Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 443–447

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Potent Antagonists of Gonadotropin Releasing Hormone Receptors Derived from Quinolone-6-Carboxamides

Thomas F. Walsh, [a,*] Richard B. Toupence, [a] Jonathan R. Young, [a] Song X. Huang, [a] Feroze Ujjainwalla, [a] Robert J. DeVita, [a] Mark T. Goulet, [a] Matthew J. Wyvratt, Jr., [a] Michael H. Fisher, [a] Jane-Ling Lo, [b] Ning Ren, [b] Joel B. Yudkovitz, [b] Yi Tien Yang, [b] Kang Cheng [b] and Roy G. Smith [b]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA*
[b]*Department of Biochemistry & Physiology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA*

Received 23 November 1999; accepted 22 December 1999

Abstract—SAR studies which focused upon the C-6 position of a recently described series of quinolone gonadotropin releasing hormone antagonists are reported. Synthetic access to diverse quinolone-6-carboxamides was achieved via the palladium-catalyzed amino-carbonylation reactions of iodide **4** with various amines. Amides related to **9y** were especially potent, functional antagonists of rat and human GnRH receptors.

Introduction

Peptidic antagonists of gonadotropin releasing hormone (GnRH) receptors are of current interest for their ability to block the release of the anterior pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which results in suppressing the biosynthesis of gonadal steroid hormones. The resulting gender-independent, reversible blockade of the pituitary–gonadal axis has been shown to reduce gonadal steroid hormone concentrations to castrate levels, which has utility in the management of some disease states exacerbated by the presence of those hormones.[1–3] The discovery of the first non-peptidic GnRH receptor antagonist was reported last year by workers from Takeda.[4] In previous communications from this laboratory, preliminary structure–activity relationships (SAR) for a new series of non-peptide GnRH antagonists based upon a quinolone screening lead were disclosed.[5,6] In this letter we present further SAR studies surrounding the quinolone 6-position substituent which have afforded highly potent, functional antagonists of rat and human GnRH receptors.

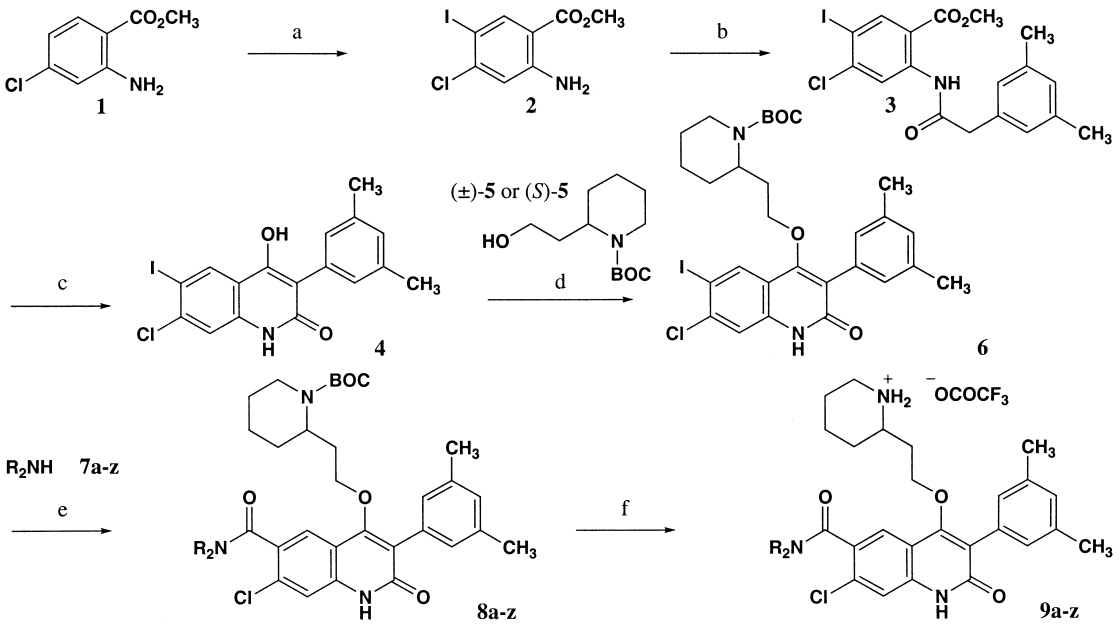
Chemistry

At the time we initiated this study, SAR studies for the quinolone series had established the importance of a substituted phenyl at the C-3 position, the 6-nitro and 7-chloro substituents, and a 4-*O*-alkyl ether derived from a piperidinyl substituted ethanol. Parallel efforts within our laboratory indicated that potency could be further enhanced through derivatization of the 6-position nitro group.[7] In particular, certain 6-ureido derivatives bearing nitrogen-containing heteroaromatic substituents had been found to have low nanomolar affinity in the rat GnRH receptor binding assay. We sought to further extend the SAR for this series by examining 6-amido substituted quinolones wherein the amide formally derives from a quinolone-6-carboxylic acid. To this end, it was recognized that palladium-catalyzed amino-carbonylation reactions[8] of 6-iodoquinolones with various amines would provide convenient access to diverse quinolone-6-carboxamides.

