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IDENTIFICATION AND INITIAL STRUCTURE-ACTIVITY RELATIONSHIPS OF A NOVEL NON-PEPTIDE QUINOLONE GnRH RECEPTOR ANTAGONIST

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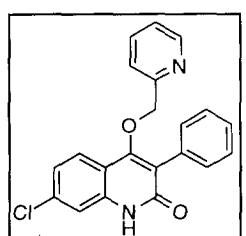
Abstract: Screening of the Merck sample collection for non-peptide compounds with binding affinity for the rat GnRH receptor led to the identification of the substituted quinolone (**1**) as a lead compound in the search for a non-peptide GnRH receptor antagonist. Substantial improvements in potency (~300 fold) were achieved by addition of an alkyl amine at the 4-position, a 3,5-dimethylphenyl group at the 3-position and 6-nitro-7-chloro-substitution of the 1H-quinolone core.

Introduction: Gonadotropin releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide released by the hypothalamus and identified in the early 1970's by Schally.[1] GnRH binds to gonadotropes in the pituitary activating the GnRH receptor, a G-protein coupled receptor,[2] causing the release of signaling hormones (FSH, LH) which act on the gonads to induce sex hormone production. A variety of disease conditions such as endometriosis and prostate cancer can be treated by suppression of the pituitary-gonad hormonal axis. Super agonists are used to over-stimulate the pituitary GnRH receptor which leads to down regulation of that receptor and shut down of the hormonal pathway.[3,4] The initial over production of sex hormones leads to an exacerbation of symptoms known as the "flare-effect." There is recent clinical evidence that peptidic GnRH antagonists directly lower sex hormone levels alleviating disease symptoms without the concomitant flare effect.[5] Peptidyl agonists and antagonists of the GnRH receptor have been used in the clinic for the treatment of sex hormone related conditions.[6] A non-peptidyl GnRH receptor antagonist has been recently reported.[7] In this letter, we decribe the identification and initial structure-activity relationships of a novel lead structure for a non-peptidyl GnRH receptor antagonist.

Lead Identification

The search for a non-peptidyl GnRH receptor antagonist began with the development of an assay to identify compounds which bound competitively to GnRH receptors (from rat pituitary membranes) using a radio-labeled GnRH-agonist (^{125}-I-buserelin).[8] Screening of the Merck sample collection led to the identification of the 4-(3-pyridylmethyl)oxyquinolone **1**, prepared for an earlier project,[9] as an active (IC_{50} = 10μM). After verifying the activity of the structure by re-synthesis and assay, we began a medicinal chemistry program to optimize the structure-activity relationships of this non-peptidyl lead compound.



1

IC_{50} = 10μM

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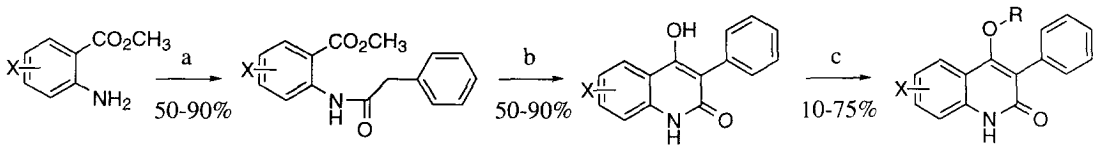
Chemistry

The 4-alkylated quinolones were prepared according to literature procedures[9] as outlined in Scheme 1 (range of yields shown). Anthranilic acid esters[10] **2** were treated with phenylacetyl chloride in refluxing 1,2-dichloroethane. After removal of the reaction solvent, the crude products were recrystallized from MeOH. The dried phenylacetamides **3** were treated with excess sodium bis(trimethylsilyl)amide (NaHMDS) in THF at 0°C then quenched with aq. 6N HCl. The crude products were filtered, washed with water, then ice cold acetonitrile to provide the 4-hydroxyquinolone derivatives **4** in excellent yields. After drying the products at 50°C in a vacuum oven overnight the 4-hydroxyl group was alkylated with a variety of alkyl halides. Aminoalkylchloride hydrochloride salts in particular were useful electrophiles and were generally commercially available or prepared from the corresponding aminoalcohols.[11,12] The final 4-O-alkylated quinolone products **5** were isolated either by quenching the reaction with water, filtering the resulting solids or extractive workup followed by purification by recrystallization or silica gel chromatography, respectively.[13] Similarly, the 3-(3,5-dimethylphenyl)quinolones were prepared using the acid chloride of 3,5-dimethylphenylacetic acid.

