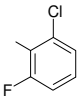
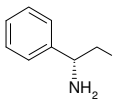
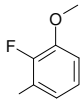
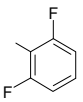
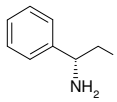
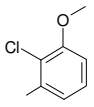
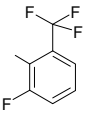
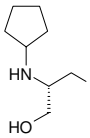
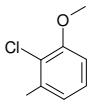
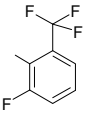
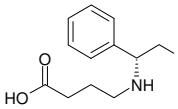
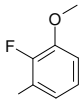
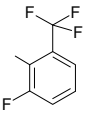
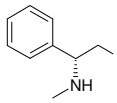
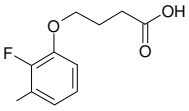
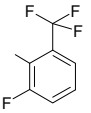
Previously, Neurocrine Biosciences had introduced the pyrrolo[1,2-a]pyrimidin-7-one (**20**-**22**) and imidazolo[1,2-a]pyrimidin-5-one (**23**-**26**) derivatives as potent human GnRH receptor antagonists.[116,121] Based on the SAR of these compounds, it was postulated that the five-membered ring of the scaffold was not necessary for receptor binding. The cyano- or imidazole-nitrogen was replaced by a carbonyl moiety resulting in pyrimidine-2,4-dione derivatives, which were also referred to as uracil-based GnRH receptor antagonists (Table 2.8).[130] Similar substituents as on the previous scaffolds resulted in a GnRH receptor antagonist with reasonably high affinity (**34**; K_i = 34 nM). The bioavailability of **34** was only 1.6% due to the high lipophilicity and poor metabolic stability. Further efforts were, therefore, undertaken to improve the metabolic stability. Introduction of an α- (**36**) or β-methyl (**35**) at the N-3 position increased the affinity, with the (*R*)-isomers being more active than the (*S*)-isomers.[131,132] The increase in binding affinity was explained by a receptor model

Table 2.8 Binding affinities of pyrimidin-2,4-dione derivatives (**34**-**45**) at the human GnRH receptor.



Compound	R^1	R^2	Ar	X	K_i (nM)[a]	Ref
34				CH_3	34	130
35				CH_3	5.5	131
36				CH_3	20	132

(Continued)

37				CH_3	1.1	[133]
38				CH_3	0.6 ± 0.1	[134]
39				CH_3	2[b]	[135]
40				CH_3	0.7	[136]
41				CH_3	0.56 ± 0.03	[137]
42				H	0.45	[138]
43				CH_3	0.30	[139]
44				H	2.2	[140]
45				CH_3	1.2	[141]

[a] The ability to inhibit the binding of des-Gly^{10}[^{125}I-Tyr^5,DLeu^6,NMeLeu^7,Pro^9-NEt]-GnRH to the cloned human GnRH receptor stably expressed in HEK293 cells.[119]

[b] The ability to inhibit the binding of ^{125}I-[His^5, D-Tyr^6]GnRH to the cloned human GnRH receptor stably expressed in RBL cells.[142]

in which the pyridyl side chain of the R-isomer is oriented towards the aspartic acid in TM VII (Asp302) and the ring itself towards the phenylalanine in TM VII (Phe309) of the receptor. When the rotational freedom of the 5-(3-methoxy)phenyl group was restrained with the introduction of a 2-fluoro substituent a significant increase in binding affinity was obtained (**37**; K_i = 1.1 nM).[133] Furthermore, **37** was a highly potent functional antagonist with

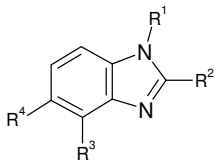
an IC_{50}-value of 0.5 nM. The metabolic stability of these compounds was still poor and therefore branched primary amines were introduced at the N-3 side chain.[134] Compound **38** had the highest affinity (K_i = 0.6 nM) reported thus far and showed increased metabolic stability in an *in vitro* liver microsomes assay. However, the half-life was still too short for acceptable pharmacokinetics. In another attempt to improve pharmacokinetic and metabolic properties, the 5-phenylgroup was substituted with a thiazole or thienyl ring.[135] In addition, one of the nitrogen-carbon bonds from the N-3 substituent was removed, which was known to be easily cleaved and oxidized by liver enzymes, by replacing it with (*R*)-phenylglycinol. With these modifications the potency was maintained (see **39)** and the intrinsic clearance drastically decreased. Subsequently, the N-1 substituent was optimized. Although the substituent at position 5 was replaced by a (fluoro) substituted phenyl again, the substitution of a 2,6-difluorobenzyl for a 2-chloro-6-fluorobenzyl group resulted in an increased binding affinity (**40**; K_i = 0.7 nM).[136] In this study it was indicated once more that the electron-deficient N-1 benzyl group possibly interacts with a tyrosine residue in TM VI (Tyr283 or Tyr284). These two amino acids have also been implicated in binding of the endogenous ligand GnRH as the mutated receptor could not be activated by GnRH.[143] Combination of the optimized substituents resulted in the highly potent (K_i = 0.56 nM) and orally available compound **41**, which is also referred to as NBI 42902.[137] Compound **41** was pharmacologically characterized in a second study, where it was shown to provide novel opportunities to control the hypothalamic-pituitary-gonadal axis.[144] Similar to the compounds described above, the affinity of compound **41** was very low for the rat GnRH receptor (IC_{50} < 10 µM). *In vivo* studies were, therefore, performed in monkeys, where the affinity for the GnRH receptor was only 6-fold lower (K_i = 3.5 nM).[137,144] Oral administration of 100 mg/kg **41** completely suppressed LH blood levels over 24 h in castrated male macaques. A final optimization study was performed to improve the manufacturing reproducibility, as **41** showed atropisomerism (rotational stereoisomerism) of the 5-aryl group due to the 6-methyl.[138] Therefore another study was conducted to modify 1- and 5-substituents of the desmethyl analogs (**42** and **44**), while maintaining a high potency. The resulting compound **42** had a high receptor affinity (K_i = 0.45 nM) and did not possess stereo isomeric properties. Molecular modeling indicated that the 5-phenyl ring could interact with a tyrosine residue in TM V (Tyr211) through π-π stacking. In addition, an asparagine residue in TM V (Asn212) could form a hydrogen-bond with the 3-methoxy group on the phenyl ring. Both residues have also been implicated in GnRH binding.[145] Further studies were performed to obtain

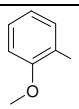
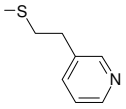
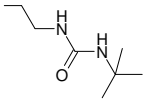
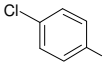
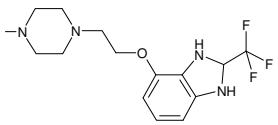
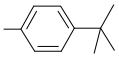
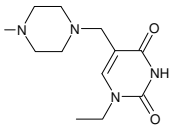
thermally stable single isomers.[139] Previously, pyrimidin-2,4-dione derivatives with an *N*-alkyl aminoalkyl side chain (e.g. **38**) were reported as potent GnRH receptor antagonists.[134] Therefore, compound **43** was synthesized and its isomers separated.[139] The *R*-isomer of **43** was stable at room temperature, either dry or in DMSO solution, and 15-fold more potent than the *S*-isomer. X-ray crystallography of another derivative confirmed that the *R*-isomer is preferred for receptor interactions. Although compounds **41**-**43** had high affinity and potency for the human GnRH receptor, their metabolic profile was poor. Therefore, additional efforts were made to decrease CYP3A4 metabolism of these compounds. A close analog of **42** (2-F in stead of 2-Cl) inhibited this metabolic liver enzyme with an IC_{50} of 0.1 μM.[140] Neurocrine Biosciences reasoned that introduction of polar groups would be tolerated, due to the presence of several basic residues in the ligand binding pocket of the human GnRH receptor.[146] The attachment of an acidic group to either the amine (**44**)[140] or to the 3-methoxyphenyl group (**45**)[141] was further investigated. The location of the acidic group proved important. Both compound **44** and **45** had over 100-fold decreased CYP3A4 potency, with an IC_{50} value of 36 μM and 13 μM, respectively. The pharmacokinetic profiles were investigated and it was shown that bioavailability was low in rats. However, reduction of the amount of hydrogen bond donors by methylation of the basic amine group increased oral bioavailability (**44**: %F = 2; **45**: %F = 13). Notably, oral bioavailability of **44** was 10-fold higher in monkeys than in rats.[140]