The synthesis (Scheme 1) begins with the silver(I)-mediated iodination [9] of methyl 4-chloroanthranilate (**1**) to afford its 5-iodo derivative **2**. *N*-Acylation of **2** with 3,5-dimethylphenylacetyl chloride affords amide **3** which cyclizes under basic conditions to afford the 4-hydroxyquinolone **4**. *O*-Alkylation of quinolone **4** with either (±)-**5** or (*S*)-**5** using our previously described Mitsunobu

*Corresponding author. Tel.: +1-732-594-5232; fax: +1-732-594-2210.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00024-X

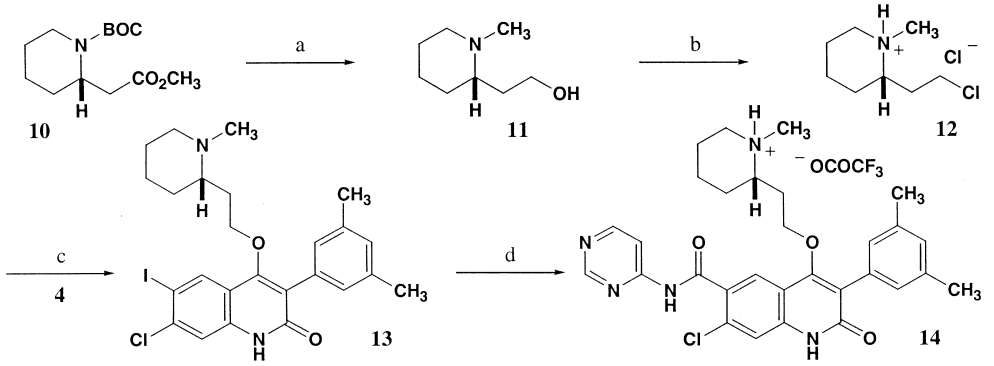


Scheme 1. Reagents and conditions: (a) I_2, Ag_2SO_4, EtOH, rt, 1 h, 100% crude yield; (b) 3,5-dimethylphenylacetyl chloride, 1,2-dichloroethane, 80 °C, 3 h, 85%; (c) $LiN(TMS)_2$, THF, 0 °C, 2 h, 88%; (d) PPh_3, DEAD, THF, 0 °C to rt, 4 d, 55%; (e) amines **7a–z**, $(PPh_3)_2PdCl_2$, CO (1 atm), Et_3N, DMF, 95 °C, 16 h, 50–70%; (f) CF_3CO_2H:CH_2Cl_2 (1:1), rt, 3 h, 100%.

protocol[5] affords the 4-*O*-alkyl ethers (±)-**6** and (*S*)-**6**. Palladium-catalyzed amino-carbonylation of iodo compound **6** with a variety of primary and secondary amines (**7a–z**) led to the protected quinolone-6-carboxamides **8a–z** in 55–70% yield.[10] Final deprotection of the BOC-protected piperidines **8a–z** using trifluoroacetic acid furnished the targeted amides **9a–z**.[11] Evaluation of the *N*-methylpiperidine derivative (**14**) of one of the more potent antagonists **9y** discovered in this series was also undertaken, since it was known from our previous studies that *N*-methylation of the heterocyclic substituent at C-4 also afforded potent compounds. Synthesis of **14** was accomplished as shown in Scheme 2 by alkylating the 4-hydroxyquinolone **4** with (*S*)-2-(2-chloroethyl)-1-methylpiperidine (**12**) followed by amino-carbonylation of intermediate **13** with 4-aminopyrimidine. Ester **10**, derived from (L)-pipecolic acid as previously described,[6] provided the requisite alkylating agent **12** in two steps as shown.