Scheme 1

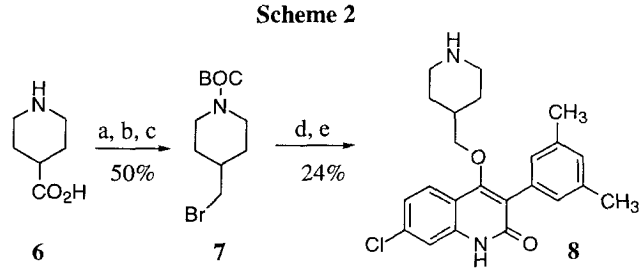


2 **3** **4** **5**

Reagents and conditions: a) phenylacetyl chloride, 1,2-dichloroethane, reflux, 3 hr; b) 3 equiv NaHMDS, THF, 0°C, 3hr; excess HCl quench, filter; c) 2.2 equiv K_2CO_3, R-X; cat NaI; DMF, 80°C, 16hr.

The 4-piperidinemethanol analog was prepared as outlined in Scheme 2 from 4-piperidinecarboxylic acid **6**. The piperidine nitrogen was protected with a BOC-group followed by reduction of the acid to the alcohol *via* the mixed anhydride.[14] Conversion of the alcohol to the bromide **7** went smoothly with carbon tetrabromide/triphenylphosphine. O-Alkylation of the quinolone with bromide **7** followed by standard TFA deprotection gave the 4-piperidine methyl analog **8**.

Scheme 2



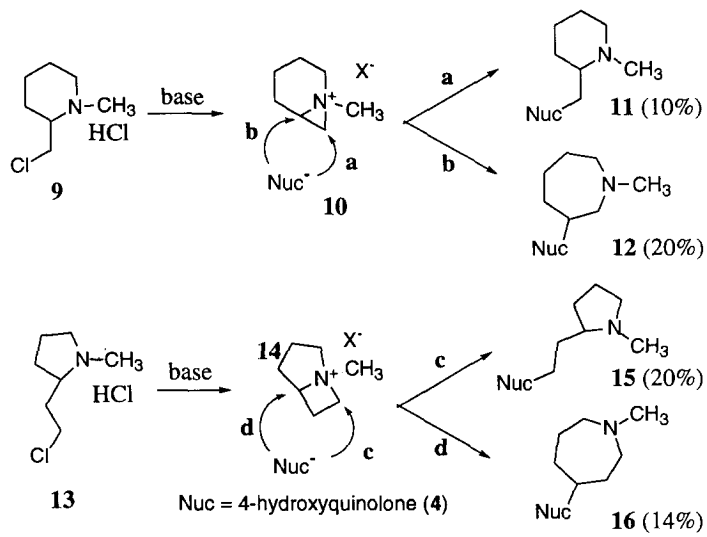
6 **7** **8**

Reagents and conditions: a) BOC_2O, aq NaOH, DME; b) isobutyl chloroformate. N-methylmorpholine, DME, 0°C; filter, aq $NaBH_4$; c) CBr_4, PPh_3, CH_2Cl_2; d) K_2CO_3, cat NaI, quinolone **4**, DMF, 80°C; e) TFA, anisole, CH_2Cl_2.

Two aminoalkyl chlorides most likely reacted via their quaternary ammonium salts as outlined in Scheme 3.[15] For example, reaction of N-methyl-2-chloromethylpiperidine hydrochloride (**9**) with the

4-hydroxyquinolone derivative **4** gave two major isolable products. Internal quaternization goes presumably to aziridinium salt **10**, which can be alkylated productively via two pathways (or as a methylating agent; not determined). Alkylation of the 4-quinolone alkoxide along path **a** affords the desired 2-methylpiperidine derivative **11**, while ring opening via path **b** gives the 3-substituted seven-membered amine product **12**. Both products were isolated in moderate yields. Similarly, 2-(2-chloroethyl)-1-methylpyrrolidine **13**, upon reaction with base, forms the quaternary bicyclo-[3.2.0]-ammonium salt **14** which gives quinolone product **15** via path **c** and product **16** via path **d**, respectively.

