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INVESTIGATION OF THE 4-O-ALKYLAMINE SUBSTITUENT OF NON-PEPTIDE QUINOLONE GnRH RECEPTOR ANTAGONISTS

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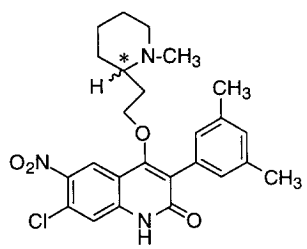
Abstract: Synthesis and *in vitro* activity of the enantiomers of quinolone GnRH antagonist (±)-**1** are reported. Chiral amino alcohols were prepared from the appropriate cyclic D- or L-amino acids by the Arndt-Eistert homologation followed by reduction of the resulting esters. Incorporation of these pharmacophores was achieved via a novel Mitsunobu alkylation of 4-hydroxyquinolones. The key amine pharmacophore for binding to the rat GnRH receptor was most active in the S-configuration. Ring size was not important for potency with 4, 5, 6, and 7-membered ring amines exhibiting similar potency.

Introduction:

In the previous letter,[1] we described the identification of a novel 1*H*-quinolone structure as a GnRH receptor antagonist and the intial structure activity relationships of this non-peptide lead class. Modifications of the lead established that key substitutions of the 6- and 7-positions of the quinolone core, 3,5-dimethylphenyl group at the 3-position and 2-ethylpiperidine at the 4-position resulted in a potent non-peptide antagonist of the rat GnRH receptor (compound **1**, IC_{50} = 32nM). In this letter, we describe the synthesis of both enantiomers of compound **1** and related analogs and their evaluation as antagonists of the rat GnRH receptor.



(±)-1

rat GnRH Binding IC_{50} = 32nM

Chemistry:

Enantiospecific synthesis of the desired protected 2-piperidinethanols was achieved using the Arndt-Eistert homologation[2] as outlined in Scheme 1. N-BOC Protected (L)-pipecolic acid (**2**) was converted to the diazoketone by formation of the mixed anhydride followed by addition of excess ethereal diazomethane. The resulting ketone was purified on silica gel and rearranged by treatment with Ag(I)benzoate and triethylamine in methanol to afford the N-BOC-piperidineacetic acid derivative **3**. Simple reduction with lithium aluminium hydride in ether at 0°C gave the desired (S)-alcohol **4**. Similarly, this route efficiently provided the R-aminoalcohol **5** from D-pipecolic acid, the (R)- and (S)-pyrrolidine derivatives **6** and **7** from D- and L-proline, respectively, and the (S)-azetidinethanol **8** from (S)-azetidinecarboxylic acid. The rearrangement of the azetidinyl diazoketone was performed at carefully controlled temperatures (-10° – 0°C) in order to prevent base catalyzed β-elimination of the N-BOC amine of the azetidineacetic ester, which would result in ring opening of the azetidine.

The racemic seven membered ring analog (2-azepinethanol) was prepared based on similar literature precedent[3] as outlined in Scheme 2 (eq. 1). Ethyl 2-cyclohexanoneacetate **9** was treated with hydrazoic acid to

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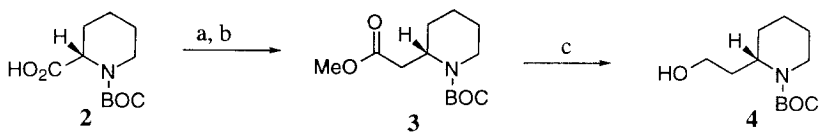
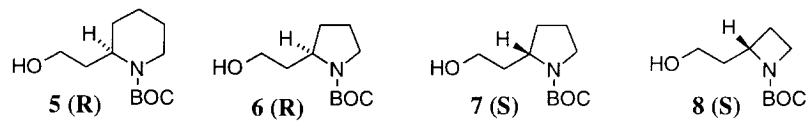
provide azepinone **10**. Complete reduction of the lactam and the ester with lithium aluminium hydride in refluxing tetrahydrofuran followed by N-protection of the crude aminoalcohol with di-*t*-butyl dicarbonate afforded the azepinethanol **11**.

