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Quinolones as Gonadotropin Releasing Hormone (GnRH) Antagonists: Simultaneous Optimization of the C(3)-Aryl and C(6)-Substituents

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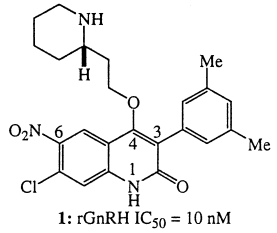
Abstract—A series of 3-arylquinolones was prepared and evaluated for their ability to act as gonadotropin releasing hormone (GnRH) antagonists. A variety of substitution patterns of the 3-aryl substituent are described. The 3,4,5-trimethylphenyl substituent (**23h**) was found to be optimal.

Introduction

Recent reports from these laboratories described the GnRH activity of a series of 3-arylquinolones.[1] These earlier efforts detailed modifications of the C(4)-substituent,[1a,b] culminating in **1** which was 1000 times more potent than the screening lead. A subsequent communication detailed studies on the optimization of the C(6)-position.[1c] In this letter, we wish to report on the continued advancement of the quinolone structure–activity relationships (SAR) with emphasis on the C(3)- and C(6)-positions.



1: rGnRH IC_{50} = 10 nM

Synthesis

The syntheses of 3-arylquinolones **10**, **12**, and **14** began with nitration of 5-chloroanthranillic ester **2** followed by coupling with acid chloride **5** (Scheme 1). The acid chlorides[2] were generally prepared by Arndt–Eistert[3] homologation of benzoic acids **4** followed by exposure to oxalyl chloride. Treatment of **6** with base afforded the Claisen condensation product **7**, which upon alkylation with alcohol **8**[4] under Mitsunobu conditions gave ether **9**. Acid catalyzed *N*-BOC deprotection provided the target compounds **10**. Those compounds with encouraging in vitro activities were converted to a variety of urea and amide derivatives. This involved treatment of **9** with a combination of hydrazine and catalytic Fe(III), followed by either isocyanate generation and in situ trapping with amines, or EDC mediated amide formation with carboxylic acids. Deprotection of the BOC moiety with TFA furnished ureas **12** and amides **14**, respectively.

To further expand our investigation, we next examined the incorporation of polar substituents into **1**. Thus, radical bromination (NBS, benzoyl peroxide, CCl_4) of **9** under high-dilution conditions gave a 4:1 mixture of mono- and di-bromo adducts (**15** and **16**, respectively) accompanied by recovery of some unreacted starting material (Scheme 2). Treatment of **15** with acetate or cyanide[5] produced **10w** and **10aa**, after removal of the *N*-BOC protecting group. Hydrolysis of nitrile **17** to amide **10y** was achieved with basic peroxide.[6] Several sequences were investigated for conversion of **15** to alcohol **18** of which the most efficient route involved Kornblum[7] oxidation followed by $NaBH_4$ reduction.[8] Re-oxidation of **18** with tetrapropylammonium perruthenate (TPAP) provided the aldehyde, which after subjection to either alkyltitanium[9]