2.2.9 Benzimidazole Derivatives

In 2005, Bayer reported benzimidazole derivatives as a new class of non-peptidic GnRH receptor antagonists (Table 2.9).[147] A screen of approximately one million compounds resulted in a first hit, compound **46**. This compound had low micromolar potency at both rat and human GnRH receptors (hIC_{50} = 3.4 μM). Electron withdrawing *para*-substituents on the sulfonamide phenyl ring improved the potency of this compound. In addition, introduction of a flexible side chain with a basic moiety, as seen in most GnRH receptor antagonists, resulted in compound **47** (hIC_{50} = 120 nM). Notably, the potency at the rat receptor was retained. For further studies, *in vivo* low nanomolar antagonists were a prerequisite. Therefore, compound **47** was further optimized to compound **48** that had an almost 30-fold increased potency and showed no selectivity against the rat GnRH receptor. It was shown that two hydrogen-bond

Table 2.9 Binding affinities of benzimidazole derivatives (**46-50**) at the human GnRH receptor.



Compound	R^1	R^2	R^3	R^4	IC_{50} (nM)[a]	Ref
46			H		3400	147
47			H		120	147
48			H		4.2	148
49	H	CF_3		H	1540[b]	149
50	H			H	1.7 ± 0.65[b]	149

[a] IC_{50} in a CHO-hGnRHR-Ca^{2+} assay.[147]

[b] The ability to inhibit the binding of ^{125}I-(D-trp^6)LHRH to the cloned human GnRH receptor in recombinant cells.[149]

donors at the 2-position increased the potency, for example by introducing an urea-linker. In addition, the spatial orientation of the bulky aliphatic group attached to the linker was of great importance.[148] The findings were in correspondence with Sasaki and coworkers for the thieno[2,3-d]pyrimidin-2,4-dione derivatives.[124] Recently, Wyeth published on a screen of a library of approximately 2200 compounds that was rich in GPCR ligands.[149] This resulted in two lead compounds (e.g. **49**) that were also potent in binding the human serotonin (5-HT-1) receptor subtypes. Compound **49** had low micromolar affinity for the human GnRH receptor (IC_{50} = 1.54 μM) and a much higher affinity at the 5-HT-1 receptor subtypes (e.g. IC_{50} = 0.55 nM at 5-HT_{1D}). Therefore, efforts were made to optimize binding potency for the human and rat GnRH receptors, as well as selectivity over the 5-HT-1 receptor subtypes. Optimization of the piperazine linker and one of the benzimidazole groups resulted in compound **50** that had a

900-fold increased affinity for the human GnRH receptor, a greatly enhanced rat receptor affinity and selectivity over other drug-targets.[149] Compound **50** had a high oral bioavailability in rats (%F = 74) and oral administration of 30 mg/kg completely suppressed serum LH levels over 6 h in castrated rats.

2.2.10 1,3,5-Triazine-2,4,6-trione Derivatives

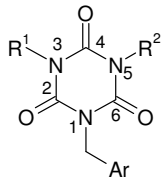
In 2005 Neurocrine Biosciences introduced another monocyclic class of non-peptidic GnRH receptor antagonists, the 1,3,5-triazine-2,4,6-trione derivatives (Table 2.10).[150] Replacement of the 6-methyl group of **34** with a carbonyl moiety resulted in a compound with micromolar affinity (**51**; K_i = 4.2 μM). Introduction of (*R*)-phenylglycinol at the 3-position yielded compound **52**, which exhibited a substantially higher affinity, K_i = 37 nM. This proved that the 6-methyl group is not essential for human GnRH receptor binding. The advantage of this novel scaffold was that the synthetic route was a convenient two-step, one-pot cyclization procedure with high yields.[150] Receptor affinity of **52** was 5-fold improved by the introduction of a 2-fluoro substituent at the 5-phenyl group.[151] Substitution of the 2-fluoro by a bromo substituent at the N-1 benzyl group resulted in a 20-fold overall increase in potency (**53**; K_i = 2 nM). Compound **53** showed functional antagonism in an inositol phosphate accumulation assay (IC_{50} = 33 nM) and its metabolic stability was comparable to that of compound **39**. These compounds also showed species selectivity with a 10- and 1000-fold lower binding affinity at the monkey and rat GnRH receptor for **53**, respectively.

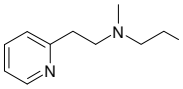
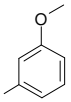
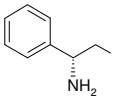
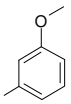
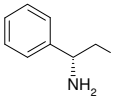
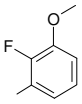
2.2.11 Various

Neurocrine Biosciences has recently published four other classes of non-peptidic compounds as human GnRH receptor antagonists (Figure 2.1). The scaffolds of compounds **54**-**56** are derived from the pyrimidin-2,4-dione class (**34**-**45**).

In case of **54** a thiazole ring was introduced, resulting in thiazolino[3,2-c]pyrimidin-5,7-dione derivatives. Also different compounds with an oxazole instead of a thiazole ring were tested. The sulfur derivatives were equipotent to the oxo-derivatives. A bicyclic system rigidifies the N-1 benzyl substituent and apparently yielded more potent ligands, such as compound **54**, which showed high receptor affinity (K_i = 4.5 nM).[152] Similar to 1,3,5-triazine-2,4,6-trione

Table 2.10 Binding affinities of 1,3,5-triazine-2,4,6-trione derivatives (**51**-**53**) at the human GnRH receptor.



Compound	R^1	R^2	Ar	K_i (nM)[a]	Ref
51				4200	150
52				37	150
53				2	151

[a] The ability to inhibit the binding of des-Gly^{10}[^{125}I-Tyr^5,DLeu^6,NMeLeu^7,Pro^9-NEt]-GnRH to the cloned human GnRH receptor stably expressed in HEK293 cells.[119]

derivatives (**51**-**53**), the synthesis of this class of compounds was straightforward and proceeded with high yields. Pontillo *et al.* showed that the presence of a 6-carbonyl moiety was more important than the 2-carbonyl moiety of the triazinedione derivatives.[153] Removal of the 6-carbonyl resulted in a reduction of receptor affinity that could be recovered by the introduction of a 2-chloro substituent at the 3-methoxyphenyl ring and replacement of a fluoro by a trifluoromethyl group at the benzyl ring (**55**; K_i = 2.3 nM). Although **55** had the same substituents as **42**, the latter had a 5-fold higher affinity, which indicates that the third nitrogen in the ring is unfavorable. Another scaffold with similar substituents was introduced by Lanier *et al.*, the tetrahydropyrido[4,3-d]pyrimidin-2,4-dione derivatives.[154] Compounds with low nanomolar binding affinity emerged in this class (**56**; K_i = 5 nM).

Chen and coworkers studied a series of tetrahydropyrrolo[3,2-*c*]pyridines as GnRH receptor ligands.[155] These compounds were based on the indole derivatives (**10**-**19**) that were described previously.[115] The indole-based ligands were less species-selective, and could therefore be tested on the more convenient and cost effective *in vivo* castrated rat model.

