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Identification of neutral 4-*O*-alkyl quinolone nonpeptide GnRH receptor antagonists

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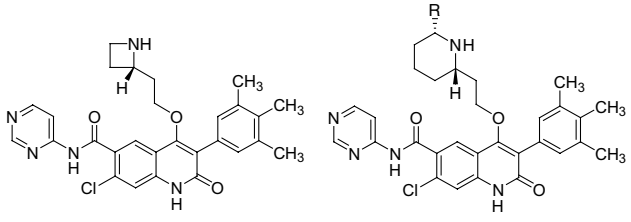
Abstract—A series of neutral, nonbasic quinolone GnRH antagonists were prepared via Mitsunobu alkylation of protected and unprotected 4-hydroxy quinolone intermediates. The synthetic route was improved by utilization of unique reactivity and convergency afforded by the use of mono and bis-trimethylsilylethyl protected quinolones. Potent neutral GnRH antagonists were identified, including ether and lactam derivatives, that show similar in vitro binding affinity and functional activity as compared to the earlier basic 4-aminoalkyl quinolone series of nonpeptide GnRH antagonists.

1. Introduction

Gonadotropin releasing hormone (GnRH) is a decapeptide released by the hypothalamus, which binds to GnRH receptors on pituitary.[1,2] The activation of this G-protein coupled receptor causes the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which regulate downstream gonadal steroid hormone production.[3] A variety of hormone dependent disease conditions such as prostate cancer may be treated clinically with peptidyl GnRH agonists, which act by over stimulation, then desensitization of this pathway, resulting in suppression of the hypothalamic-pituitary-gonadal axis.[4] Peptidyl antagonists, which act by directly blocking the effects of GnRH, are currently being explored in the clinic.[5] Over the last decade, there have been many reports of nonpeptidyl small molecule GnRH antagonists, which may offer some advantages such as oral dosing.[6]

A series of recent reports from this laboratory described the syntheses and structure–activity relationships (SAR) of 3-aryl quinolones, such as compound **1**, as nonpeptide GnRH antagonists.[7] A cyclic *basic* amine moiety, for example, an azetidine at the 4-position of the quinolone, was found to contribute significantly to GnRH receptor binding affinity, functional activity, and in vivo efficacy in primates.[7c] Other cyclic amines exhibited equivalent binding affinity, most notably the 2-piperidinyl side chain at the 4-position (i.e., compound **2**) for much of the reported SAR.



1 **2, R = H** **3, R = CF_3**

Compound	h GnRH IC_{50} (nM)	h PI Turn IC_{50} (nM)
1	0.44	0.9
2	0.3	2.2
3	2.0	18

We were extremely surprised to find that the 6-trifluoromethylated piperidine analog **3**, did not suffer from a large decrease in binding affinity in spite of the 3-orders

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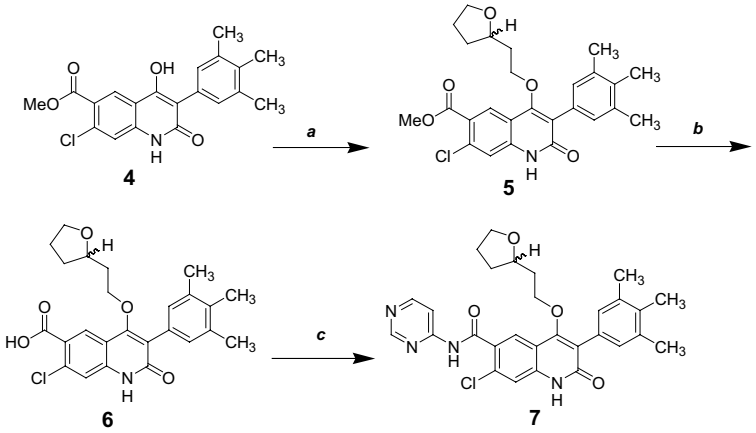
of magnitude difference in pK_a as compared to compound **2**.[7f] We sought to extend the SAR by examining a variety of analogs containing neutral (nonbasic) side chains at the 4-position. In this letter,[8] we disclose SAR studies for neutral substituents at the quinolone 4-position leading to the identification of nonbasic GnRH antagonists possessing high affinity and functional antagonist activity for cloned human receptors. In addition, new synthetic routes were devised, which afforded intermediates that allowed late stage substitution at the 4-position of the quinolone for rapid analog synthesis.

