Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 317–320

Parallel solid-phase synthesis of disubstituted (5-biphenyltetrazolyl) hydantoins and thiohydantoins targeting the growth hormone secretagogue receptor

Rune Severinsen,[a] Jesper F. Lau,[a] Kent Bondensgaard,[b] Birgit S. Hansen,[a] Mikael Begtrup[c] and Michael Ankersen[a,*]

[a]*Medicinal Chemistry, Novo Nordisk A/S, Novo-Nordisk Park 2760 Maaloev, Denmark*
[b]*Protein Engineering, Novo Nordisk A/S, Novo-Nordisk Park 2760 Maaloev, Denmark*
[c]*The Danish University for Pharmaceutical Sciences, Universitetsparken 2, 2100 Copenhagen O, Denmark*

Received 5 August 2003; revised 22 October 2003; accepted 10 November 2003

Abstract—An efficient solid-phase protocol for the synthesis of substituted (5-biphenyltetrazolyl)-hydantoins and -thiohydantoins has been developed. Suzuki cross-coupling between resin-bound 2-(tetrazol-5-yl)-phenylborinane and 4-bromobenzaldehyde gave the corresponding tetrazolylbiphenyl aldehyde. Subsequent reductive amination using amino acid esters gave the pivotal resin bound amino acid esters which were transformed to hydantoins or thiohydantoins via two routes: (i) treatment with isocyanates or isothiocyanates or (ii) successive treatment with triphosgene and primary amines. Using molecular modeling, we were able to jump from L-692,429, a well known non-peptidyl growth hormone secretagogue (GHS), to biphenyltetrazolyl hydantoins, obtaining compounds with IC_{50} values below 600 nM after two iterative cycles only.

1. Introduction

Growth hormone (GH) is synthesized and released from somatotrophs (i.e., GH releasing cells) in the anterior pituitary.[1] Both GH synthesis and GH release from somatotrophs are under tight control by two hypothalamic hormones; growth hormone releasing hormone (GHRH)[2] which stimulates the synthesis and release of GH, and somatostatin[3] which inhibits GH release. In addition to the effect of GHRH, a new class of compounds, called growth hormone secretagogues (GHS), is able to induce GH release from the pituitary.[4] An endogenous ligand of the GHS pathway is Ghrelin,[5] a 28 amino acid *O*-*n*-octanoylated peptide, which was identified more than 20 years later than the first synthetic compounds. Ghrelin has been shown to be released from the stomach and is able to release GH in vitro as well as in vivo. It is widely believed that ghrelin and the synthetic GHSs elicit their effect at both the hypothalamic and pituitary level and work synergistically with GHRH.[6] Several non-peptidyl mimics of Ghrelin have been reported, notably the compound L-692,429 from Merck (Fig. 1).[7] Here we report a new series of nonpeptidyl compounds containing the biphenyltetrazole moiety of L-629,429 combined with disubstituted hydantoins and thiohydantoins in the general structure **1**. All compounds were prepared on solid support using a parallel synthesis strategy.

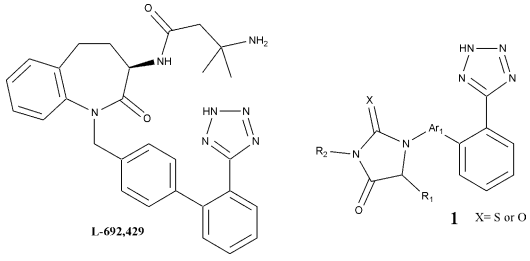


Figure 1. L-629,429 and general library scaffold.

2. Modeling

Previously, we have reported a model of L-692,429 docked in a receptor model of the GHS receptor.[8] L-

Keywords: Biphenyltetrazole; Hydantoins; Cross-coupling reactions; Growth hormone.
* Corresponding author. Tel.: +45-4443-2181; fax: +45-4443-4547; e-mail: miak@novonordisk.com

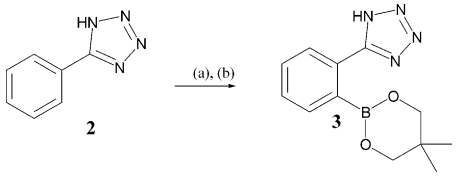
0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.012

