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Modeling Directed Design and Biological Evaluation of Quinazolinones as Non-Peptidic Growth Hormone Secretagogues

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Abstract—Quinazolinone derivatives were synthesized and evaluated as non-peptidic growth hormone secretagogues. Modeling guided design of quinazolinone compound **21** led to a potency enhancement of greater than 200-fold compared to human growth hormone secretagogue affinity of a screening lead **4**.

Introduction

Growth hormone (GH) secretagogues have received considerable attention in the last several years based on promising applications of recombinant GH in animals and in humans. Potential therapeutic indications for GH secretagogues in human health include idiopathic GH deficiency states in children and adults, stimulation of anabolic processes in the elderly and supportive therapy in catabolic wasting conditions including AIDS and osteoporosis.[1,2] After extensive studies of the growth hormone releasing peptide GHRP-6 (**1**, hGHSr[3] $IC_{50} = 6$ nM, rat pit.[4] $EC_{50} = 10$ nM, Fig. 1)[5] had established its GH releasing efficacy in animals and in man, efforts were made in a number of laboratories to identify peptidomimetic GH secretagogues as potential alternatives to GH replacement therapy.[6] Several of GH secretagogue peptidominetics entered clinical studies including MK-0677 (**2**, hGHSr $IC_{50} = 0.3$ nM, rat pit. $EC_{50} = 1.3$ nM)[7] and CP-424,391 (**3**, rat pit. $EC_{50} = 3$ nM).[8] In this paper we present the design, synthesis and biological activities of quinazolinone-based compounds as a new class of non-peptidic human GH secretagogue receptor agonists that evolved from a directed screening lead **4** (hGHSr $IC_{50} = 3.5$ μM, Fig. 2).

Chemistry

The quinazolinones were prepared in a straightforward fashion as outlined in Scheme 1. The cyclization of the 2-amino-5-bromobenzoic acid **5** with 2-naphthoyl chloride **6** was accomplished by heating the mixture with DMAP and triethyl amine in DMF to yield the quinoxazolone intermediate **7**. After addition of glycine ethyl ester **8**, heating was continued to ensure depletion of the quinoxazolone **7** and formation of the quiazolinone ester **9**. Base-catalyzed hydrolysis of the ester **9** afforded the key quinazolinone acid **10** followed by a standard peptide coupling with mono-Boc protected diamines to furnish bromo-quinazolinones **11** and **12**. The bromo compounds were then either treated with HCl to yield the quinazolinones **13** and **14** or converted to the corresponding phenyl analogues **15** and **16** under standard Suzuki coupling conditions [$PhB(OH)_2$, $Pd(PPh_3)_4$ and Na_2CO_3] followed by removal of the Boc group. Similarly, the phenethyl analogues were synthesized from the cyclization of **5** and 3-phenylpropionyl chloride (**17**) and, by following the same route, phenethyl analogues **18**–**21** were prepared (Scheme 2).

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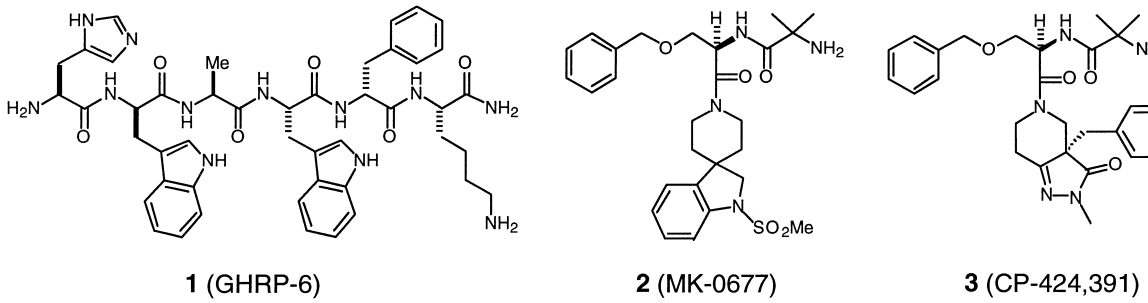


Figure 1.