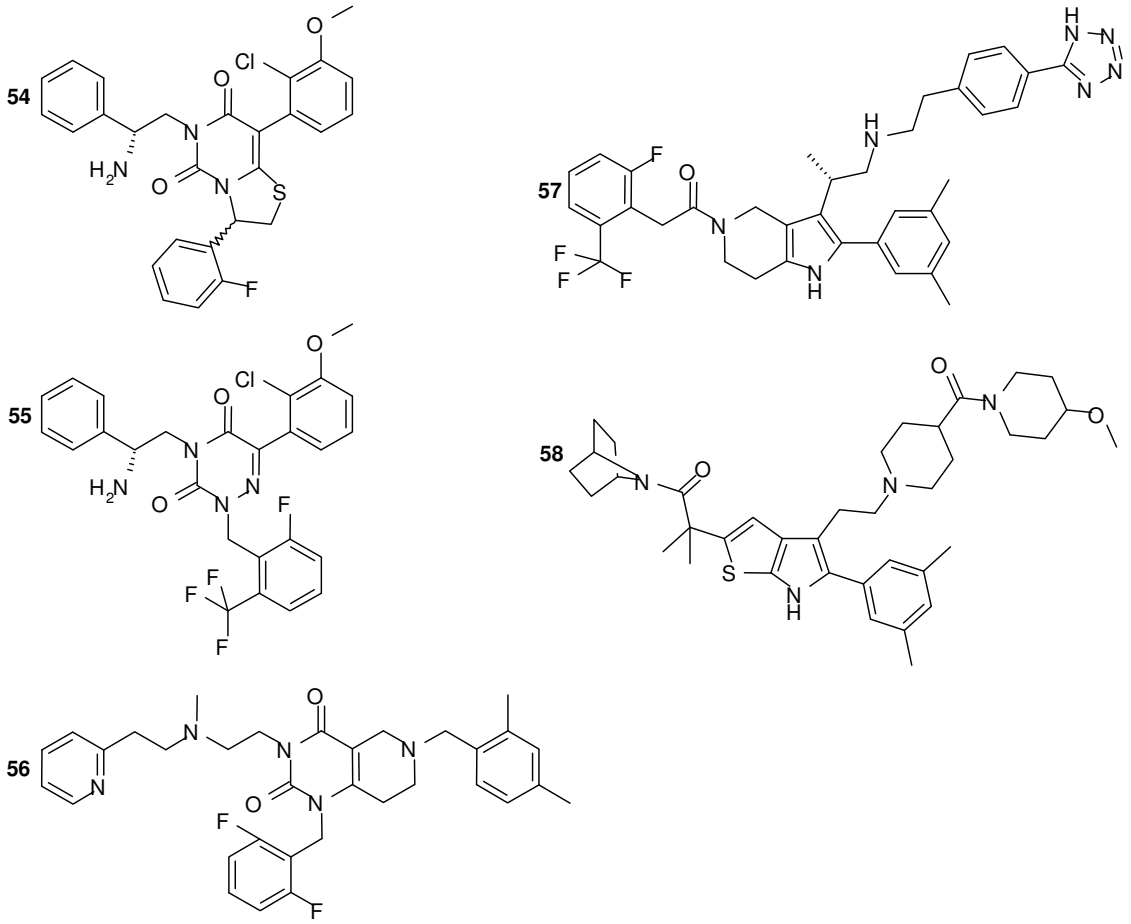


Figure 2.1 Chemical structures of the most potent analogues of thiazolino[3,2-c]pyrimidin-5,7-dione (**54**) [152], triazinedione (**55**)[153], tetrahydropyrido[4,3-d]pyrimidin-2,4-dione (**56**)[154], pyrrolo[3,2-c]pyridine (**57**)[155] and thieno[2,3-*b*]pyrrole (**58**)[156] derivatives as antagonists at the human GnRH receptor.

Compound **57** had a high rat and human GnRH receptor affinity (hIC_{50} = 1.5 nM; rK_i = 0.2 nM) and intraperitoneal administration of 15 mg/kg suppressed LH blood levels for up to 8 h in castrated rats.

Very recently, AstraZeneca reported a new class of human GnRH receptor antagonists, thieno[2,3-*b*]pyrrole derivatives (e.g. **58**).[156] This study was particularly directed towards potent orally bioavailable non-peptidic antagonists, based on previously described orally active compounds, thieno[2,3-d]pyrimidin-2,4-dione,[98] indole[115] and pyrimidin-2,4-dione derivatives.[137] Although the affinity of **58** was lower (IC_{50} = 184 nM) than reported for the other classes, it had a similar affinity value for the rat GnRH receptor (IC_{50} = 76 nM). The

pharmacokinetic profile of **58** was therefore obtained, which showed that the plasma clearance was slower (CL = 0.1 mL/min/kg) and this translated into better oral bioavailability (%F = 35) than that of previous ligands. In addition, the CYP3A4 activity was much lower for these compounds.[156]

2.2.12 Patented Ligands

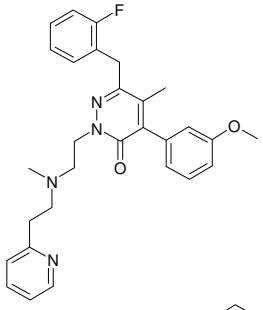
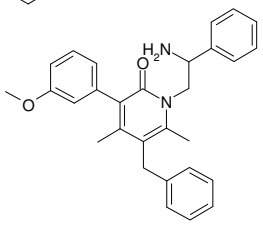
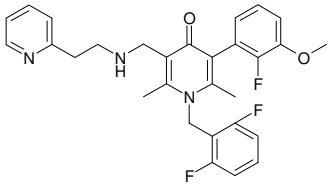
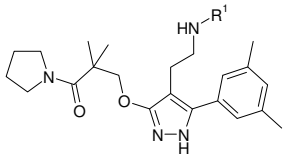
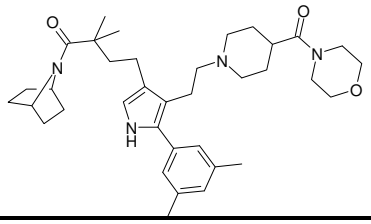
Patent literature revealed several other non-peptidic compounds classes that have been described as human GnRH receptor antagonists. In this review, these were divided into mono-, bi- and tricylic antagonists, as shown in Table 2.11-2.13, respectively.

Neurocrine Biosciences reported three monocyclic compound classes that showed antagonistic activity at the human GnRH receptor (Table 2.11), namely 3-pyrazinone,[157] pyrid-2-one[157] and pyrid-4-one derivatives.[158] AstraZeneca similarly patented pyrazole[159,160] and pyrrole derivatives,[161] while Paradigm Therapeutics showed that oxazole- and thiazole-4-carboxamide derivatives[162] are potent GnRH antagonists. Based on their substituents the latter two could be classified with the furamide analogues described above (Table 2.7).