692,429 and the general scaffold **1** share a biphenyltetrazole fragment. Using this common fragment, the core structure of hydantoin **1** was docked into a GHS receptor model such that position of the biphenyltetrazole fragment in the L-692,429–GHS receptor complex was preserved. In both cases, the biphenyltetrazole fragment is situated in a pocket between TM 5 and TM 6 spanned by Phe204(TM 5), Phe249(TM 6) and Trp253(TM 6) with the tetrazole moiety in the vicinity of Lys199(TM 5). The docked core of scaffold **1** constrains the accessible orientations of the R_1 and R_2 substitutents. R_1 is directed towards a pocket formed by a cluster of aromatic residues W104(TM 2), F119 (TM 3), F309(TM 7), F312(TM 7) and Y313(TM 7) whereas R_2 points towards Glu124(TM 3), which acts as a counterion for the amino group of L-692,429.[9] Due to the nature of these pockets, substrates with aromatic and amino group substituents were tested. Distances from the core of **1** to the complementary groups in the GHS receptor were estimated using the receptor model, and R_1- and R_2-groups were chosen accordingly. A similar arrangement of pharmacophoric elements can be achieved by swapping R_1 and R_2, and rotating the hydantoin system 180 degrees relative to the biphenyltetrazole group. However, the directionality of the aromatic and the amino substituents is somewhat different, and seems less favorable as judged by the receptor model. Likewise was the *S* stereoisomer of **1** preferred over the *R* stereoisomer.

3. Combinatorial solid-phase chemistry

In order to find an efficient method for the introduction of variable groups in the hydantoin/thiohydantoin scaffold **1**, a robust solid-phase strategy leading to **1** was developed.

Phenyltetrazole was treated with *n*-BuLi and TMEDA followed by addition of triisopropyl borate. The resulting mixture of borinanes, boronic acids and boroxines were esterified with neopentyl glycol, to give the necessary (5-tetrazolyl-2-phenyl)-5,5-dimethyl-[1,3,2]-dioxa-borinane **3** in 91% yield (Scheme 1). The tetrazole **3** was readily immobilized on a 2-chloro trityl resin in the presence of DIPEA. Subsequent treatment with 4-bromobenzaldehyde under standard Suzuki cross-coupling conditions[10] yielded the biphenyl aldehyde **5**. Amino acid esters were introduced via reductive amination using $NaBH_3CN$ in a mixture of DMF–MeOH–trimethyl orthoformate containing 1% acetic acid to give the resin bound amino acid ester **6**. Subsequent reaction with isothiocyanates or isocyanates in the presence of DIPEA followed by cleavage with 5% TFA in DCM yielded the corresponding thiohydantoins **9** or hydantoins **10** in high purity (>90%) and moderate yields (40–70%).

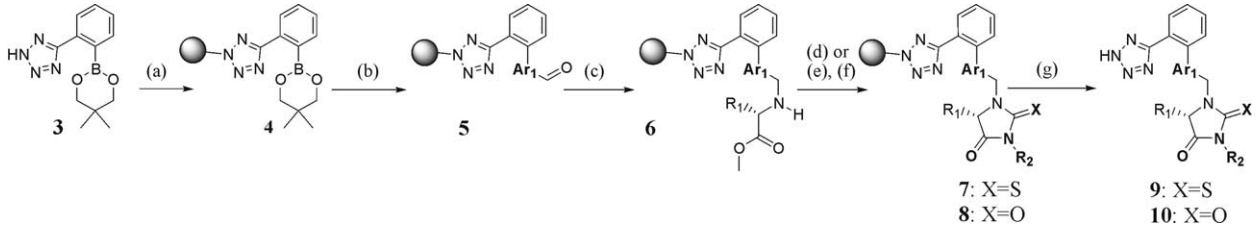


Scheme 1. (a) **2** in TMEDA, THF at −78 °C, then BuLi in hexanes and stirred 1 h to −10 °C, then $B(OiPr)_3$ at −78 °C; (b) neopentylglycol, ethyl acetate, 4 h.

To extend the scope of the scaffold, another strategy was investigated. Resin bound amino acid ester **6** was treated with triphosgene[11] in the presence of DIPEA followed by reaction with primary amines. Upon cleavage using 5% TFA in DCM, the hydantoins **10** was isolated in high purities ~90% and in moderate to good yields (40–80%).[13,14] In the case of the sterically hindered 4-amino-1-Boc-piperidine, cleavage from the resin yielded the uncyclicized derivative, which was treated with 10% acetic acid/THF at 50 °C to give **10a** in 45% yield.