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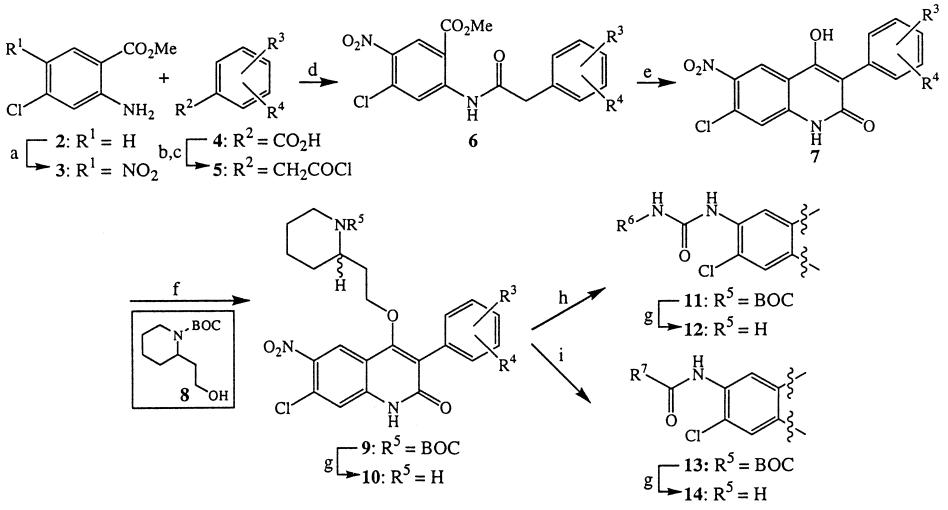
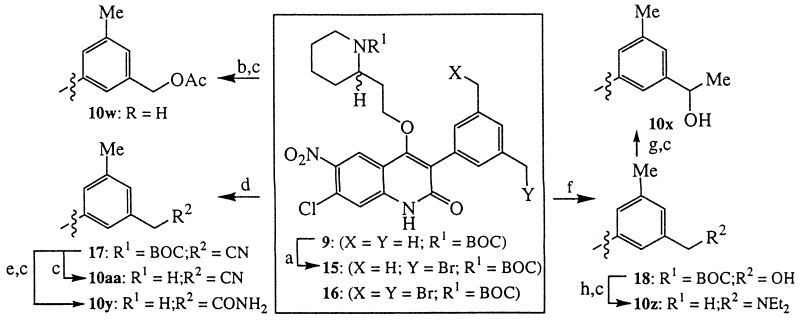


Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4; (b) $(COCl)_2$, DMF(cat), CH_2Cl_2; CH_2N_2, Et_2O, 0 °C; Ag_2O, dioxane, H_2O; (c) $(COCl)_2$, DMF(cat), CH_2Cl_2; (d) **3**+**5**, DCE, 80 °C; (e) NaHMDS, THF, 0–25 °C; (f) **8**, Ph_3P, DEAD, THF; (g) TFA, CH_2Cl_2; (h) $Cl_3Fe{\cdot}6H_2O$, hydrazine, MeOH; Cl_2CO, pyridine, CH_2Cl_2; R^6NH_2; (i) $Cl_3Fe{\cdot}6H_2O$, hydrazine, MeOH; EDC, HOBT, R^7CO_2H, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, 80 °C (**9** (32%); **15** (41%); **16** (10%)); (b) NaOAc, 18-C-6, MeCN, 60 °C (38%); (c) TFA, CH_2Cl_2; (d) KCN, TFA, DMSO, 10 °C (29%); (e) H_2O_2, K_2CO_3, DMSO, 50 °C (76%); (f) 2,4,6-collidine, DMSO, 80 °C; $NaBH_4$, MeOH (51%); (g) TPAP, NMO, CH_2Cl_2; $TiCl_4$, MeMgBr, Et_2O, −78 °C (41%); (h) TPAP, NMO, CH_2Cl_2; Et_2NH, HOAc, $NaCNBH_3$, MeOH (21%).

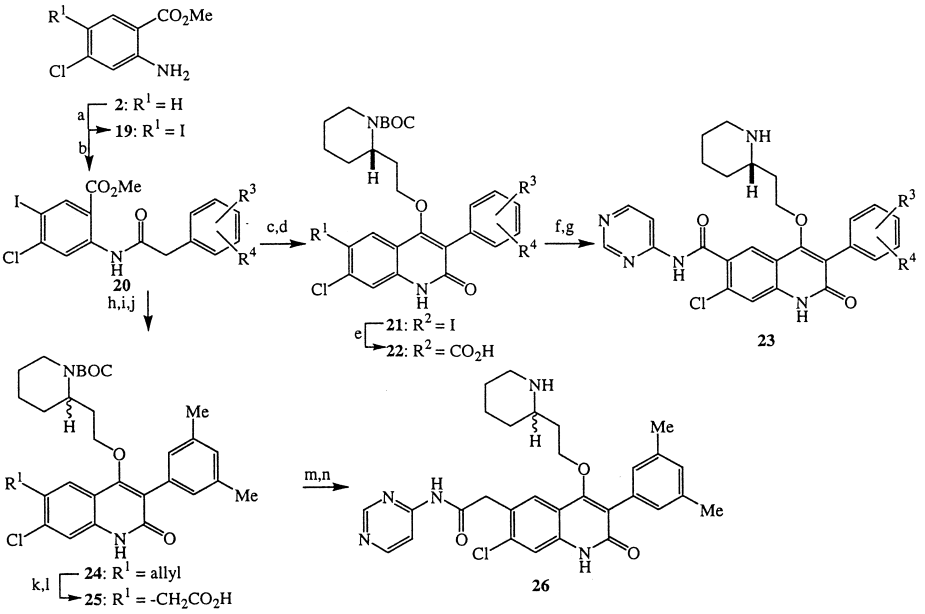
reagents or reductive-amination conditions gave **10x** and **10z**, respectively.