Discussion

During the course of this investigation, a radioligand binding assay in CHO cell-expressed cloned human GnRH receptors[12] supplemented the rat pituitary membrane binding assay[5,6] which had been previously used in our program. Additionally, new compounds were evaluated for their ability to functionally antagonize GnRH-stimulated phosphatidyl inositol (PI) hydrolysis in human cloned receptors[13] and LH release[14] from rat primary pituitary cells. Inspection of the in vitro binding and functional antagonism for examples **9a–p** (Table 1) selected from a broad survey of racemic quinolone-6-carboxamides revealed that the amides derived from aliphatic amines (**9a–b**) are weakly potent antagonists.[15] Furthermore, heterocyclic amines yielding basic amides (i.e. **9f–h**) afforded derivatives that were generally more potent than those derived from amines which yielded neutral amide sidechains (**9c–e**).



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF reflux, 90%; (b) $SOCl_2$, HCl (1 atm), $CHCl_3$, reflux, 5 h, 62%; (c) quinolone **4**, K_2CO_3, DMF, 80 °C, 3 h, 60%; (d) 4-aminopyrimidine, $(PPh_3)_2PdCl_2$, CO (1 atm), Et_3N, DMF, 95 °C, 16 h, 68%.

Table 1. In vitro activities for racemic quinolone-6-carboxamides **9a–p**

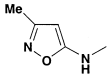
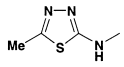
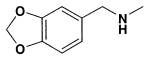
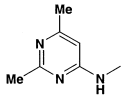
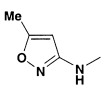
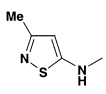
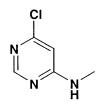
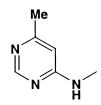
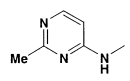
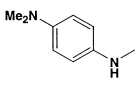
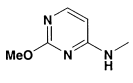
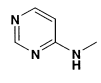
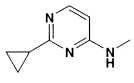
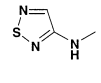
Entry	Substituent	GnRH binding IC_{50} (nM)	PI turnover IC_{50} (nM)	LH release IC_{50} (nM)	Entry	Substituent	GnRH binding IC_{50} (nM)	PI turnover IC_{50} (nM)	LH release IC_{50} (nM)
9a		190 (rat)	—	>1280	**9i**		290	>3200	>1280
9b		45 (rat)	—	>1280	**9j**		7	16	2200
9c		23	—	>1280	**9k**		320	775	>1280
9d		28	—	>1280	**9l**		3.2	8.3	240
9e		110	591	>1280	**9m**		2	6.4	292
9f		3.6	19	1265	**9n**		40	486	—
9g		3.8	10.6	1089	**9o**		32	22	—
9h		32	130	>1280	**9p**		860	977	—

Table 2. In vitro activities for (*S*)-quinolone-6-carboxamides **9q–z**

Entry	Substituent	GnRH binding IC_{50} (nM)	PI turnover IC_{50} (nM)	LH release IC_{50} (nM)	Entry	Substituent	GnRH binding IC_{50} (nM)	PI turnover IC_{50} (nM)	LH release IC_{50} (nM)
9q		2.3	77.5	513	**9v**		2.9	21.5	521
9r		1.4	14.5	79.2	**9w**		2.8	44	167
9s		1.8	33.3	87.2	**9x**		10.9	360	—
9t		9	133	—	**9y**		0.9	5.0	96.5
9u		15	250	—	**9z**		0.8	5.9	23.9

Notably, the amides derived from 4-aminopyrimidine (**9l**) and structurally related amines (i.e. **9m**) afforded the greatest potency observed in the series. Concurrent efforts in our laboratory demonstrated the dependence of GnRH binding affinity upon the (*S*)-piperidine configuration, thus we sought to focus further SAR studies of quinolone-6-carboxamides in the non-racemic series.[6] Table 2 illustrates binding and functional data for representative GnRH antagonists **9q–z** prepared from amines (**7q–z**) selected to further define the

quinolone-6-carboxamide SAR. In parallel with the racemic series, the 4-aminopyrimidine (**9y**) and 3-amino-1,2,5-thiadiazole (**9z**) derivatives were again the most potent analogues, while incorporating a variety of substituents on the heterocycle failed to effect additional improvement.

In vitro characterization of analogue **14** confirmed that *N*-methylation of **9y** affords potent antagonism (IC_{50} values: hGnRH binding = 2.5 nM, hGnRH PI hydrolysis = 16 nM, rGnRH LH release = 85 nM); albeit somewhat less potent than the parent unsubstituted piperidine. Subsequent evaluation of **14** in the rat pituitary membrane binding assay indicated that it is less potent (IC_{50} = 60 nM) at the rat receptor than at the human clones. This observation may resolve the apparent discrepancy between the functional antagonism of **14** determined in the PI hydrolysis (cloned human receptors) and LH release assays (rat primary pituitary cells).