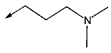
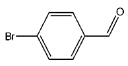
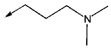
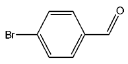
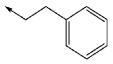
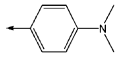
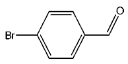
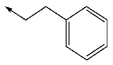
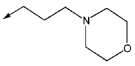
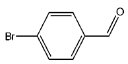
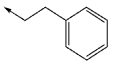
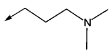
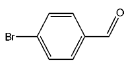
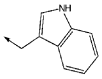
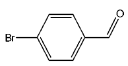
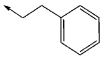
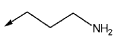
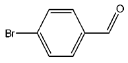
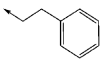
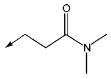
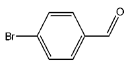
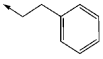
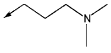
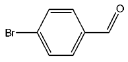
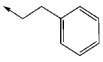
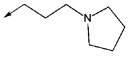
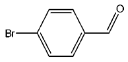
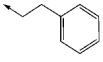
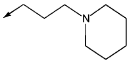
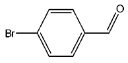
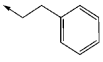
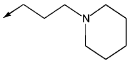
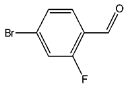
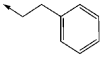
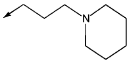
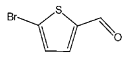
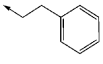
The scope of the strategy outlined in Scheme 2 was further extended by the usage of different aromatic bromo aldehydes in the Suzuki reaction. Thiophenes and fluoro substituted benzenes reacted with high purity and good yields, while furans yielded poor results in the Suzuki reaction. Pyridines worked well in the Suzuki reaction and in the reductive amination reaction, but did not cyclize to form hydantoins.

The compounds synthesized following the strategy outlined in Scheme 2, were tested for displacement of ^{125}I-Ghrelin to the human GHS-receptor expressed in BHK-cells.[12] Selected results are shown in Table 1. In the initial library R_1 and R_2 were varied, taking the pharmacophore model described earlier into consideration. When comparing the IC_{50} values of thiohydantoins **9** and hydantoins **10**, the results suggest that hydantoins fits the binding-pocket better than thiohydantoins (**9e** and **10d**). The results further suggested that the distance between the aromate in R_1 and the hydantoin ring had significant effect on the binding. When the aromatic ring was attached directly to the hydantoin ring, or when separated by one carbon atom, the binding was significantly lower than when separated by two carbon atoms (**9a**, **9b** and **9e**).



Scheme 2. Reagents and conditions: (a) 2-chlorotrityl resin, 3 equiv DIPEA, DCM, rt 2 h; (b) 4-bromobenzaldehyde, aqueous NaOH, toluene/ethanol, $Pd(PPh_3)_4$; (c) amino acid ester, (TMOF/MeOH/DMF/AcOH)/(33:33:33:1), 2 h, $NaBH_3CN$, MeOH/DMF, 4 h; (d) R_2NCO, DIPEA, DCM, 4 h, 45 °C; (e) DIPEA, DCM, $CO(OCCl_3)_2$, (f) R_2NH_2, DIPEA, DCM then 45 °C 4 h; (g) 5% TFA/DCM.

Table 1. Selected results from the library synthesized following the route outlined in Scheme 2

Product	Amino acid (*S*)-isomer (R_1)	Isothiocyanate, isocyanate or amine (R_2)	Aromatic bromo aldehydes Br-Ar_1-CHO	Purity LC–MS ELS (%)	IC_{50} GHS-R (μM)	Yield after purification
9a				99	35	74
9b				97	35	65
9c				89	> 100	45
9d				96	45	49
9e				99	8.2	57
10a				45	50	39
10b				94	> 100	82
10c				91	> 100	52
10d				97	4.0	76
10e				89	1.1	69
10f				98	0.6	78
10g				98	1.6	85
10h				92	15	72
10i				91	1.3	70