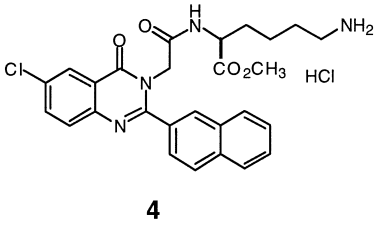
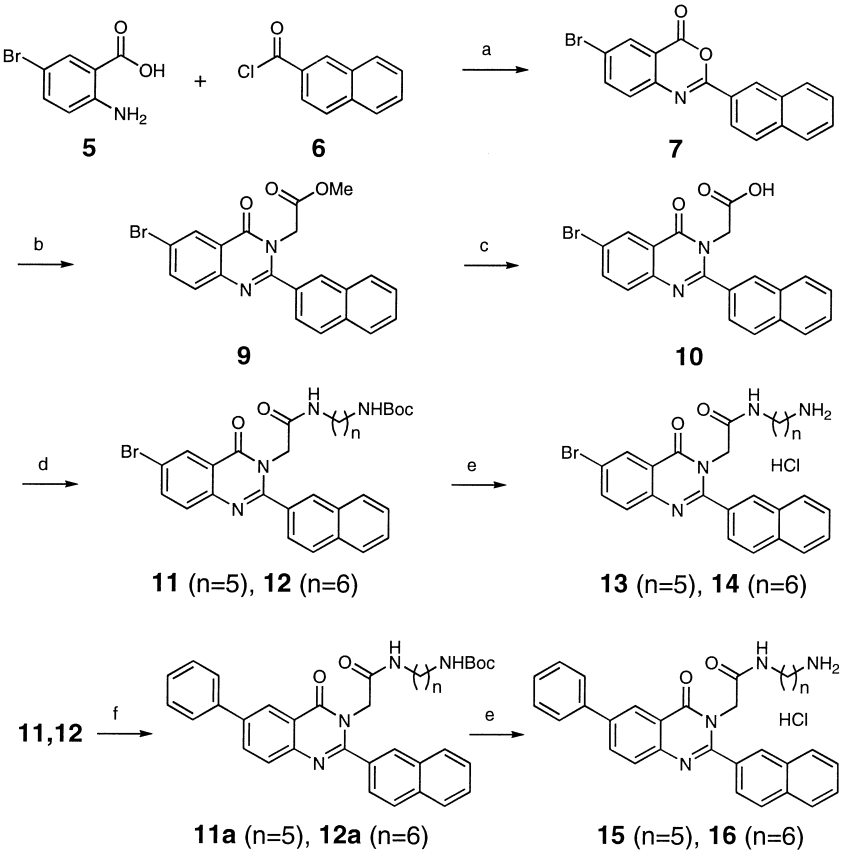


Figure 2.

Results and Discussion

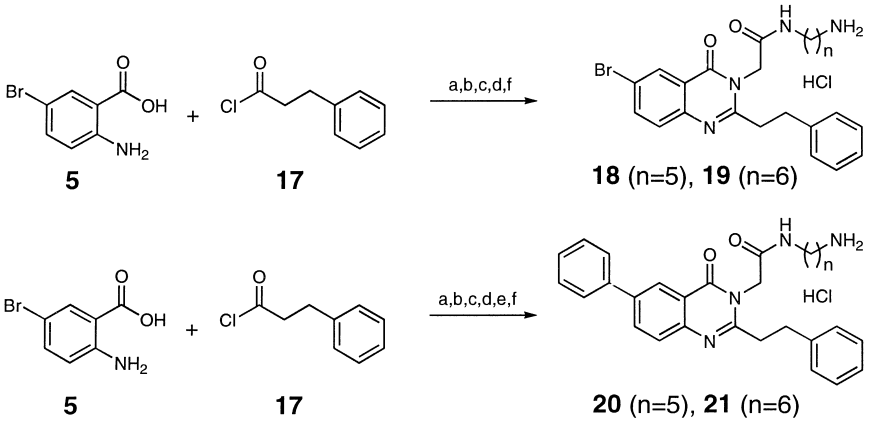
The initial screening lead compound **4** showed modest affinity toward the human GH secretagogue receptor. Modification of the amine side-chain was investigated first. Removal of the ester group (**13**, Table 1) led to a 3-fold increase in binding affinity of human GH secretagogue receptor while altering the chain length from five to six carbon atoms (**14**) provided a slight increase in potency.[9]

Previous work on GH secretagogues has suggested that the agonist pharmacophore of highly active GH secretagogue is comprised of two aryl groups and a basic amine.[10] Site-directed mutagenesis studies of the human GH secretagogue receptor indicated that the basic amine group of **2** forms a salt bridge with the negatively charged side-chain of E124 in TM3.[11] We hypothesized that the benzyl group of **2** may lie in a hydrophobic pocket formed by the side-chains of M213 in TM5 and H280 in TM6 and the spiroindane group π–π stacks with the side-chain of W274 in TM6 (Fig. 3a).



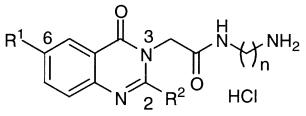
Scheme 1. (a) DMAP, Et_3N, DMF; (b) Gly-OMe (**8**), heat; (c) NaOH, H_2O, THF; (d) $NH_2(CH_2)_nNHBoc$, HOBt, EDC, NMM; (e) HCl; (f) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3.