2. Chemistry

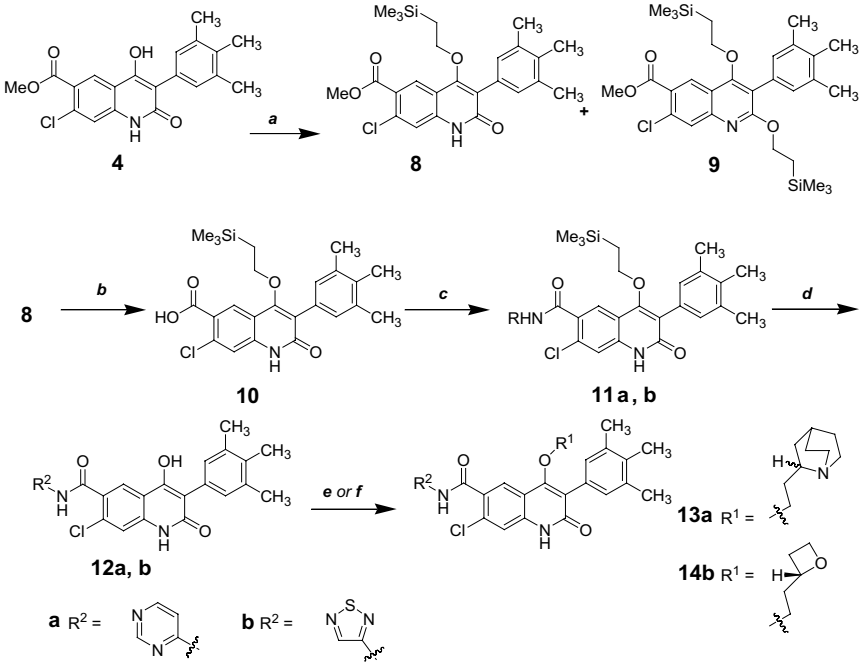
The general syntheses for quinolone compounds had been well established by the previous work.[7] Our initial efforts in synthesizing 4-substituted analogs utilized the published route (Scheme 1).[9] Thus, Mitsunobu coupling of known quinolone **4**[7c] with tetrahydrofuran-2-ylethanol cleanly provided 4-alkoxyquinolone **5**. Hydrolysis of the ester at the 6-position under basic conditions gave the carboxylic acid **6**. Standard amide bond formation with 4-aminopyrimidine gave the desired compound **7**.

This earlier route did not provide a convergent approach to the analogs of ethers at the quinolone 4-position of interest to us. Therefore, we desired a synthesis in which the 4-substituent could be introduced at a later stage with incorporation of an optimized 6-substituent at an earlier stage. Thus, the quinolone **4** was protected by Mitsunobu coupling with 2-trimethylsilylethanol, resulting in a mixture of the 4-*mono*- and 2,4-bis-protected quinolones **8** and **9**, respectively (Scheme 2). The mono-protected quinolone **8** was then treated with lithium hydroxide to afford the carboxylic acid **10**. Coupling of the carboxylic acid with 4-aminopyrimidine or 3-amino-1,2,5-thiadiazole gave the amides **11a**,**b**, which were efficiently deprotected using tetrabutylammonium fluoride to give the 6-functionalized 4-hydroxy quinolones **12a**,**b**. While the quinolone **12a** could be alkylated successfully under basic conditions to give the quinuclidine analog **13a**, Mitsunobu couplings with a variety of substituted ethanols were unsuccessful with this substrate. However, when the pyrimidine moiety was replaced with a thiadiazole as in **12b**, Mitsunobu coupling proceeded, albeit in moderate yield to give the oxetane analog **14b**. In the past, it had been observed that the yields of Mitsunobu couplings with quinolones such as compound **4** were sensitive to solubility of the quinolone in tetrahydrofuran. The solubility of the thiadiazolyl compound **12b** in tetrahydrofuran was greater than that of the pyrimidinyl **12a**, thereby enhancing its reactivity in the Mitsunobu reaction. While these useful intermediates did allow quick access to two analogs, they did not provide a general solution for preparation of 4-substituted quinolones.