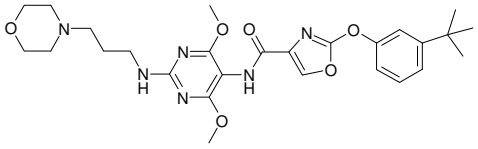
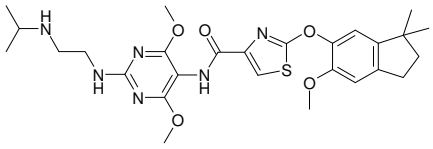
Table 2.12 summarizes all non-peptidic bicyclic GnRH receptor antagonist classes that have been patented. A wide variety of scaffolds have been contributed by several companies. Tetrahydroisoquinoline derivatives[163,164] were described by Abbott, while 1,3-dihydrobenzimidazole derivatives were patented by Yamanouchi[165] and Astellas[166,167] as potent GnRH receptor antagonists. GSK have patented bicyclic pyrrolidines[168,169] and Ortho-McNeill[170] and Schering[171,172] both described quinoline derivatives. AstraZeneca[173,174] and Wyeth[175] have both patented imidazo[1,2-*a*]pyridine-based ligands. In addition, Wyeth also described imidazo[4,5-*c*]pyridine[176,177] and triazolo[1,5-a]pyridine[175] derivatives as GnRH receptor antagonists. SCRAS[178] has patented benzimidazoles, where Wyeth has substituted an imidazole-nitrogen for sulfur or oxygen, resulting in two other compound classes, namely benzoxazoles and benzothiazoles.[179] Following thieno[2,3-*b*]pyrimidin-2,4-dione derivatives (Table 2.6), Takeda also described quinazoline-2,4-dione-based[180] and thieno[3,2-*d*]pyrimidin-2,4-dione-[181] antagonists. Recently, a slightly modified class of GnRH receptor antagonists was described by Kissei Pharmaceutical, thieno[3,4-*d*]pyrimidin-2,4-dione derivatives.[182]

Tricyclic GnRH receptor antagonists have been made by GSK and Zentaris, which patented a series of tricyclic pyrrolidine[168,169] and tetrahydrocarbazole derivatives,[183,184] respectively (Table 2.13).

Table 2.11 Binding affinities of patented monocyclic antagonists at the human GnRH receptor.

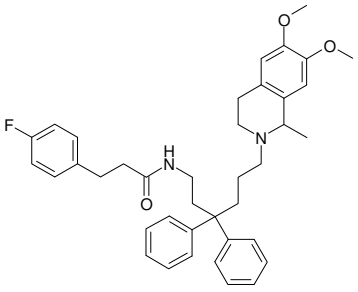
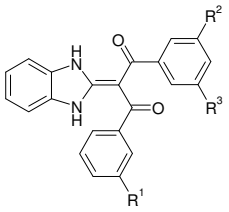
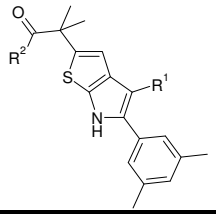
Class	Company	Structure	Potency	Ref
3-Pyrazinone	Neurocrine		n.p.	157
Pyrid-2-one	Neurocrine		n.p.	157
Pyrid-4-one	Neurocrine		K_i < 100 µM	158
Pyrazole	AstraZeneca		IC_{50} = 1-5,000 nM	159,160
Pyrrole*	AstraZeneca		IC_{50} = 1-5,000 nM	161

(Continued)

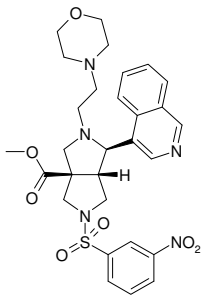
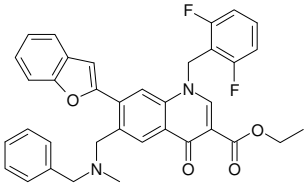
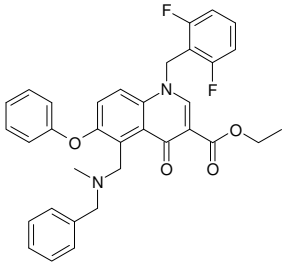
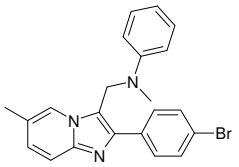
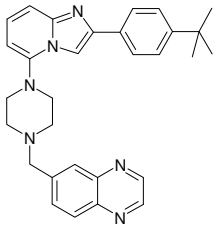
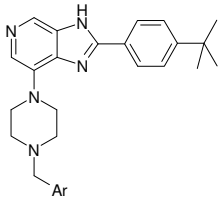
Oxazole-4-carboxamide	Paradigm		n.p.	162
Thiazole-4-carboxamide	Paradigm		IC_{50} = 4 nM	162

* The depicted chemical structure is an example from the patent. n.p. = not published

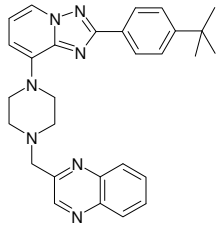
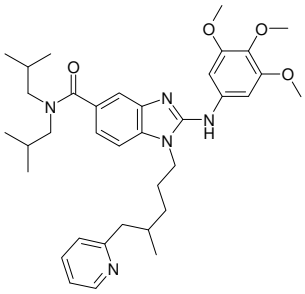
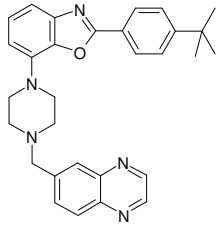
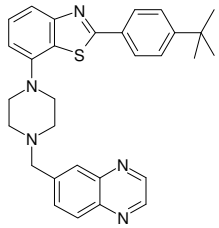
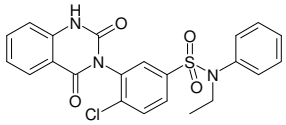
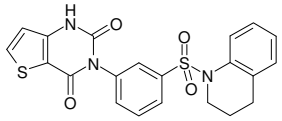
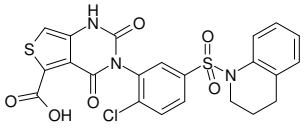
Table 2.12 Binding affinities of patented bicyclic antagonists at the human GnRH receptor.

Class	Company	Structure	Potency	Ref
Tetrahydro-isoquinoline	Abbott		K_i = 1.6 nM	163,164
1,3-Dihydrobenz-imidazoles	Yamanouchi		IC_{50} = 0.1-1 nM	165
1,3-Dihydrobenz-imidazoles	Astellas		IC_{50} = 0.058–0.24 nM	166,167

(Continued)

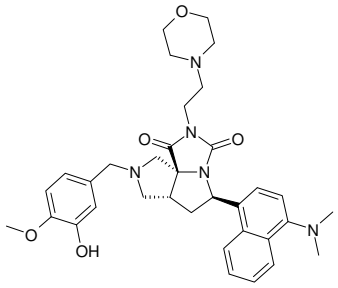
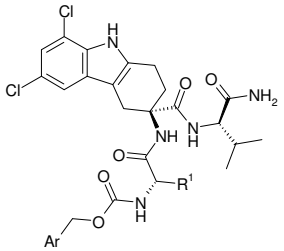
Bicyclic pyrrolidines*	GSK		IC_{50} = 35–1,500 nM	168,169
Quinolines*	Ortho-McNeill		IC_{50} = 1–32 µM	170
Quinolines	Schering		n.p.	171,172
Imidazo[1,2-a]pyridines*	AstraZeneca		IC_{50} = 1-30,000 nM	173,174
Imidazo[1,2-a]pyridines*	Wyeth		IC_{50} = 1–1,000 nM	175
Imidazo[4,5-c]pyridines	Wyeth		IC_{50} < 10 µM	176,177

(Continued)

Triazolo[1,5-a]pyridines*	Wyeth		IC_{50} = 1–1,000 nM	175
Benzimidazoles	SCRAS		n.p.	178
Benzoxazoles*	Wyeth		IC_{50} = 25–100,000 nM	179
Benzothiazoles*	Wyeth		IC_{50} = 25–100,000 nM	179
Quinazoline-2,4-diones	Takeda		IC_{50} < 10 μM	180
Thieno[3,2-d]pyrimid-2,4-diones	Takeda		> 86% inhibition	181
Thieno[3,4-d]pyrimid-2,4-diones	Kissei		IC_{50} = 2 nM	182

* The depicted chemical structure is an example from the patent. n.p. = not published

Table 2.13 Binding affinities of patented tricyclic antagonists at the human GnRH receptor.

Class	Company	Structure	Potency	Ref
Tricyclic pyrrolidines*	GSK		IC_{50} = 35–1,500 nM	168,169
1,2,3,4-Tetrahydro-carbazoles	Zentaris		IC_{50} = 0.1-15 nM	183,184

* The depicted chemical structure is an example from the patent.