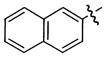
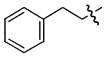
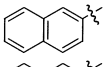
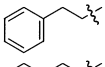
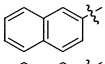
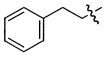
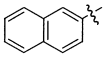
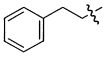
Using this hypothesis, the naphthyl quinazolinone **16** was docked[12] in the receptor model with its positively charged basic amine group interacting with E124 and the phenyl group at C-6 π–π stacking with W274 (Fig. 3b). This docking hypothesis was supported by the observation that substitution of the bromine at C-6 in **13** and **14** with a phenyl group (**15** and **16**) increased potency about 4- to 5-fold. This model indicates that the naphthyl group at the C-2 position of compound **16** may make unfavorable interactions with some of the side-chains near the top of TM4 and TM6 and not stack ideally in the hydrophobic pocket formed by M213 and H280. As shown in Figure 3c, the replacement of the rigid naphthyl group with a flexible aryl group should improve its orthogonal stacking. The phenethyl analogue (**21**) exemplifies this better fit in the hydrophobic



Scheme 2. (a) DMAP, Et_3N, DMF; (b) Gly-OMe (**8**), heat; (c) NaOH, H_2O, THF; (d) $NH_2(CH_2)_nNHBoc$, HOBt, EDC, NMM; (e) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3; (f) HCl.

Table 1. SAR trends in the quinazolinone leads



No.	R^1	R^2	*n*	hGHSr IC_{50} (μM)	No.	R^1	R^2	*n*	hGHSr IC_{50} (μM)
13	BR		5	1.4	**18**	Br		5	0.34
14	Br		6	0.96	**19**	Br		6	0.067
15			5	0.34	**20**			5	0.048
16			6	0.20	**21**			6	0.016

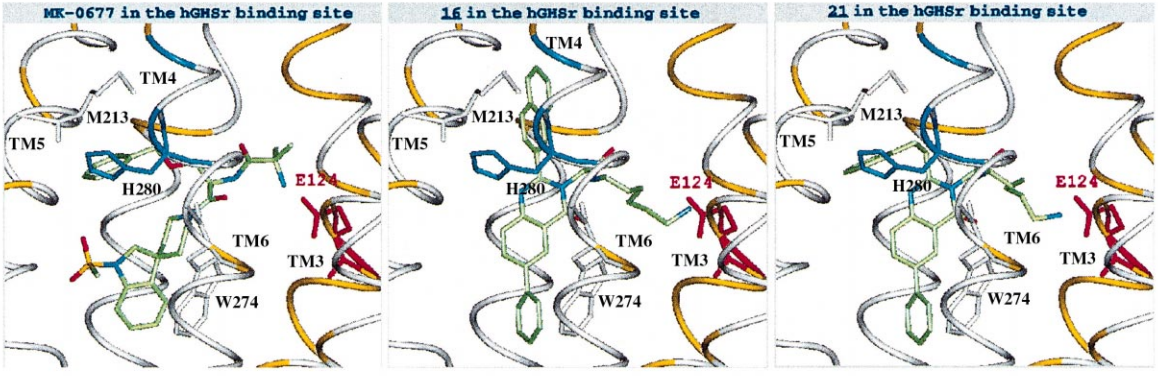
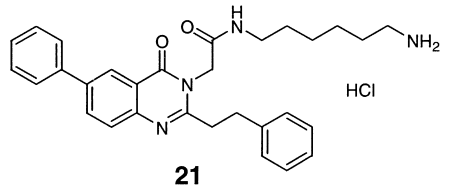


Figure 3a Figure 3b Figure 3c

Figure 3.



21

hGHSr IC_{50} = 16 nM
rat pit. EC_{50} = 0.63 nM

Figure 4.

binding pocket and should, therefore, have enhanced affinity.

Based on this modeling hypothesis, the phenethyl compounds **18**–**21** were synthesized and, as predicted, they were 8- to 15-fold more potent than the naphthyl analogues. The most active of this new class of human GH secretagogues was compound **21** (Fig. 4), which is 200-fold more potent than compound **4**.

Conclusion

Compound **21** is the first reported non-peptidic agonist of the human GH secretagogue receptor. Introduction of a phenyl group at the C-6 position of the quinazolinone core and extension of the amine side-chain improved the binding activity of lead compound **4** against the GH secretagogue receptor. We found that in all quinazolinone-type analogues, activities on the human GH secretagogue receptor increased when a phenethyl group was positioned at the C-2 position. Molecular modeling proved to be an effective tool in guiding the optimization of the screening lead **4**. Quinazolinones such as **21** provide a potent alternative to the privileged structure based dipeptide design exemplified by MK-0677.

Acknowledgements

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12. Modeling of compounds **2, 16** and **21** was carried out using the MMFFs force field (ref. Halgren T. A. *J. Comput. Chem.* **1999**, *20*, 720). Conformational generation and minimization in accord with solution NMR structure of GHRP-6 (Baum, M. W., Merck Research Laboratories) were performed and low energy structures which fit the constraints were used in the docking studies.