Conclusion

SAR studies for a variety of quinolone-6-carboxamides revealed that the amides derived from 4-aminopyrimidine (**9l, 9y**) and 3-amino-1,2,5-thiadiazole (**9m, 9z**) afford highly potent functional antagonists of rat and human GnRH receptors. As anticipated from earlier studies, *N*-methylation of the piperidine substituent at C-4 (**14**) retains antagonist potency. The advent of binding and functional assays using both rat and cloned human receptors revealed that our quinolone-derived antagonists appear to have somewhat higher affinity towards human receptors. This phenomenon was also observed with the thieno[2,3-*b*]pyridin-4-one class of GnRH antagonists reported by Takeda.[4] Additional SAR and pharmacological characterization for the quinolone series of GnRH antagonists will be reported from these laboratories in the near future.

Acknowledgements

We thank Dr. George Doss for helpful discussions regarding NMR assignments, J. Leone, J. Pisano, S. Fabian and G. Reynolds for synthetic services and A. Bernick for FAB-mass spectrometry analysis.

References and Notes

1. Roy, R. A. *Curr. Opin. Obstet. Gy.* **1994**, *6*, 262.
2. Behre, H. M.; Nordhoff, V.; Nieschlag, E. In *Ovul. Induct. Update '98, Proc. World Conf., 2nd, Meeting*, Date 1997; Filicori, M.; Flamigni, C., Eds.; Parthenon Publishing: Carnforth, UK, 1998, pp 107.
3. Goulet, M. T. In *Annual Reports in Medicinal Chemistry;* Bristol, J. A., Ed.; Academic Press: New York, 1995; Vol. 30, pp 169.
4. Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S.; Furuya, S.; Suzuki, N.; Okubo, S.; Ogi, K.; Endo, S.; Onda, H.; Fujino, M. *J. Med. Chem.* **1998**, *41*, 4190.
5. DeVita, R. J.; Hollings, D. D.; Goulet, M. T.; Wyvratt, Jr., M. J.; Fisher, M. H.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2615.
6. DeVita, R. J.; Goulet, M. T.; Wyvratt, Jr., M. J.; Fisher, M. H.; Lo, J.-L.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2621.
7. Young, J. R.; Chen, I.; Walsh, T. F.; DeVita, R. J.; Wyvratt Jr., M. J.; Fisher, M. H.; Goulet, M. T.; Ren, N.; Lo, J.-L.; Yang, Y. T.; Yudkovitz, J. B.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.*, manuscript in preparation.
8. Schoenberg, A.; Heck, R. F. *J. Org. Chem.* **1974**, *39*, 3327.
9. Sy, W.-W. *Synthetic Commun.* **1992**, *22*, 3215.
10. A representative procedure for the amino-carbonylation reaction: an oven-dried, two-necked 25 mL round-bottom flask was equipped with a magnetic stir bar, a septum and a three-way stopcock fitted with a balloon. The flask was charged with 0.233 g (0.37 mmol) of iodo compound (*S*)-**6**, 0.174 g (1.83 mmol) of 4-aminopyrimidine, 102 μL (0.73 mmol) of triethylamine, 0.013 g (0.018 mmol, 5 mol%) of dichlorobis(triphenylphosphine)palladium(II) and 2 mL of anhydrous DMF. The atmosphere in the flask was alternately evacuated in vacuo and flushed with carbon monoxide three times. The balloon was next filled with carbon monoxide, and the reaction mixture was then magnetically stirred and heated with an oil bath at 95 °C for 24 h. At the end of this period, the reaction mixture was cooled to room temperature and partitioned between EtOAc (15 mL) and 10% aqueous $NaHSO_4$ (15 mL). The organic layer was separated, washed with additional 10% aqueous $NaHSO_4$, saturated NaCl, then dried ($MgSO_4$), filtered and evaporated. The residual oil was purified on a silica gel flash chromatography column using 2.5% MeOH in $CHCl_3$ as eluent. Evaporation of the purified fractions and drying in vacuo afforded 0.125 g (54%) of compound **8y** as an amorphous pale yellow powder.
11. All final compounds were obtained as trifluoroacetic acid salts following purification by reversed-phase HPLC. Intermediates and final products afforded satisfactory 400 or 500 MHz ^{1}H NMR and ESI mass spectral characterization. Data for **9y** (TFA salt): ESI-MS *m*/*z* 532 ($M^+ + 1$); ^{1}H NMR (CD_3OD, 500 MHz) δ 1.15–1.22 (m, 1H), 1.34–1.45 (m, 111), 1.52–1.86 (m, 7H), 1.97–2.06 (m, 1H), 2.37 (s, 6H), 2.82–2.92 (m, 1H), 2.98–3.05 (m, 1H), 3.27–3.34 (m, 1H), 3.73–3.87 (m, 2H), 7.06 (s, 2H), 7.09 (s, 1H), 7.51 (s, 1H), 8.10 (s, 1H), 8.34 (dd, J = 1.0, 6.0 Hz, 1H), 8.71 (d, J = 6.0 Hz, 1H), 8.89 (d, J = 1.0 Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 21.46 (2C), 23.03, 23.33, 28.95, 34.76, 46.02, 55.38, 70.19, 111.80, 117.14, 117.56, 123.55, 126.00, 129.43 (2C), 130.68, 130.99, 133.38, 134.85, 139.21 (2C), 140.95, 158.79, 159.18, 159.63, 161.14, 165.65, 168.10. Data for **14** (TFA salt): ESI-MS *m*/*z* 546 ($M^+ + 1$); ^{1}H NMR (CD_3OD, 500 MHz) δ 1.22–1.80 (m, 7H), 2.35–2.40 (m, 1H), 2.37 (s, 6H), 2.77 (s, 3H), 2.86–2.90 (m, 2H), 3.42–3.46 (m, 1H), 3.73–3.86 (m, 2H), 7.06 (s, 2H), 7.10 (s, 1H), 7.51 (s, 1H), 8.15 (s, 1H), 8.36 (dd, J = 1.0, 6.0 Hz, 1H), 8.72 (d, J = 6.0 Hz, 1H), 8.91 (d, J = 1.0 Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 21.47 (2C), 22.95, 24.28, 29.53, 32.23, 41.63, 57.58, 64.13, 70.67, 111.78, 117.07, 117.60, 123.64, 126.18, 129.49 (2C), 130.48, 131.01, 133.45, 134.93, 139.26 (2C), 140.95, 158.26, 158.84, 159.90, 161.32, 165.62, 168.02.
12. Crude membranes prepared from rat pituitary glands or Chinese hamster ovary K1 cells stably expressing human GnRH receptors were used as the sources for GnRH receptors. 5-[^{125}1-Tyr]-Buserelin (a peptidyl GnRH agonist obtained from Woods assays) having specific activity of 1000 Ci/mmol was used as the radiolabelled ligand. Competitive binding was measured in a 50 mM Tris–HCl based buffer (pH 7.5) containing 2 mM $MgCl_2$ and 0.1% bovine serum albumin. The binding activity is reported as an IC_{50} value which is the antagonist concentration required to inhibit the specific binding of [^{215}1]buserelin to GnRH receptors by 50%.
13. Chinese hamster ovary cells stably expressing human GnRH receptors functionally coupled to phospholipase C