2.2.13 Overview GnRH receptor antagonists

A comparison of the GnRH receptor antagonists presented here shows that within each structural class high-affinity and bioavailable compounds have been designed. This indicates that the substituents rather than the scaffolds are the key determinants, which is not surprising as similar substituents often prove optimal for each class. For four compound classes possible interaction sites were predicted and/or visualized by molecular modeling using the architecture of rhodopsin as a template for the GnRH receptor structure. It seems that the binding sites of non-peptidic GnRH receptor antagonists overlap with each other, but also (in part) with the orthosteric binding site of GnRH itself. Figure 2.2 represents a summary of the optimal pharmacophore extracted from the data described above, including the possible interaction sites in the receptor pocket. The clinical use of these GnRH receptor antagonists is uncertain as selectivity data with respect to other drug targets (e.g. GPCRs) is often lacking. In addition, species differences and the interaction with cytochrome P450 activity of these compounds add to this uncertainty.

Although a wide range of GnRH receptor antagonists has been described, non-peptidic agonists have not thus far. The only agonistic 'starting point' currently available is the

endogenous peptide ligand and some of its analogues. As the binding site of non-peptidic ligands seems to overlap with GnRH, a pharmacophore model of the peptide may give further clues. Moreover high-throughput screening of large compound libraries may yield lead compounds too, as there is no upfront reason why this successful approach would not work in this specific situation.

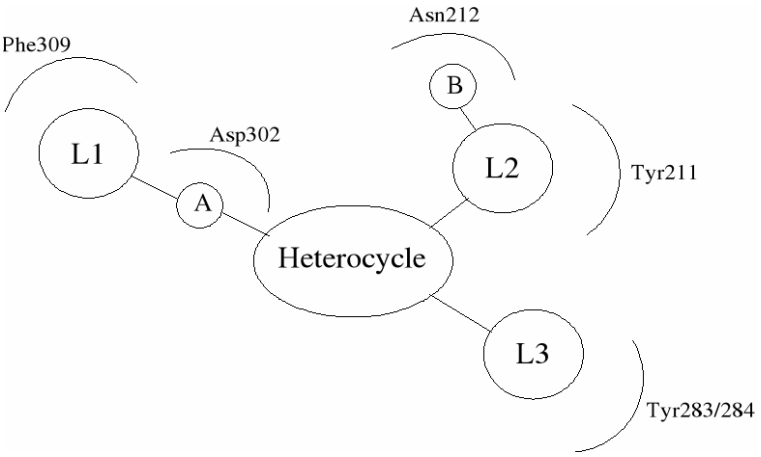


Figure 2.2 Schematic diagram that summarizes the non-peptidic GnRH receptor antagonist pharmacophore obtained from literature survey, including the possible interactions with the receptor pocket. A and B represent a basic nitrogen (H-bond donor) and an ester (H-bond acceptor), respectively. $L_{1\text{-}3}$ represent (heterocylic) aromatic rings.

2.3 LH RECEPTOR AGONISTS

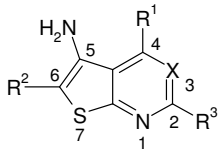
The LH receptor recognizes two endogenous ligands, namely LH and human chorionic gonadotropin (hCG). These gonadotropins are closely related in structure and consist of a conserved α-subunit and a hormone specific β-subunit.[185] It has been shown that these hormones bind to the large N-terminal domain of the LH receptor.[186] Infertility treatment has traditionally been based on the administration of urine-derived gonadotropins. More recently recombinant hormones have also been developed with a similar activity profile.[187] Deglycosylated hCG has been described as an antagonist of the LH receptor.[188] Recently, another hormonal antagonist was identified, which was generated by fusing two hCG β-subunits.[189] Antagonists of the LH receptor could lead to novel contraceptive treatments. However, protein hormones need to be administered by intramuscular or subcutaneous injection. Research is therefore aimed at the development of non-peptide, orally active gonadotropins. For the LH receptor, the first low molecular weight agonists have now been identified by Organon Biosciences (currently Schering-Plough) and Merck Serono, respectively, which will be described here.

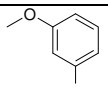
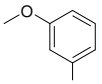
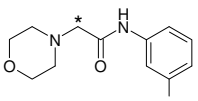
2.3.1 Thieno[2,3-b]pyri(mi)dine Derivatives

In 2002, Van Straten and coworkers revealed the first two classes of orally active non-peptidic LH receptor agonists.[190] Both thienopyridines and -pyrimidines were able to activate the receptor (Table 2.14). Substitution of the amide group at position 6 with a branched alkyl group resulted in a dramatic increase in potency. The potency was further enhanced by the introduction of a 3-methoxyphenyl moiety at position 4. The most potent thienopyridine and –pyrimidine compounds were **59** and **60**, with EC_{50}-values of 160 nM and 20 nM, respectively. Although the two compound classes showed similar SAR, the overall potency of thienopyrimidines was higher, which indicates the importance of the additional nitrogen atom in the ring. Compound **60** showed *in vivo* efficacy, namely oral administration of 50 mg/kg resulted in 40% ovulating FSH-primed mice.[190] Notably, these ligands were not able to displace ^{125}I-LH binding.[21] This indicates that the binding site of low molecular weight ligands is located in the 7-TM domain. Therefore these compounds can be considered as allosteric agonists. Jäschke and coworkers further examined the binding pocket of **60** by molecular modeling.[191] Compound **60** was shown to be a partial agonist at the thyroid-stimulating hormone (TSH) receptor (EC_{50} = 7700 nM). Based on the predicted binding

pocket different mutant receptors were constructed, where residues in the binding pocket of the LH receptor were introduced at corresponding positions in the TSH receptor. As expected, **60** was a full agonist with increased potency in some of these constructs. As a

Table 2.14 Potencies of thieno[2,3-b]pyri(mi)dines (**59-61**) at the human LH receptor.



Compound	X	R_1	R_2	R_3	EC_{50} (nM)[a]	Ref
59	CH				160 ± 20	21
60	N				20 ± 5	21
61	N				1.7 ± 0.2	*Chapter 4*

[a] EC_{50} in the CHO-hLHR-luciferase assay.[192,193]

* Position of the tritium substitution in [^{3}H]Org 43553 (*Chapter 4*).

follow-up, Moore and colleagues examined a key residue for receptor-ligand interaction, Glu3.37, which is conserved in LH and TSH receptors.[194] Different analogues of **60** were prepared, which were aimed at increasing the selectivity toward the LH receptor. Compounds with increased steric bulk at the amide portion of **60** showed enhanced selectivity, while maintaining their potency and efficacy at the LH receptor. This corresponded to the finding that the binding pocket of the TSH receptor is smaller than the pocket of the LH receptor.[191,194] Interestingly, compound **60** was shown to act as a chaperone molecule by increasing the membrane expression of wild-type FSH receptors, while it did not have intrinsic efficacy at this receptor.[195] Recently, a few other thienopyrimidines were reported as described in *Chapter 4*. The most potent compound (**61**; EC_{50} = 1.7 nM) was labeled with tritium and characterized for its receptor binding. It was shown that there was a high correlation between the affinity and potency of low molecular weight LH receptor agonists. Notably, a high concentration of the endogenous hormone LH only modestly displaced the

radioligand, confirming an allosteric binding site for the thienopyridimines. Furthermore, Organon Biosciences (currently Schering-Plough) recently published some additional data on compound **61** (i.e. Org 43553).[36] Chimeric TSH/LH receptors were prepared and it was confirmed that **61** activates the LH receptor through the 7-TM domain. In addition, it was shown that this low molecular weight agonist is signaling-selective, as it only stimulates the cAMP pathway and not the phospholipase C pathway, unlike LH.