Concurrent with our efforts to improve the C(6)-urea substituent, we made the observation that simplified tethers such as benzamide derivatives[1c] or the reversed amides resulted in improved in vitro activity. During this investigation, 4-aminopyrimidine was found to be superior to cyclopropylamine (i.e., **12A** vs **12hh**; Table 1). In addition, the more potent enantiomer of the piperidine was found to possess the *S*-stereochemistry.[1c] These results prompted a re-examination of the C(3)-aryl position in this more potent series. Our synthetic efforts began with electrophilic iodination of aniline **2** (Scheme 3) which was then transformed to the fully elaborated 6-iodoquinolone (**21**) in close analogy to **9**, as described above. Carboxylation of iodide **21** under the mild palladium catalyzed conditions of Cacchi et al,[10] subsequent EDC mediated amide formation and *N*-BOC deprotection afforded the target compounds **23a**–**h**. Acetamides such as **26**, were prepared as potential isosteric replacements for the urea substituent. The acetamide side chain was masked in the form of an allyl group which was installed via a Stille cross-coupling reaction between allyltin and iodide **20**. After Claisen condensation and Mitsunobu alkylation, the allyl moiety was converted to acetamide **26** by oxidative cleavage of the derived diol followed by amide formation as described above.

Biological Results and Discussion[11]

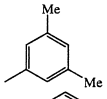
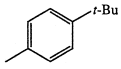
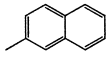
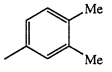
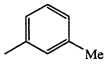
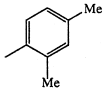
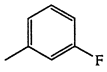
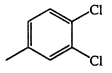
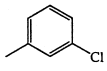
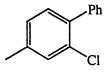
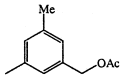
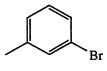
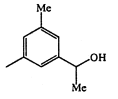
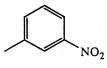
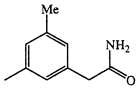
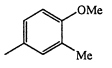
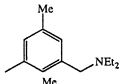
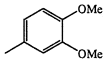
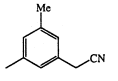
The lead compound **1** bound with high affinity to the rat GnRH receptor (IC_{50} = 10 nM).[1c] Absence of the methyl groups (**10a**) established the importance of *meta*-substitution, as the binding affinity of this analogue was reduced 40-fold, although most of the potency could be restored from a single *meta*-methyl (**10b**) or halogen (**10c**–**e**) substituent. The *meta*-electron withdrawing nitro group (**10f**) was also beneficial albeit to a lesser degree. Comparison of the binding activities of the regioisomeric chlorophenyls **10d**, **10g**, and **10h** revealed that the optimum order of activity was *meta* > *para* > > *ortho*. The bulky *t*-butyl analogue **10i** was nearly as potent as the sterically smaller chloro analogue **10h** suggesting a fairly large steric latitude in the receptor pocket where the aryl substituent resides.

We turned our attention towards increasing the substitution around the aryl ring in order to optimize this hydrophobic interaction. The 3,4-dimethyl analogue **10j** and dichloro analogues **10l** and **10n** were nearly equipotent to



Scheme 3. Reagents and conditions: (a) I_2, $AgSO_4$, MeOH; (b) **4**, $(COCl)_2$, DMF, CH_2Cl_2; **19**, DCE, 80 °C; (c) NaHMDS, THF, 0–25 °C; (d) **8**, Ph_3P, DEAD, THF; (e) $Cl_2Pd(dppf)$, CO, DMSO, 60 °C; (f) 4-aminopyrimidine, EDC, DMAP, NEt_3, CH_2Cl_2; (g) TFA, CH_2Cl_2; (h) allyltributyltin, $(Ph_3P)_2PdCl_2$, DMF, 95 °C; (i) NaHMDS, THF, 0–25 °C; (j) **8**, Ph_3P, DEAD, THF; (k) OsO_4, NMO, *t*-BuOH-THF-H_2O; (l) $Pb(OAc)_4$, pyridine-MeOH; (m) 4-aminopyrimidine, EDC, DMAP, NEt_3, CH_2Cl_2; (n) TFA, CH_2Cl_2.