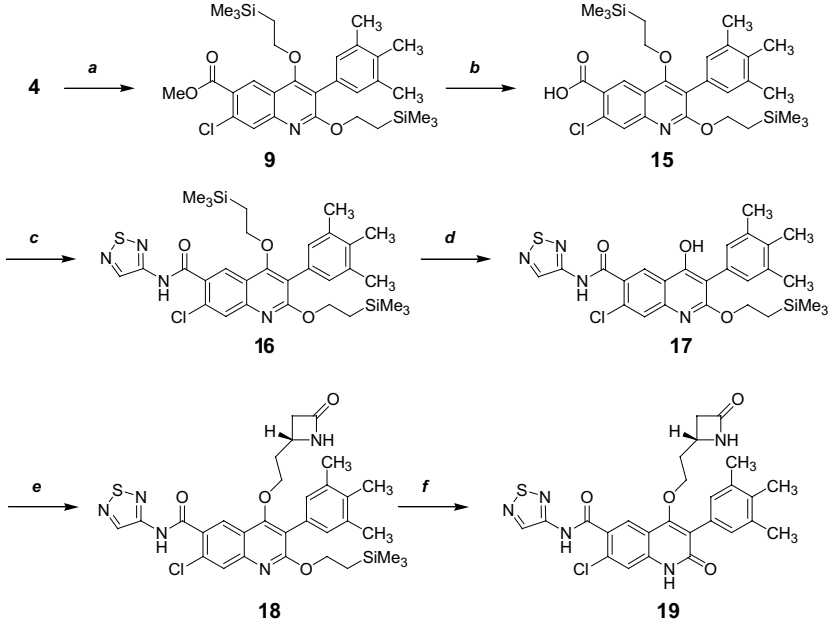
We then sought an intermediate that would allow late-stage introduction of 4-position substituents with improved solubility over compounds **12a**,**b**. To that end, the Mitsunobu coupling of the quinolone **4** with 2-trimethylsilylethanol was re-examined (Scheme 3) and it was discovered that use of excess reagents in the protection provided exclusive formation of the 2,4-bis-protected quinolone **9** in nearly quantitative yield. In addition, the nonpolar nature of this intermediate allowed for easy purification from the reaction by-products, thereby removing a difficulty of the earlier routes. Subsequent ester hydrolysis to carboxylic acid **15**, followed by carboxamide formation provided the intermediate **16** in a facile manner. We found our original conditions, tetrabutylammonium fluoride in tetrahydrofuran, afforded completely selective deprotection of the 4-trimethylsilylethoxy group of bis-protected quinolone **16**. The resulting intermediate **17** was versatile in Mitsunobu couplings, exemplified by reaction with 2-(azetidinon-4-yl)ethanol to give the azetidinone ether **18**. The subsequent deprotection of the 2-(2-trimethylsilylethoxy)quinolone was effected with trifluoroacetic acid completing a novel and efficient route to 4-substituted quinolones such as lactam analog **19**. The 2-trimethylsilylethyl protected quinolone **17** provided a general improvement in the Mitsunobu reaction as compared to quinolones **4** and **12a**,**b** presumably due to its improved solubility in tetrahydrofuran and facile purification of subsequent reaction products.



Scheme 1. (a) 2-Tetrahydrofuran-2-ylethanol, PPh_3, DEAD, THF, rt, 80%; (b) LiOH, aq EtOH, rt, 90%; (c) 4-aminopyrimidine, EDAC, HOBt, CH_2Cl_2, rt, 95%.



Scheme 2. (a) 2-TMS–EtOH (1.1 equiv), PPh_3, DEAD, THF, **8**, 35%, **9**, 20%; (b) LiOH, EtOH (aq), 85%; (c) 4-aminopyrimidine (for **a**) or 3-amino(1,2,5)thiadiazole·HCl (for **b**), DiPEA, EDAC, HOBt, CH_2Cl_2, 90%; (d) TBAF, THF, 90%; (e) for **13a**, 2-(2-chloroethyl)quinuclidine, $KHCO_3$, DMF, 55%; (f) for **14b**, 2-(2-hydroxyethyl)oxetane, PPh_3, DEAD, THF, 75%.



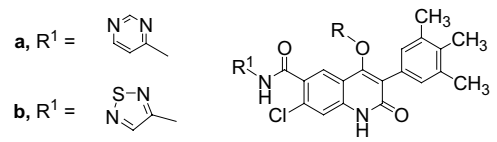
Scheme 3. (a) 2-TMS–EtOH (3 equiv), PPh_3, DEAD, THF, 99%; (b) $LiOH·H_2O$, EtOH (aq), 90%; (c) 3-amino(1,2,5)-thiadiazole·HCl, DiPEA, EDAC, HOBt, CH_2Cl_2, 85%; (d) TBAF, THF, 90%; (e) 4-(2-hydroxyethyl)-2-azetidinone, PPh_3, DEAD, THF, 95%; (f) TFA, CH_2Cl_2, 90%.