2.3.2 Pyrazole Derivatives

Recently, Merck Serono reported a new class of low molecular weight LH receptor agonists (Figure 2.3).[196] Pyrazole derivatives were identified from an in-house library screen. Subsequently, a new pyrazole library was synthesized to examine the SAR of these new ligands. The most potent agonist (**62**) had an EC_{50} value of 20 nM in an *in vitro* cAMP assay and was able to partially activate the receptor (E_{max} = 70% of maximal LH response). Notably, compound **62** did not compete for ^{125}I-hCG binding to the LH receptor,[196] as was also described for the thienopyrimidine derivatives.[21] In an *in vivo* testosterone-induction model in rats, **62** was able to dose-dependently increase testosterone levels after subcutaneous injection.

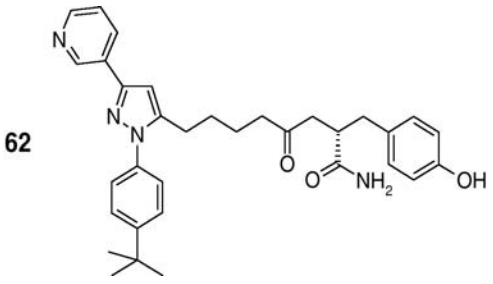


Figure 2.3 Chemical structure of the most potent pyrazole (**62**) derivative as an agonist at the human LH receptor.[196]

2.3.3 Patented ligands

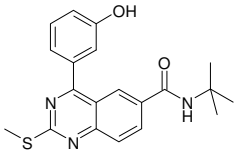
Organon Biosciences (currently Schering-Plough) patented another class of LH receptor agonists, the quinazoline-6-carboxamides (Table 2.15).[197] The apparent similarity with the

thienopyrimidine-based ligand is due to the presence of a pyrimidine ring and the substituted amide group.

2.3.4 Overview LH receptor agonists

Research of non-peptidic LH receptor agonists has started only recently. With four reported compound classes, there is still a large field to be explored. In addition, non-peptidic antagonists would be of great interest, as they could possibly be used as non-hormonal contraceptives. Hopefully, the first thienopyrimidine radioligand, [^{3}H]Org 43553 (**56**), will aid in the discovery of other allosteric ligands for the LH receptor (*Chapter 4*).

Table 2.15 Potency of a patented agonist at the human LH receptor

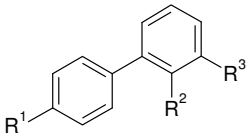
Class	Company	Structure	Potency	Ref
Quinazoline-6-carboxamides*	N.V. Organon		IC_{50} = 10-100 nM	197

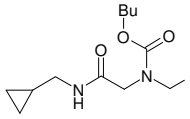
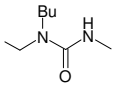
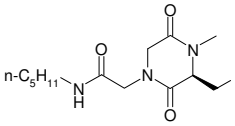
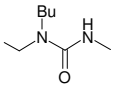
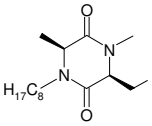
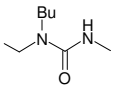
* The depicted chemical structure is an example from the patent.

2.4 FSH RECEPTOR LIGANDS

FSH is a glycoprotein hormone that consists of an α- and a β-subunit, like LH and hCG.[18] In 2005 the crystal structure of FSH bound to the large extracellular domain of the receptor was reported.[198] Hence, it was shown that FSH could form a stable complex with the N-terminal domain of the receptor in the absence of the 7-TM domain. Urinary and recombinant FSH is also used in the treatment of infertility.[187] Recombinant gonadotropins need to be administered daily and therefore effort was made to develop gonadotropins with a longer half-life. Long-acting FSH may be produced by fusing the C-terminal extension of the β-subunit of hCG to the β-subunit of FSH or by additional glycosylation of FSH.[77,78] Sustained-release formulations of FSH are also in development that could result in less frequent dosing.[199] In contrast to the LH receptor, both low molecular weight agonists and antagonists have been identified for the FSH receptor. FSH receptor antagonists may be used as non-hormonal contraceptives. The different compound classes will be discussed separately, including patented ligands.

Table 2.16 Potencies of biphenyl derivatives (**63**-**65**) at the human FSH receptor.



Compound	R^1	R^2	R^3	EC_{50} (nM)[a]	Ref
63			H	3900	200
64		H		1700	200
65		H		1.2	201

[a] EC_{50} in the CHO-hFSHR-luciferase assay.[192,193]

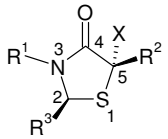
2.4.1 Biphenyl-based agonists

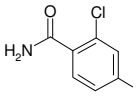
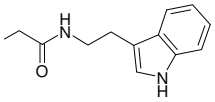
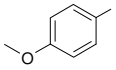
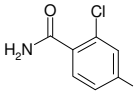
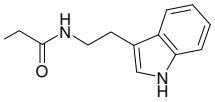
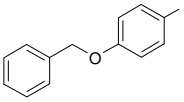
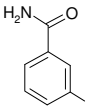
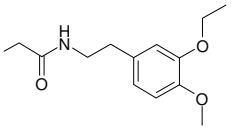
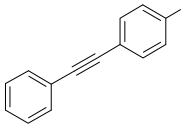
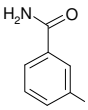
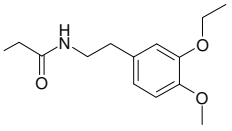
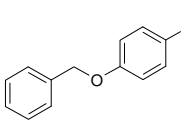
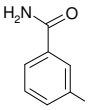
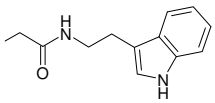
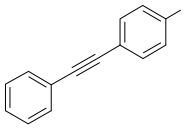
A high-throughput screen at Pharmacopeia of two million compounds yielded the first non-peptidic FSH receptor agonist.[200] Compound **63** consists of a biphenyl scaffold (Table 2.16) with low micromolar potency (EC_{50} = 3.9 μM). A combinatorial chemistry approach was used to synthesize a wide variety of compounds. Different substituents were placed on the R^1-R^3 groups, where the amide function in R^1 was retained. Through this approach over 30,000 distinct compounds were obtained of which 72 were active (EC_{50} < 10 μM), for example **64**.[200] The actives were generally the R^3-*meta*-substituted compounds. Guo *et al.* further optimized the biphenyl compounds into potent FSH receptor agonists via parallel synthesis.[201] Removal of the R^1 amide function proved beneficial and an octyl substituent on the N-4 position appeared optimal for high potency. In addition, introduction of a second (*S*)-methyl group at the diketopiperazine ring resulted in a dramatic increase in potency. The most potent compound in this series was **65** (EC_{50} = 1.2 nM; luciferase-reporter-gene assay), which displayed similar (partial) agonistic activity in a cAMP accumulation assay.

2.4.2 Thiazolidin-4-one-based agonists

A second low molecular weight FSH receptor agonist class was described by Affymax.[202] From a screen, a thiazolidinone derivative (**66**) was discovered, which had only moderate potency (EC_{50} = 5 μM) in a luciferase reporter gene assay (Table 2.17). A similar combinatorial chemistry approach was used as described for the biphenyl-based ligands, which resulted in a library of more than 42,000 compounds with a thiazolidinone scaffold. A modification of the R^3 substituent, addition of a phenyl ring, resulted in a more than 150-fold increase in potency (**67**; EC_{50} = 32 nM). This thiazolidinone library described by Maclean and coworkers also resulted in several hits against other biological targets (data not shown).[202] Another (encoded) library of thiazolidinone analogs (e.g. **66**) was prepared by Yanofsky and coworkers, consisting of more than 40,000 compounds.[54] The library was screened in an FSH receptor reporter gene assay, as none of the compounds was able to displace [^{125}I]FSH. Further optimization by parallel synthesis resulted in the identification of a highly potent FSH receptor agonist (**68**; EC_{50} = 2 nM).[54] Compound **68** fully activated the native FSH receptor in rat ovarian granulosa cells with an EC_{50} value of 10.5 nM. In addition, chimeric human FSH/TSH receptors were constructed. It appeared that the thiazolidinone

Table 2.17 Potencies of thiazolidinone derivatives (**66-70**) at the human FSH receptor.