Table 1.[11]

Entry	Aryl	rGnRH IC_{50} (nM)	Entry	Aryl	rGnRH IC_{50} (nM)	Entry	Aryl	rGnRH IC_{50} (nM)
1		10	**10i**		200	**10r**[1]		53
12a		1				**12r**[1]		68
10a		410	**10j**		18	**10s**		220
			12j[1]		6			
10b		27	**10k**		400	**10t**		900
12b[a]		23						
10c		53	**10l**		29	**10u**		23
			12l[1]		18			
10d		40	**10m**		900	**10v**		45
10e		40	**10n**		24	**10w**		63
12e[1]		6						
10f		100	**10o**		71	**10x**		71
10g		360	**10p**		40	**10y**		270
10h		160	**10q**		600	**10z**		18

[a]R^6 for compounds **12** (Scheme 1) is cyclopropyl.

1, which were all 3–4 times more potent than the electron-withdrawing bis-trifluoromethyl compound **10o**. Electron-releasing groups also proved to be detrimental. For example, sequential replacement of the methyl groups of **10j** with a methoxy substituent (**10p–q**) resulted in a 2-fold decrease in GnRH binding affinity for one methoxy group and a 10-fold loss for the second. Incorporation of larger aromatic substituents such as the naphthyl

derivative **10r** or a biaryl ring system **10m** served to further define the emerging SAR. Replacement of the phenyl ring with the isosteric thiophene residue showed a marked preference for attachment at the C(2)-position (**10s** vs **10t**) in which **10s** was modestly more potent than the simple phenyl (**10a**) case. Further improvements in this design included the pseudo *meta*-chloro analogue **10u** whose activity was consistent with the phenyl SAR.

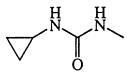
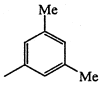
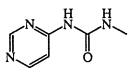
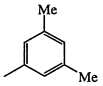
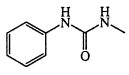
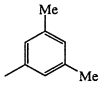
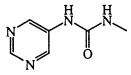
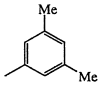
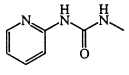
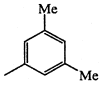
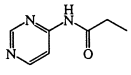
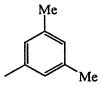
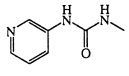
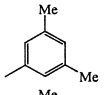
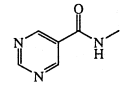
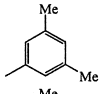
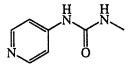
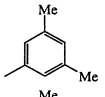
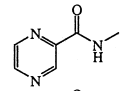
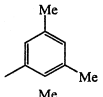
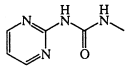
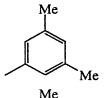
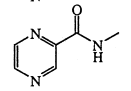
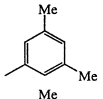
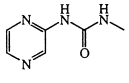
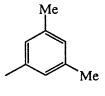
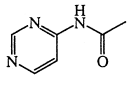
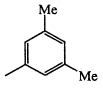
At this juncture, a preference for 3,4- or 3,5-dialkyl or dihalogenated phenyls was established. Although electron-releasing/withdrawing groups were not beneficial when directly attached to the aryl ring, we pondered the effect of these groups if deployed off the methyl groups of **1**. The results were quite clear. With the exception of the cyanomethyl analogue (**10z**), all other variations reduced receptor-binding affinity (cf. acetate **10v**, alcohol **10w**, amide **10x**, or amine **10y**). In conclusion, the results from Table 1 suggest that the C(3)-aryl group is occupying a hydrophobic binding region within the GnRH receptor in which alkyl or halogen substituents are preferred.