were used to evaluate the functional GnRH antagonism of test compounds. Clones were seeded at a concentration of 60,000 cells/mL/well in inositol-free F12 medium containing 10% dialyzed fetal bovine serum, 1% Pen/Strep, 2 mM glutamine, 500 μg/mL G418 and 1 μCi [^{3}H]inositol in 24-well tray. Forty-eight hours after seeding, cells were washed with 3×1 mL of PBS containing 10 mM LiCl and treated with various concentrations of test compounds for 2 h at 37 °C before addition of 0.5 nM GnRH. After incubation at 37 °C for an additional 60 min, the medium was removed and the cells were lysed with 1 mL of 0.1 M formic acid. The trays were freeze–thawed once and the cell extract was applied to a Dowex AG1-X8 column. The column was washed with 2×1 mL H_2O to remove free [^{3}H]inositol and [^{3}H]inositol phosphates were eluted with 3×1 mL 2 M ammonium formate in 1 M formic acid. The eluate was counted in a scintillation counter. The results are reported as an IC_{50} value which is the antagonist concentration required to inhibit the GnRH stimulated PI hydrolysis by 50%.

14. Rat primary pituitary cells were isolated by enzymatic digestion with a mixture of collagenase and hyaluronidase. The suspended cells were then cultured for 4 days in 24-well tissue culture plates. On the day of the assay, the cells were washed and then treated with medium containing 2 nM GnRH and the compound of interest at concentrations between 5 and 5120 nM. After a 3 h incubation, the medium was removed and centrifuged. The supernatant was then analyzed for content of LH using a RIA kit specific for rat LH. The results are reported as an IC_{50} value which is the antagonist concentration required to inhibit the GnRH stimulated LH release by 50%.

15. The IC_{50} values for GnRH receptor binding shown for **9a** and **9b** in Table 1 were determined using rat pituitary membranes. See refs 5 or 6 for a description of the assay.