Scheme 3



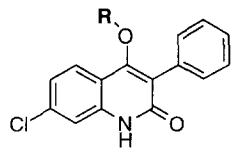
Discussion

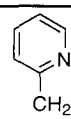
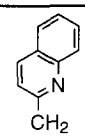
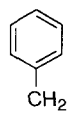
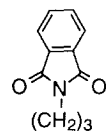
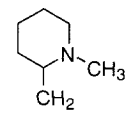
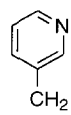
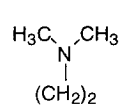
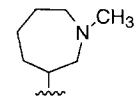
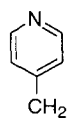
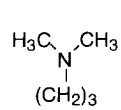
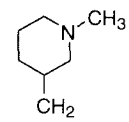
The compounds prepared were tested in the rat GnRH receptor binding assay for the ability to displace [^{125}-I]-radiolabeled buserelin.[8] All compounds with binding affinity better than 10 μM were titrated on a 4-point curve and their data are reported as IC_{50} values. The rat GnRH receptor binding data for initial analogs prepared around the original screening lead are reported in Table 1. The O-benzyl analog **17**, the 3-pyridyl analog **18** and the 4-pyridyl analog **19** are all less active in the GnRH binding assay at a concentration of 10μM indicating the importance of the nitrogen atom as well as its ring position in the screening hit **1**. Similarly, the larger 2-quinolylmethyl compound **20** and the non-basic phthalimide derivative **21** were less active. The dimethylaminoethyl analog **22** also had poor binding affinity, however, higher homologue **23** (9μM) was equipotent with the lead. Removal of the methyl groups reduced potency as demonstrated by compound **24**. The piperidine derivative **11a** (9μM) and the seven membered amine derivative **12** (7μM) showed slightly improved binding affinity to the lead compound. The 3-piperidinylmethyl derivative **25** (4μM) was over two fold more potent than the lead indicating that an alicyclic amine at an appropriate ring position can give compounds with greater binding potency at the rat GnRH receptor.

A variety of cyclic amines were evaluated in a new more potent series in which the 3,5-dimethylphenyl group has been incorporated at the 3-position of the the quinolone[16] (Table 2). The 3-piperidinylmethyl analog **26** is over 10-fold more active as a result of incorporation of the 3,5-dimethyl groups when compared to compound **25**. Removal of the N-methyl group affords a slightly less potent analog **27** while again ring position

is important as demonstrated by the large decrease in potency observed for the 2-piperidinemethyl analog **11b** and 4-piperidinemethyl analog **8**. Removal of the methylene spacer as in the 4-piperidine analog **28**, places the amine 3-carbon atoms from the quinolone core as with compound **26**, and improves affinity relative to compound **8**.

Table 1[a]



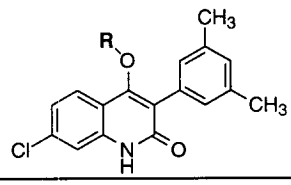
Analog	R	IC_{50}	Analog	R	IC_{50}	Analog	R	IC_{50}
1		10	**20**		NA	**24**		NA
17		NA	**21**		NA	**11a**		9
18		NA	**22**		NA	**12**		7
19		NA	**23**		9	**25**		4

[a]Rat GnRH receptor binding assay. All data reported in μM. NA: <50% inhibition @10μM

Other analogs which maintained the 3-carbon linker between the basic amine and the 4-quinolone oxygen also provide good GnRH binding affinity as demonstrated by compounds **29**, **30** (5-membered more potent than 7-membered) and **16** (7-membered more potent than 6-membered **28**). The 1-propylpiperidinyl analog **31** was much less potent showing that the incorporation of the ring in the tether is beneficial for binding activity. The 2-pyrrolidinylethyl analog **15** (n = 0) and ring homolog **32** (n = 1) were of similar potency to the 3-piperidylmethyl analog **26**, while incorporation of a ring oxygen as in morpholine **33** or ring cleavage to acyclic analog **34** greatly decrease GnRH binding activity.