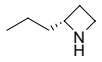
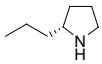
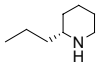
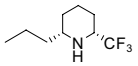
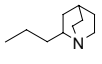
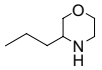
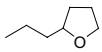
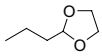
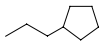
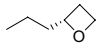
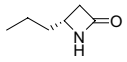
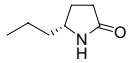
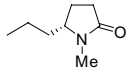
3. Biological results and discussion

Compounds were evaluated for their ability to compete with GnRH receptor agonist [^{125}I]buserelin for binding to the human GnRH receptor in the presence of 0.1% BSA. Functional antagonism in vitro was also determined via inhibition of GnRH-stimulated phosphatidyl inositol (PI) hydrolysis by cloned Chinese hamster ovary (CHO) cells stably expressing the human GnRH receptor.[7c,d]

A number of 4-substituted quinolones were examined, as shown in Table 1. The azetidine, pyrrolidine, and piperidine analogs, entries 1–3, show the close range of binding affinity and functional activity with respect to amine ring size and carboxamide heterocycle in this

Table 1. Inhibition of human GnRH receptor binding and PI turnover[a]



Entry	R^1	R	hGnRH IC_{50} (nM)	PI turn IC_{50} (nM)
1[b]	**b**		0.32	0.8
2[b]	**a**		0.8	2.2
3[b]	**b**		0.55	1.6
4[b]	**a**		2	18
5[c]	**b**		0.6	5.8
6[d]	**b**		0.7	32
7[b]	**a**		0.6	3.3
8[c]	**b**		0.6	67
9[b]	**b**		243	1150
10[c]	**b**		0.9	49
11[d]	**b**		0.1	1.3
12[d]	**b**		0.3	0.7
13[d]	**b**		1.4	11

[a] In vitro data represent the average of at least two experiments.
[b] Synthesis of compounds: employed original route (Scheme 1).
[c] Synthesis of compounds: utilized route Scheme 2 synthesis.
[d] Synthesis of compounds: utilized route Scheme 3.

series GnRH antagonists. Reduction of the amine basicity by addition of the CF_3-group (entry 4), formation of a tertiary amine such as quinuclidine (entry 5), or introduction of a heteroatom for the morpholine analog (entry 6) resulted in similar binding affinity (hGnRH binding) but diminished functional activity in the PI turnover assay. All these analogs necessarily upheld the previous SAR requiring a basic site, though modified, at the 4-position of the quinolone. However, the tetrahydrofuran analog (entry 7) was essentially equipotent in binding and function as compared to the pyrrolidine analog, the first indication that a nonbasic heterocycle at the quinolone 4-position can also serve as an effective GnRH antagonist.

We continued to explore nonbasic heterocycles at the 4-position. When another heteroatom is introduced as in the acetal analog (entry 8), binding affinity is maintained but a 30-fold loss in functional activity is observed. A similar effect is observed for the morpholine analog indicating diminished functional activity when more than one heteroatom is incorporated in the heterocyclic ring. When all heteroatoms are removed, as in cyclopentyl analog (entry 9), a dramatic loss in binding and function is observed, with a 300- and 500-fold loss in activity, respectively, as compared to the pyrrolidine analog. This further refined the requirement of a polar group such as a heteroatom, resulting in the creation of a Bronsted or Lewis basic site, to achieve high binding affinity in this series.

Decreasing the size of the tetrahydrofuran ring to the oxetane analog (entry 10) resulted in a 15-fold loss in functional potency while maintaining nearly equal binding affinity as compared to the tetrahydrofuran analog. When the cyclic ether is replaced with a cyclic amide, as in β-lactam (entry 11) or pyrrolidinone (entry 12), binding affinity is improved and functional activity is restored matching that of the fully basic azetidine. Methylation of the lactam results in 10-fold decrease in functional activity indicating the importance of the lactam N–H to provide functional antagonist activity for nonbasic heterocycles.

While it had been previously established that a cyclic basic amine was important for activity on the quinolone 4-position side chain, we have found nonbasic heterocycles, such as cyclic ethers and cyclic lactams, are also effective pharmacophores for binding and functional antagonist activity on the human GnRH receptor. The original synthesis of quinolones was modified to provide a general and facile late stage synthesis of 4-substituted analogs. Useful intermediates for Mitsunobu alkylation of the 4-hydroxyl group were prepared by bis-protection at the 2- and 4-positions, installation of the 6-carboxamide and subsequent selective deprotection at the 4-position. The efficiency of Mitsunobu alkylation of the 4-hydroxy quinolone was generally improved with 2-trimethylsilylethoxy intermediates due to their increased solubility in tetrahydrofuran and the ease of purification of resulting ether reaction products.

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9. All compounds characterized by LC–MS and/or NMR.