Scheme 1



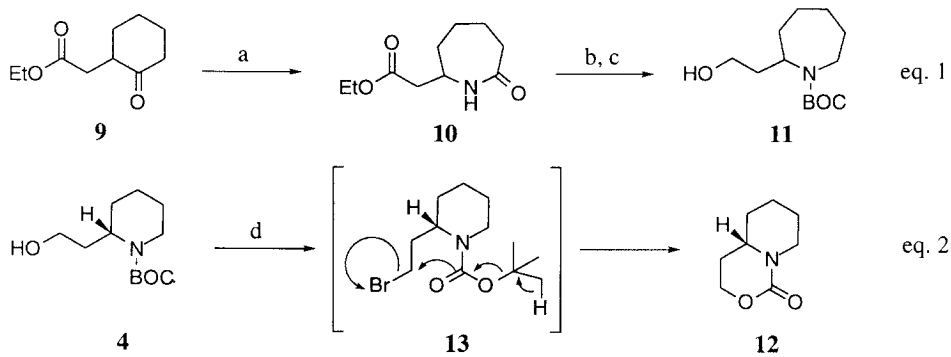
2 **3** **4**

Reagents and Conditions: a) isobutylchloroformate, TEA, THF/ether (1/1), 0°C, 1 hr; add excess ethereal diazomethane, 0°C, 3 hr; b) cat AgOBz, TEA, MeOH, 0°C–RT, 3 hr; c) 0.75 equiv $LiAlH_4$, ether, 0°C, 30 min.



5 (R) **6 (R)** **7 (S)** **8 (S)**

Scheme 2



9 **10** **11** **4** **13** **12**

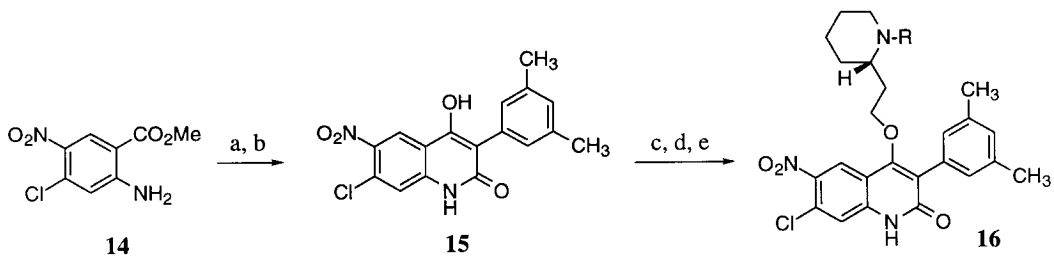
Reagents and Conditions: a) NaN_3, aq H_2SO_4; $CHCl_3$, 0°C–RT, 16 hr; b) 2.1 equiv $LiAlH_4$, THF, reflux, 24 hr; c) BOC_2O, CH_2Cl_2, 0°C–RT, 6 hr; d) CBr_4/Ph_3P, CH_2Cl_2, RT, 16 hr.

Previously, O-alkylated quinolones were prepared by reaction of the 4-hydroxy group of the quinolone with aminoalkyl halides under basic conditions;[1,4] consequently, conversion of alcohol **4** to the bromide was attempted (Scheme 2, eq. 2). Treatment of alcohol **4** with the reagent system of carbon tetrabromide/triphenyl phosphine (16 hrs) gave a new *more* polar compound which after purification was identified as the bicyclic carbamate **12**. More careful monitoring of the reaction found that a *less* polar compound was initially observed by thin layer chromatography after several hours, presumably the desired bromide **13**. This intermediate is unstable and undergoes conversion to the carbamate upon further stirring. Similarly, the corresponding methanesulfonate was prepared under basic conditions and isolated, but it also cyclizes to carbamate **12** on standing.

A new O-alkylation procedure was developed as outlined in Scheme 3. As previously described,[1,4] the 6-nitro-7-chloroanthranilic ester **14** was converted in two steps to the 4-hydroxyquinolone derivative **15** in excellent yield. The well known Mitsunobu reaction[5] was attempted on the novel quinolone substrate[6] since the acidic 4-hydroxyl group of the quinolone, a vinylogous carbamic acid, is within the appropriate pKa range for Mitsunobu reactants. Treatment of a well-stirred mixture of powdered, insoluble quinolone **15**, alcohol **4** and

triphenylphosphine in THF at room temperature with diethylazodicarboxylate dropwise led to slow dissolution of the quinolone. Stirring the yellow reaction mixture at RT overnight followed by removal of solvent and column chromatography afforded the desired O-alkylated quinolone derivative in high yield (60–80%). The synthesis was completed by standard TFA deprotection of the amine. N-Methylated amines were easily prepared by reductive amination with aq. formaldehyde and $NaBH_4$. Similarly, the Mitsunobu procedure routinely provided analogs of differing ring size from the previously described aminoalcohols **5**, **6**, **7**, **8**, **11** as well as N-BOC-2-piperidinepropanol and 2-pyridinethanol.[7]