Compound	R^1	R^2	R^3	X	EC_{50} (nM)[a]	Ref
66				H	5,000	202
67				H	32	202
68				H	2	54
69				CH_3	51 ± 42	203
70				H	1,700[b]	204

[a] EC_{50} in the CHO-hFSHR-luciferase assay.[205]

[b] IC_{50} in a phFSH-induced rat aromatase assay.[206]

compounds activated the FSH receptor through an allosteric site located in the region formed by TM I/II and the first extracellular loop. Wyeth continued the optimization of the thiazolidinone compounds to facilitate the synthesis of the active trans isomer.[203] The addition of a 5-methyl substituent locked both scaffold side chains into the trans orientation. FSH receptor activity of compound **69** was similar to the 5-hydrogen analog **67**. Recently, four thiazolidinone compounds were pharmacologically characterized by Arey and coworkers.[204] Among these four compounds were **68** and a demethylated analog of **69** (X = H), which were shown to be a full and partial agonist in a cAMP assay, respectively. Another derivative (**70**) was shown to be an antagonist in this assay. Together, these data demonstrated that thiazolidinone compounds can have different pharmacological effects,

ranging from full agonists to partial agonists and antagonists. In addition, it was shown that compound **70** was capable of inducing association of the human FSH receptor to the G_i, next to the G_s signaling pathway, unlike the endogenous hormone FSH.[204] These LMW ligands are able to activate additional G protein-signaling pathways, providing another way of modulation of the action of glycoprotein hormones.

2.4.3 Various Agonists

Recently, Wyeth described an analog of **69** as a potent FSH receptor agonist, where the thiazolidinone scaffold was replaced by a γ-lactam ring (Figure 2.4).[207] The γ-lactam derivative **71** showed similar FSH receptor potency (EC_{50} = 24.8 ± 11.8 nM) and selectivity over the TSH receptor.

Van Straten *et al.* identified compound **72** as an FSH receptor agonist through a high-throughput screen in a luciferase reporter gene assay (Figure 2.4).[208] Compound **72** was a selective agonist with an EC_{50} of 4.4 μM. In an optimization study, the pharmacological profile of analogues of **72** shifted from functional agonists to antagonists, which was further exploited and will be discussed in one of the next paragraphs.

2.4.4 Patented Agonists

Several companies have patented FSH receptor agonists containing different scaffolds (Table 2.18). ARS Holding has patented carbazole derivatives[209] that show low nanomolar potency on the receptor, while Serono has patented pyrazole-based agonists.[210] Besides tetrahydroquinoline-based agonists, Organon (currently Schering-Plough) has described two additional low molecular weight compound classes, namely thieno[2,3-*b*]pyrimidine[211] and hexahydroquinoline derivatives.[212-214] Notably, the former class was also identified as LH receptor agonists by this company.[21] Lastly, Arena patented isoxazolylthiazole-based compounds as FSH receptor agonists.[215]

2.4.5 Various Antagonists

More than a decade ago, it was shown that suramin inhibited FSH binding to its receptor.[216] However, suramin *per se* is a non-selective agent that also interacts with other

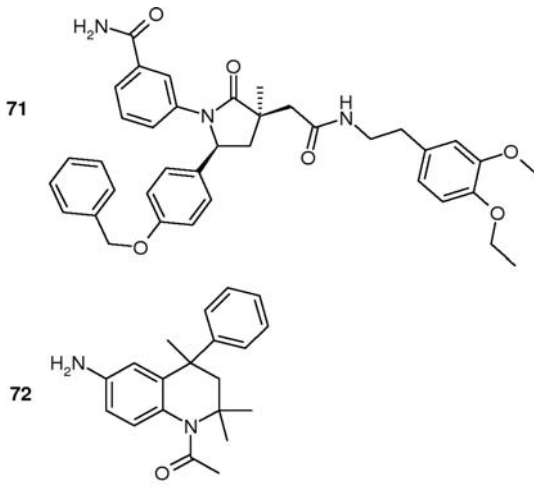


Figure 2.4 Chemical structure of the most potent γ-lactam (**71**)[207] and tetrahydroquinoline (**72**)[208] derivative as an agonist at the human FSH receptor.

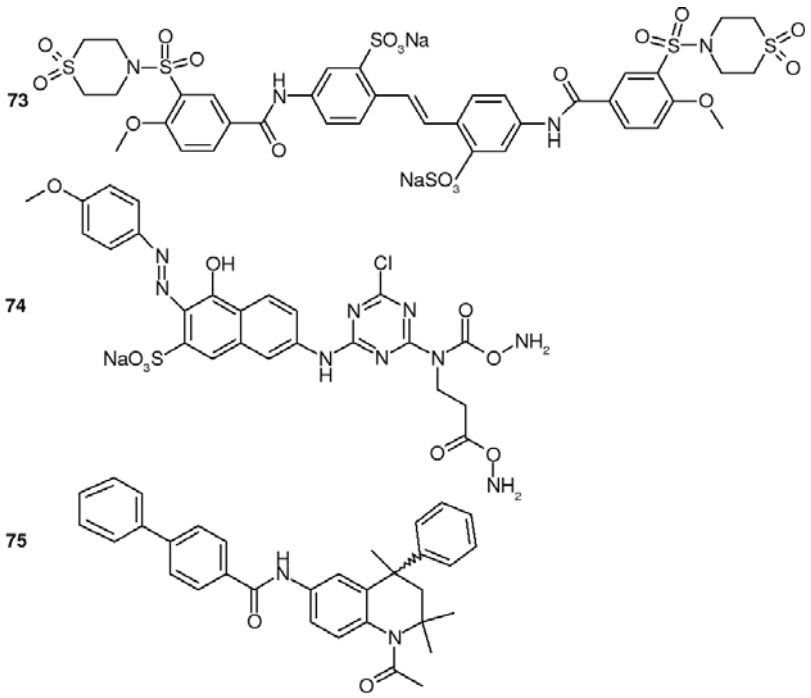
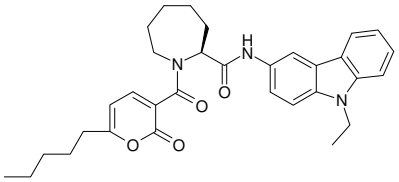
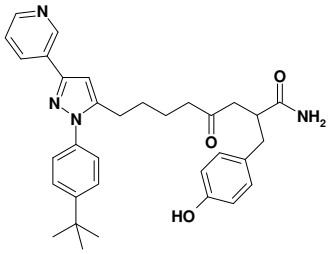
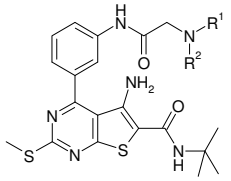
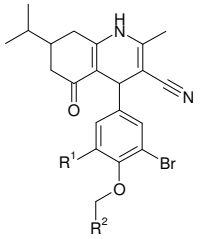
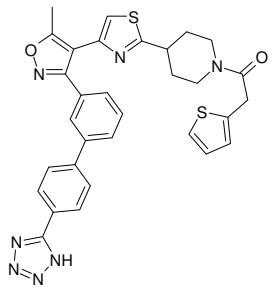


Figure 2.5 Chemical structures of the most potent analogues of suramin (**73**)[217], diazonaphtylsulfonic acid (**74**)[218] and c) tetrahydroquinoline (**75**)[208] derivatives as antagonists at the human FSH receptor.