A concomitant investigation directed at the C(6) position, revealed that the cyclopropyl-urea analogue (**12a**) had a 10-fold potency advantage over the nitro variant (**1**) (Tables 1 and 2). Naturally, we decided to survey some of the more potent C(3)-aryl substituents with this urea modification. As expected, **12b**, **e**, **j**, and **l** demonstrated improved binding activity over the corresponding nitro analogues. However, the naphthyl analogue **12f** did not benefit from this modification. A more detailed account of the C(6) SAR is provided in Table 2.

At this stage of the program, several new developments occurred that impacted our lead development. First, the individual enantiomers of the piperidine side chain were independently prepared and incorporated into our lead design, wherein it was determined that the *S*-configuration was desired. Secondly, the cloned human GnRH receptor was now available in both a binding (hGnRH) and functional (hPI) assay, both of which would now serve as our primary tools to screen new analogues. Table 2 will illustrate this transition.

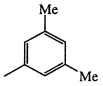
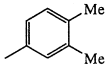
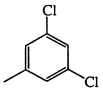
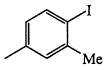
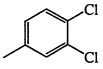
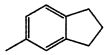
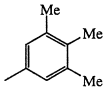
Of the simple alkyl ureas prepared, cyclopropyl was clearly the most potent and displayed good functional antagonism in both the rat primary pituitary cell assay (rLH: $IC_{50} = 375$ nM) and in CHO cells expressing the human receptor (hPI; $IC_{50} = 12$ nM). The single enantiomer of **12a** with the *S*-configuration showed improved functional antagonism against the human clone (Table 2). The superiority of the cyclopropyl urea motif in the functional assays over isosteric analogues such as *i*-propyl (rGnRH: $IC_{50} = 25$ nM; rLH: $IC_{50} = 4500$ nM), suggested that the sp^2 character of the ring may contribute to the functional potency. Indeed, phenylurea **12bb** was also active in the rLH assay albeit with 10-fold less potency. Evaluation of the pyridine regioisomers in the rat assays revealed a potency preference wherein the order was 2→3→4-aminopyridine (**12cc** >**12dd** >**12ee**); moreover, **12cc** was comparable to **12a** in functional potency. These results led us to consider that other basic heteroaromatics may lead to further improvements. Indeed, incorporation of pyrimidine and pyrazine heterocycles into the urea design provided a major breakthrough in functional potency. For example, pyrazine **12gg** and pyrimidine

Table 2.

Entry	C(6)	C(3)	GnRH[a] IC_{50} (nM)	rLH/hPI IC_{50} (nM)	Entry	C(6)	C(3)	GnRH[a] IC_{50} (nM)	rLH/hPI IC_{50} (nM)
12a			1.1 0.8/**2.3**[b]	375/12 682/5	**12hh**			0.6 0.4/**1.8**[b]	235/na 52/na
12bb			10	3300/na[c]	**12ii**			**57**[b]	na/897
12cc			15	463/na	**26**			7.9	2500/227
12dd			20	1300/na	**14a**[12]			**25**[b]	na/225
12ee			40	na/na	**14b**			1.8/**10**	1920/na
12ff			6	na/na	**14c**[13]			**2.0**[b]	na/23
12gg			0.3/**1.1**[b]	56/9	**23a**			**0.9**[b]	97/5

[a]GnRH binding in rat pituitary cell are listed first and GnRH binding using human cloned receptors are in bold.
[b]Denotes compounds bearing the (*S*)-piperidine configuration.
[c]Data not available.

Table 3.

23	Aryl	hGnRH IC_{50} (nM)	hPI IC_{50} (nM)	23	Aryl	hGnRH IC_{50} (nM)	hPI IC_{50} (nM)
a		0.9	5.0	e		0.7	6.5
b		1.1	8.5	f		0.5	7.1
c		1.0	7.7	g		0.7	16.0
d		3.9	53	h		0.3	2.2

12hh had binding affinities <1 nM in the rat and both analogues were 7 times more potent than **12a** in the rLH assay. Other pyrimidine isomers, such as **12ff** and **12ii**, which have no net dipole, were not active in the rLH assay (at concentrations of 1 μM). Replacement of the urea nitrogen directly attached to the quinolone core with a methylene (**26**) was not potency enhancing, although removal of the urea nitrogen attached to the heterocycle led to amides **14a–c**, which were equipotent to or better than their corresponding urea counterparts. Finally, reversing the amide connectivity of **14c** furnished **23a**, which was 2 times more potent in the binding assay (hGnRH) and 4 times more potent in the PI assay than **14c**. This pyrimidine-carboxamide was found to be the superior C(6)-substituent.