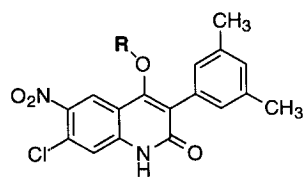
Scheme 3

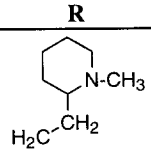
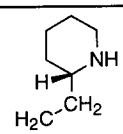
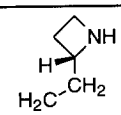
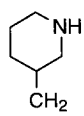
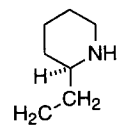
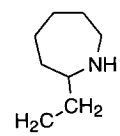
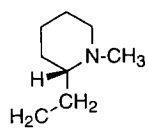
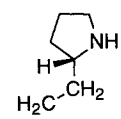
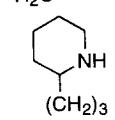
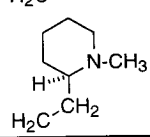
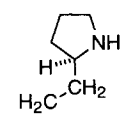
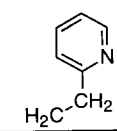


14 **15** **16**

Reagents and Conditions: a) 3,5-dimethylphenylacetyl chloride, 1,2-dichloroethane, reflux, 3 hr; b) 3 equiv NaHMDS, THF, 0°C, 3 hr; excess HCl quench, filter; c) Ph_3P, alcohol **4**, DEAD, THF (0.1M), RT, 16 hr; d) TFA, anisole, CH_2Cl_2, RT, 3 hr; e) for N-methyl analogs: aq formalin, $NaBH_4$, HOAc, MeOH.

Table 1[a]



Analog	R	IC_{50}	Analog	R	IC_{50}	Analog	R	IC_{50}
1		32	**20**		10	**24**		25
17		36	**21**		150	**25**		36
18		20	**22**		15	**26**		1420
19		85	**23**		100	**27**		NA

[a]Rat GnRH receptor binding assay.[8] All data reported in nM. NA: not active @10μM

Discussion

The compounds were tested in a rat pituitary membrane binding assay for their ability to displace [^{125}-I]-radiolabeled buserlin.[8] All compounds were titrated on a 4-point curve and activities reported as an IC_{50} value. (±)-2-Ethylpiperidine derivative **1** and (±)-3-piperidine analog **17** are included for comparison with the pure enantiomers. The majority of GnRH binding activity resides in the (S)-enantiomer as demonstrated by compound **18** which is approximately two–fold more potent than racemic **1** and four-fold more potent than R-isomer **19**. Similarly, (S)-secondary amines **20** and **22** are fifteen- and seven-fold more potent than the corresponding R-isomers (**21**, **23**) respectively. Ring size is not a critical determinant of binding potency with piperidine **20**, pyrrolidine **22** and azetidine **24** being essentially equipotent and the racemic azepine **25** of comparable activity to the earlier racemic compounds. Extension of the tether linking the amine to the quinolone is detrimental to binding activity as shown with the racemic 3-carbon linked piperidine **26** (forty-fold decrease). The pyridine derivative **27** is significantly less active demonstrating the importance of the basicity and chirality of the 4-substituent in the quinolone series of non-peptidyl GnRH receptor antagonists.

Conclusion

The synthesis of chiral 4-alkoxyamino quinolones was accomplished by a novel application of the Mitsunobu reaction to couple 4-hydroxyquinolones with N-BOC protected amino alcohols. Enantiospecific synthesis of the desired amino alcohols was achieved by Arndt-Eistert homolgation of the corresponding D- or L-amino acids followed by reduction. Assay of the enantiomerically pure analogs demonstrated that the S-amine enantiomers were the more active antagonists at the rat GnRH receptor. Ring size was not an important factor in potency as 4-, 5-, 6- and 7-membered amines were of similar potency but changes in tether length and ring saturation greatly affected GnRH binding activity. Progress in the development of the structure activity relationships of this novel non-peptidyl GnRH receptor antagonist lead will be reported in the future.

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References and Notes

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7. All products and intermediates were fully characterized by TLC, MS and ^{1}H NMR.
8. Crude membranes prepared from rat pituitary glands were the GnRH receptor source and [^{125}-I]buserelin (a peptidyl GnRH analog) was used as the radiolabeled ligand. The competitive binding was conducted in a Tris-HCl based buffer at 4°C for 90 min. The activities are presented as the IC_{50} for the inhibition of [^{125}-I]buserelin binding to the receptors. Functional antagonist activity of these and related compounds will be reported in the future.