Table 2.18 Potencies of patented agonists at the human FSH receptor.

Class	Company	Structure	Potency	Ref
Carbazoles	ARS Holding		EC_{50} = 3 nM	209
Pyrazoles	Serono RBI		EC_{50} = 62 nM	210
Thieno[2,3-d]-pyrimidines	N.V. Organon		EC_{50} < 10 µM	211,219
Hexahydro-quinolines	N.V. Organon		EC_{50} < 10 nM	212-214
Isoxazolyl-thiazoles	Arena		EC_{50} = 1,100 nM	215

(glycoprotein hormone) receptors. Therefore, Wrobel and coworkers developed suramin derivatives as selective FSH receptor antagonists.[217] Compound **73** (Figure 2.5) was shown to inhibit the binding of [^{125}I]FSH (IC_{50} = 2.0 ± 0.21 µM) and antagonized the production of

cAMP (EC_{50} = 1.5 ± 0.1 μM). It should be noted, however, that suramin has also been shown to inhibit agonist receptor binding by disturbing the receptor's interaction with the G protein.[220,221] A large compound library was screened for displacement of [^{125}I]FSH from the FSH receptor expressed in 3D2 cell membranes, which resulted in compound **74** (IC_{50} = 10 ± 2.8 nM; Figure 2.5), a diazonaphthylsulfonic acid derivative.[218] Scatchard analysis of the binding of hFSH in the presence of **74** showed that inhibition of binding was non-competitive in nature. In addition, this compound was selective for the FSH receptor, as no effect was detected on other glycoprotein hormone receptors and other unrelated GPCRs.

Organon Biosciences (currently Schering-Plough) identified another class of non-peptidic FSH receptor antagonists.[208] The lead tetrahydroquinoline compound (**72**) was identified as a selective agonist. Introduction of a 6-phenyl substituent via an amide linkage yielded antagonistic compounds. Especially, compounds with phenyl substituents with electron-donating groups showed high potencies. Compound **75** (Figure 2.5) was the most potent (IC_{50} = 5 nM) and contained a biphenyl substituent, indicating the presence of a large lipophilic pocket in the receptor.[208] None of the reported compounds was able to displace ^{125}I-FSH. The authors therefore postulated that the lipophilic non-peptidic antagonists bind in the seven transmembrane (7-TM) domain of the FSH receptor.

2.4.6 Patented Antagonists

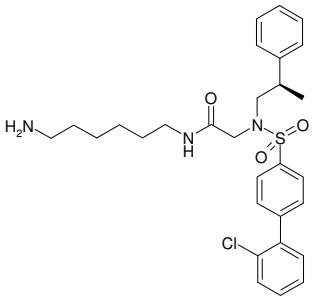
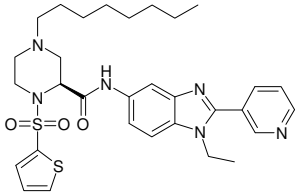
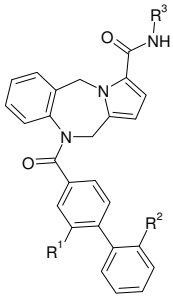
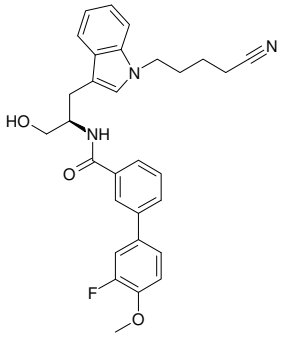
Four additional compounds classes have been reported in patent literature as non-peptidic human FSH receptor antagonists (Table 2.19). Ortho-McNeill and ARS Holding have described aminoalkylamides[222] and piperazines[223] as antagonists, respectively. Pyrrolo[2,1-*c*]benzodiazepine[224-227] and indole derivatives[228] have been patented by Wyeth en Schering. Notably, all compounds classes, except for piperazines, contain the biphenyl moiety that has been described as a scaffold for FSH receptor agonists.

2.4.7 Overview FSH Receptor Ligands

In comparison with the LH receptor, more compound classes, including agonists and antagonists, have been described. However, the potency of these ligands needs to be improved. Further insight into the high affinity ligand binding criteria may be obtained from a molecular pharmacophore study. It appeared from the tetrahydroquinoline derivatives that

there is a delicate balance whether a compound behaves like an agonist or antagonist. This should be further investigated using the tetrahydroquinoline-based ligands as a starting point. In addition, data concerning oral bioavailability of these non-peptidic ligands is lacking.

Table 2.19 Potencies of patented antagonists at the human FSH receptor.

Class	Company	Structure	Potency	Ref
Aminoalkyl-amide	Ortho-McNeill		IC_{50} = 50 nM	222
Piperazines	ARS Holding		IC_{50} = 13 nM	223
Pyrrolo[2,1-c]-benzodiazepine	Wyeth		IC_{50} = 41-6,820 nM	224-227
Indole	Schering		IC_{50} = 100 nM	228

2.5 GPR54 RECEPTOR LIGANDS

In 1999 the cDNA of a novel GPCR was isolated and named GPR54.[87] Only very recently, the endogenous ligand for GPR54 was discovered, metastin.[90] Ohtaki *et al.* showed that the biological activity of metastin was retained when all but ten residues (45-54) were removed.[229] In the past two years, this peptide ligand, also referred to as KP-10 or KiSS-1, has been investigated. Niida and coworkers performed both an alanine and D-amino acid scan of KP-10 to identify important residues for GPR54 agonistic activity.[230] It was shown that five amino acids (50-54) were important for receptor binding and activation. Incorporation of a basic group, e.g. guanidine, and substitution of Phe with Trp resulted in the first significantly downsized peptide GPR54 agonist with similar potency as KP-10 (EC_{50} = 1.4 nM). A 2-fold improvement in potency was obtained when Phe-50 was replaced with the more lipophilic 3-(2-naphthyl)alanine.[231] A similar approach was used by Orsini *et al.*, which led to the development of three metastin pharmacophore models.[232] In short, the models consisted of four-point queries; two phenyl/hydrophobic and one isopropyl feature in combination with either a positively charged or amide contribution. Virtual screening of the in-house library led to the identification of the first non-peptidic (partial) agonist, **76** (Figure 2.6) with a K_i value of 708 nM.

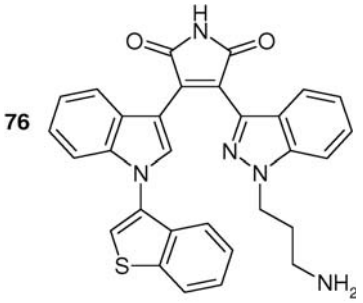


Figure 2.6 Chemical structure of the most potent non-peptidic GPR54 agonist (**76**).[232]

2.6 PERSPECTIVES

For the GnRH receptor large progress has been made over the past decade in the identification of potent non-peptidic antagonists. Different scaffolds yield compounds with high affinity, functionality and oral bioavailability. In several cases, it has been proven possible to activate the LH and FSH receptor (and antagonize the latter) via an allosteric site with non-peptidic ligands. However, only little SAR-knowledge has been obtained for these receptors, in comparison to LMW ligands for the GnRH receptor. In addition, the first non-peptidic agonist for GPR54 was identified very recently. Possibly with the help of this pharmacophore, novel agonists and antagonists will be reported in the near future.

With several series of ligands available, the different receptors of the HPG-axis can be studied more extensively. Although species (rat/mouse vs. human) and target selectivity, metabolic stability (cytochrome P450) and bioavailability data is often lacking, non-peptidic ligands offer the promise of oral bioavailability. These compounds may therefore emerge as useful drugs for these receptors, mainly for the treatment of infertility and for contraception.