Anilines, primary amines, secondary amines, amides and tertiary amines were all tested in the R_2 position, but only the tertiary amines yielded significant binding. Based on these results a second library was made in which different R_2 groups were combined with the most potent R_1 (homophenylalanine) resulting in several compounds with IC_{50} values below 2 μM. The most potent compounds all contained a tertiary amine, situated three carbon atoms from the hydantoin ring (**10d**, **10e** and **10f**). When the tertiary amine was constrained in a ring system, the binding was further enhanced. The *R*-isomer of **10b**, **10d**, and **10f** was synthesized to explore the statement about the *S*-isomer being preferred over the *R*-isomer. The *R*-isomer all had IC_{50} values above 100 μM, thus supporting the results obtained by modeling.

The introduction of heteroaromatic or fluorosubstituted rings, did not have any significant influence on the binding (**10g**, **10i** and **10f**), even though **10h** displayed a substantial lower binding.

In summary R_1 in the model should contain an aromatic ring separated from the hydantoin-ring by at least two carbon atoms. R_2 should contain a tertiary amine, separated from the hydantoin ring by three carbon atoms and preferable the amine nitrogen should be part of a constraining ring. The SAR obtained is in good

agreement with both the chemical nature and spatial arrangement of the R-groups of the pharmacophoric model developed using the receptor model.

4. Conclusion

In conclusion, an efficient solid-phase protocols for the synthesis of biphenyltetrazolyl thiohydantoins **9** and hydantoins **10** from simple building blocks has been developed. Employing these strategies, libraries targeting GHSR were produced yielding compounds with IC_{50} values below 1 μM. A SAR described, based on the results, is in good agreement with the initial pharmacophoric model.

Acknowledgements

The authors would like to thank Corporate Research Affairs at Novo Nordisk A/S and The Danish Academy of Technical Sciences for financial support.