The activity of analogs which evaluate substitution of the quinolone ring are reported in Table 3 with the **a**-series containing the 3-piperidine and the **b**-series containing the 2-piperidine at the 4-position. The position of the 7-chlorine atom is extremely important with the 5, 6 and 8-chloro analogs (**35**, **36**, **37**) all being inactive. Other substituents at the 6-position (nitro-**38**; methoxy-**39**; methyl-**40**) as well as 7-position (carbomethoxy-**41**) are all inactive. The 7-methyl analog **42** and the 7-nitro analog **43** were 20- and 7-fold less active, respectively, (compared to compound **26**) while 7-fluoro analog **44** was surprisingly not active. Compounds **45** (7-Br) and **46** (7-CF_3) were of similar potency to the 7-chloro derivative **32** demonstrating that some combination of size, electronic properties and lipophilicity are important for activity at the 7-position of the quinolone.

Table 2[a]



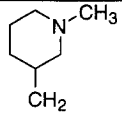
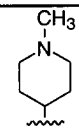
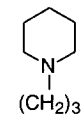
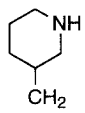
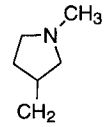
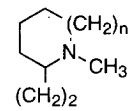
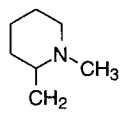
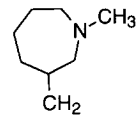
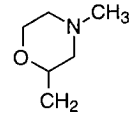
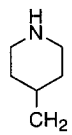
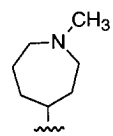
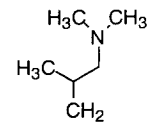
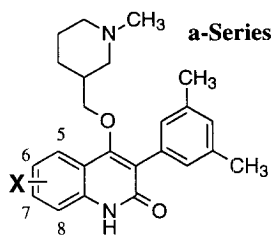
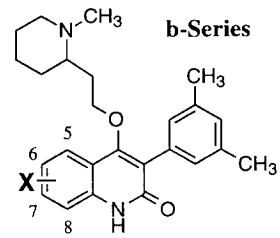
Analog	R	IC_{50}	Analog	R	IC_{50}	Analog	R	IC_{50}
26		0.3	**28**		1.3	**31**		7.5
27		0.5	**29**		0.5	**15** (n = 0) **32** (n = 1)		0.2 0.2
11b		4	**30**		1.3	**33**		1.4
8		NA	**16**		0.3	**34**		6.3

Table 3[a]





Analog	X	IC_{50}	Analog	X	IC_{50}	Analog	X	IC_{50}
26-a	**7-Cl**	0.3	**39-a**	**6-CH_3O**	NA	**44-a**	**7-F**	NA
35-a	**5-Cl**	NA	**40-a**	**6-CH_3**	NA	**32-b**	**7-Cl**	0.2
36-a	**6-Cl**	NA	**41-a**	**7-CO_2Me**	NA	**45-b**	**7-Br**	0.15
37-a	**8-Cl**	NA	**42-a**	**7-CH_3**	3.9	**46-b**	**7-CF_3**	0.1
38-a	**6-NO_2**	NA	**43-a**	**7-NO_2**	1.4	**47-b**	**6-NO_2, 7-Cl**	0.032

[a]Rat GnRH receptor binding assay. All data reported in μM. NA: <50% inhibition @10μM

Incorporation of both the 6-nitro group and the 7-chlorine atom (compound **47**) gave a 6-fold increase in potency over **32** (over 300-fold improvement compared to lead **1**). The role of the nitro group in **47** could be

to modulate the electron density of the quinolone ring or perhaps create a new binding contact with the receptor.[16] Surprisingly, the 6-nitro analog **38** is inactive demonstrating the importance of the 7-chlorine atom as well as the synergistic increase in potency by combining the two substituents at the 6- and 7-positions of the quinolone ring system.

Conclusion

The GnRH rat receptor binding activity of the quinolone screening lead **1** was improved by incorporation of alkyl cyclic amines at the 4-position with analogs possessing 3-carbon atoms between the quinolone oxygen and the basic amine being most active. The 7-chlorine atom is extremely important for binding affinity while incorporation of both 6-nitro and 7-chloro groups affords a greater increase in potency as compared to each individual substitution. Compound **47**, which possesses the 4-(2-piperidinylethyl)group and 3-(3,5-dimethylphenyl) group on the optimized quinolone core is over 300-fold more potent in GnRH binding activity as compared to the inital screening lead at the rat receptor. Further structure activity relationships of this lead will be reported in the following paper[17] and in the future.[16]

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References and Notes

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