With the C(6) position optimized, we returned to the C(3)-aryl position for further refinement. Compounds **23b–e** are hybrids of the best aryl substitution patterns found in Table 1 with the preferred C(6)-substituent from Table 2. The SAR in this series correlated with previous observations, in which the 3,5- and 3,4-dimethyl (**23a** and **23e**) and dichloro (**23b–c**) designs were the most potent and indeed, nearly equivalent to one another (Table 3). The activities of iodo-methyl analogue **23f** were indistinguishable from **23a**. Attempts to improve the potency of **23e** by incorporating the methyl groups in an indane framework (**23g**) resulted in a slight loss of functional antagonism. The similar in vitro profiles of the **23a** and **23e** suggested the preparation of the 3,4,5-trimethylphenyl hybrid (**23h**).[3] Gratifyingly, **23h** gave improved binding and functional antagonist activity compared to the disubstituted analogues.

In conclusion, a thorough study of the SAR at the C(3)-aryl position indicated the distinct preference for 3,4- or 3,5-dialkyl or di-halo substituted aromatics. Based upon the initial SAR, we discovered that the optimal aryl substituent was hydrophobic in nature and this ultimately led to the identification of the 3,4,5-trimethylphenyl analogue (**23h**), a potent GnRH antagonist with subnanomolar binding affinity and excellent functional antagonism.

Acknowledgements

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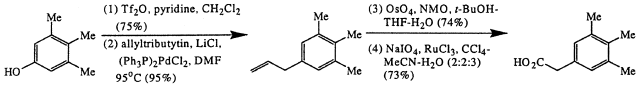
References and Notes

1. (a) DeVita, R. J.; Hollings, D. D.; Goulet, M. T.; Wyvratt, M. J., Jr.; Fisher, M. H.; Lo, J.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2615. (b) DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J., Jr.; Fisher, M. H.; Lo, J.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2621. (c) Walsh, T. F.; Toupence, R. B.; Young, J. R.; Huang, S. X.; DeVita, R. J.; Goulet, M. T.; Wyvratt, Jr., M. J.; Fisher, M. H.; Lo, J.-L.; Cui, J.; Ren, N.; Yudkovitz, J. B.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 443.

2. For the preparation of the indanephenylacetic acid and the 3,4,5-trimethylphenylacetic acid, the syntheses began with the phenols which were processed according to the following 4-step sequence.



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4. Racemic *N*-BOC-2(2′-hydroxyethyl)-piperidine **8** was prepared by protection of the free-amine using standard conditions (BOC_2O, NEt_3, CH_2Cl_2). The *S*-enantiomer of **8** was prepared by Arndt–Eistert homologation of *N*-BOC (*S*)-pipecolic acid.

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7. Wilcox, C. F. Jr.; Weber, K. A. *J. Org. Chem.* **1986**, *51*, 1088.

8. Due to difficulties encountered in purification of the initial aldehyde product, the crude aldehyde was reduced to the alcohol which was isolated cleanly.

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11. For detailed information on rat and human based assays for receptor binding and function, see ref 1c.

12. Prepared by carbomethoxylation of 5-bromopyrimidine ($(Ph_3P)_2PdCl_2$, NEt_3, DMF-MeOH, CO (1 ATM), 90 °C (44%)) followed by saponification of the ester (NaOH; HCl (85%)): Godefroi, E. F. *J. Org. Chem.* **1962**, *27*, 2264.

13. 4-Carboxypyrimidine was prepared by reaction of 4-methylpyrimidine with SeO_2/pyridine (39%): Archer, G. A.; Kalish, R. I.; Ning, R. Y.; Sluboski, B. C. *J. Med. Chem.* **1977**, *20*, 1312.