References and notes

1. Reichlin, S.; Saperstein, R.; Jackson, I. M. D.; Boyd, A. E.; Patel, Y. *Ann. Rev. Physiol.* **1976**, *38*, 389.
2. Rivier, J.; Spiess, J.; Thorner, M.; Vale, W. *Nature* **1982**, *300*, 276.
3. Krulich, L.; Dhariwal, A. P.; McCain, S. M. *Endocrinology* **1968**, *83*, 783.
4. Bercu, B. B.; Walker, R. F. *Growth Hormone Secretagogues*; Springer: New York, 1996.
5. Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. *Nature* **1999**, *402*, 656.
6. Bowers, C. Y.; Reynolds, G. A.; Durham, D.; Barrera, C. M.; Pezzoli, S. S.; Thorner, M. O. *J. Clin. Endocrinol. Metab.* **1990**, *70*, 975.
7. Schoen, W. R.; Pisano, J. M.; Prendergast, T. K.; Wyvratt, M. J.; Fisher, M. H.; Cheng, K.; Chan, W. W. S.; Butler, B.; Smith, R. G.; Ball, R. G. *J. Med. Chem.* **1994**, *37*, 897.
8. Bondensgaard, K.; Thøgersen, H.; Ankersen, M.; Hansen, B. S.; Wulff, B. S.; Bywater, R. P. *J. Med. Chem.*, in press.
9. Feighner, S. D.; Howard, A. D.; Prendergast, K.; Palyha, O. C.; Hreniuk, D. L.; Nargund, R.; Underwood, D.; Tata, J. R.; Dean, D. C.; Tan, C. P.; McKee, K. K.; Woods, J. W.; Patchett, A. A.; Smith, R. G.; Van der Ploeg, L. H. T. *Mol. Endocrinol.* **1998**, *12*, 137.
10. Blettner, C. G.; Konig, W. A.; Ruhter, G.; Stenzel, W.; Schotten, T. *Synlett* **1999**, 307.
11. Cotarca, L.; Delogu, P.; Nardelli, A.; Sunjic, V. *Synthesis Stuttgart* **1996**, *5*, 553.
12. Hansen, B. S.; Raun, K.; Nielsen, K. K.; Johansen, P. B.; Hansen, T. K.; Peschke, B.; Lau, J.; Andersen, P. H.; Ankersen, M. *Eur. J. Endocrinol.* **1999**, *141*, 180.
13. General method for preparation of biphenyl tetrazole hydantoins **10** using RNH_2. (5-Tetrazolyl-2-phenyl)-5,5-dimethyl-[1,3,2]-dioxaborinane **3** was immobilized on a 1% DVB resin, using a 2-chlorotrytil linker. Borinane **3** (26 mg, 0.1 mmol, 2 equiv) was dissolved in DCM (2 mL) and DIPEA (68 μL, 0.4 mmol, 8 equiv) and added to the resin (50 mg, 0.05 mmol, 1 equiv). The mixture was agitated for 1 h at 25 °C and subsequently washed with 3×DCM, 2×MeOH and 3×DCM yielding resin bound borinane **4**. 4-Bromobenzaldehyde (27.6 mg, 0.15 mmol, 3 equiv) was dissolved in toluene (1.8 mL), EtOH (0.2 mL) and 1 N $NaOH_{(aq)}$ (0.15 mL, 0.15 mmol, 3 equiv) and the resin was swelled in the mixture. Subsequently the mixture was purged with N_2 for 5 min, where upon $Pd(PPh_3)_4$ (6 mg, 0.005 mmol, 0.1 equiv) was added to the mixture. The reaction vessel was then sealed with a rubber septum, and agitated at 50 °C for 8 h. Subsequent washing with 3×DMF, 2×MeOH, 2×DCM and 3×DMF gave aldehyde **5**.
Amino acid esters (0.15 mmol, 3 equiv) were dissolved in a mixture of (MeOH/DMF/TMOF/AcOH 33:33:33:1), (1 mL) and added to the resin. The mixture was agitated for 1 h before $NaBH_3CN$ (25.2 mg, 0.4 mmol, 4 equiv) dissolved in DMF:MeOH/1:1, (1 mL) was added, and the mixture was agitated at 45 °C for further 5 h. The resin was then washed with 3×DMF and 3×DCM resulting in the amino acid esters **6**.
Amino acid esters **6** was then dissolved in DCM and DIPEA (1 mL DCM and 0.1 mL DIPEA) and agitated 10 min. Triphosgene (45 mg, 0.15 mmol, 3 equiv) in DCM (1 mL) were added and the mixture was agitated for additionally 2 h, and washed with 3×DCM. Primary amines dissolved in DCM (2mL) and DIPEA (25 μL, 0.15 mmol, 3 equiv) was added to the resin, agitated for 3 h and subsequently washed with 3×DCM, 2×DMF and 5×DCM yielding hydantoins **8**. Cleavage from the resin was affected using TFA/DCM 5:95, containing 1% TIS, and the products were purified by reverse phase HPLC. The products were characterized by LC–MS and NMR. Data for compound **10f**: ^1H NMR (400 MHz; $CDCl_3$): δ 1.36 (m, 1H), 1.80 (m, 3H), 1.87 (m, 2H), 2.09 (m, 3H) 2.29, (m, 1H), 2.58 (m, 1H), 2.65 (m, 3H), 2.97 (t, $J=12$ Hz, 1H), 3.07 (t, $J=12$ Hz, 1H), 3.48 (m, 1H), 3.58 (m, 3H), 3.81 (d, $J=16$ Hz, 1H), 4.02 (dd, $J_1=4$ Hz, $J_2=8$ Hz, 1H), 4.88 (d, $J=16$ Hz, 1H), 6.97 (d, $J=8$ Hz, 2H), 7.03 (d, $J=8$ Hz, 2H), 7.14 (d, $J=8$ Hz, 2H), 7.19 (t, $J=8$ Hz, 1H), 7.27 (t, $J=8$ Hz, 3H), 7.36 (dd, $J_1=2$ Hz, $J_2=8$ Hz, 1H), 7.46 (dt, $J_1=2$ Hz, $J_2=8$ Hz, 1H), 7.53 (dd, $J_1=2$ Hz, $J_2=8$ Hz, 1H). Anal. calcd for $C_{30}H_{33}N_7OS{\cdot}CF_3CO_2H{\cdot}H_2O$: C 60.42, H 5.79, N 14.09. Found: C 60.18, H 5.58, N 14.16.
14. *General method for preparation of biphenyltetrazolyl hydantoins using RNCS or RNCO*. Same procedure as above, except that triphosgene treatment was omitted and isocyanates or isothiocyanates were added in DCM/DIPEA.