Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1825–1829
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of tetrazole-based growth hormone secretagogue: The SAR studies of the *O*-benzyl serine side chain

Jun Li,[a,*] Stephanie Y. Chen,[a] Shiwei Tao,[a] Haixia Wang,[a] James J. Li,[a] Steve Swartz,[a] Christa Musial,[a] Andres A. Hernandez,[a] Neil Flynn,[b] Brian J. Murphy,[b] Blake Beehler,[b] Kenneth E. Dickinson,[b] Leah Giupponi,[b] Gary Grover,[b] Ramakrishna Seethala,[b] Paul Sleph,[b] Dorothy Slusarchyk,[b] Mujing Yan,[b] William G. Humphreys,[c] Hongjian Zhang,[c] William R. Ewing,[a] Jeffrey A. Robl,[a] David Gordon[b] and Joseph A. Tino[a,*]

[a]*Discovery Chemistry, Bristol Myers Squibb, Princeton, NJ 08543, USA*
[b]*Metabolic Disease Biology, Bristol Myers Squibb, Princeton, NJ 08543, USA*
[c]*Pharmaceutical Candidate Optimization, Bristol Myers Squibb, Princeton, NJ 08543, USA*

Received 4 January 2008; revised 7 February 2008; accepted 8 February 2008
Available online 13 February 2008

Abstract—The structure–activity relationship of the *O*-benzyl serine side chain was investigated based on the tetrazole-based growth hormone secretagogue BMS-317180 (**2**). The ortho position of the benzyl moiety was found to be favorable for introduction of substituents. A series of ortho-substituted compounds were synthesized with improved in-vitro and in-vivo activity. Among them, the biphenyl compound **2p** shows twofold improvement in potency compared to its parent compound BMS-317180 (**2**).

Since Bowers discovered a novel mechanism for releasing endogenous growth hormone (GH) from the pituitary in 1982, numerous efforts to uncover an orally active growth-hormone secretagogue (GHS) have been reported.[1] The initial focus was on small growth hormone-releasing peptides (GHRPs), including hexarelin, that produced a GH response from the pituitary in a physiologically pulsatile pattern in animals and humans.[1,2] A group from Merck disclosed a G-protein coupled receptor found in the pituitary and the hypothalamus was cloned and identified as the growth hormone secretagogue receptor (GHSR), which is distinct from the growth hormone releasing hormone (GHRH) receptor.[2c] Subsequently, small non-peptidic GHS agents including L-692429 and MK-0677 (**1**) were reported.[3] MK-0677 is the first potent GH secretagogue with excellent oral bioavailability in dogs.[3b] Subsequently, more small molecule GHSs have been reported by pharmaceutical companies.[3,4] The natural ligand ghrelin for the GHS receptor was also discovered.[7]

Recently, we disclosed a potent, orally active GHS agonist, BMS-317180, which was based on a tetrazole core and was advanced into clinical evaluation.[5] To further improve the potency and pharmacological properties of BMS-317180, extensive SAR studies were conducted. Herein we report the SAR study around the left-hand *O*-benzyl serine side chain of BMS-317180 (**2**) (Fig. 1).

Our initial approach focused on halogen-substituted benzyl analogues of **2**. The synthesis of 2, 4-difluro-benzyl-substituted BMS-317180 (**2a**) is exemplified in

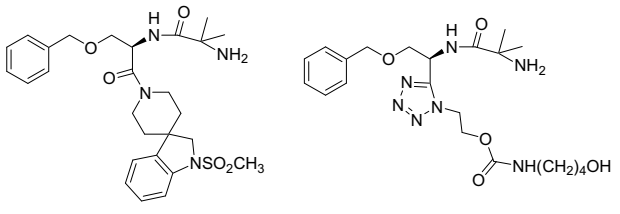


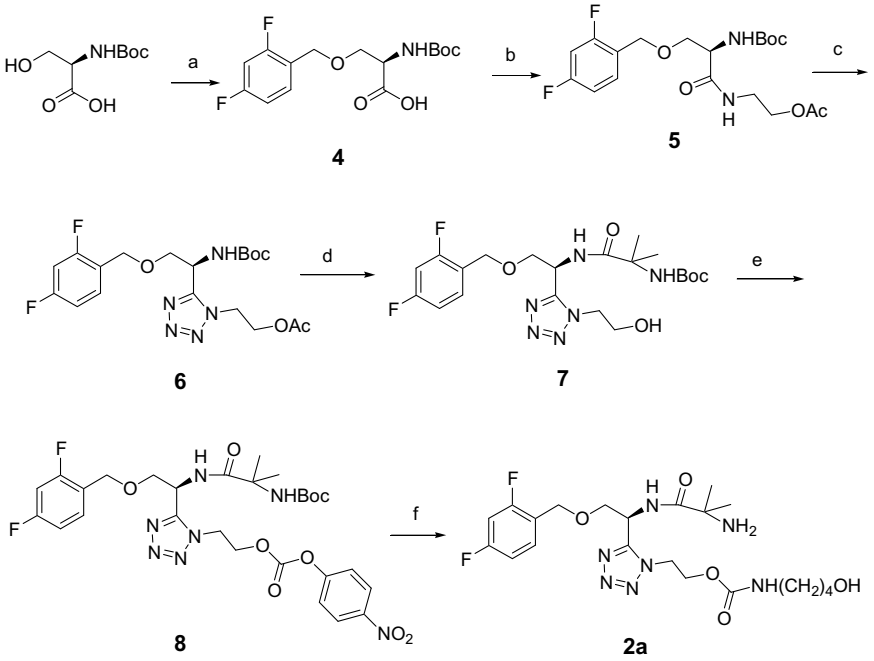
Figure 1. Small-molecule growth-hormone secretagogues: MK-677 and BMS-317180.

Keyword: Growth hormone secretagogue.
* Corresponding authors. Tel.: +1 609 818 7123; fax: +1 609 818 6810 (J.L.); e-mail addresses: jun.li@bms.com; joseph.tino@bms.com

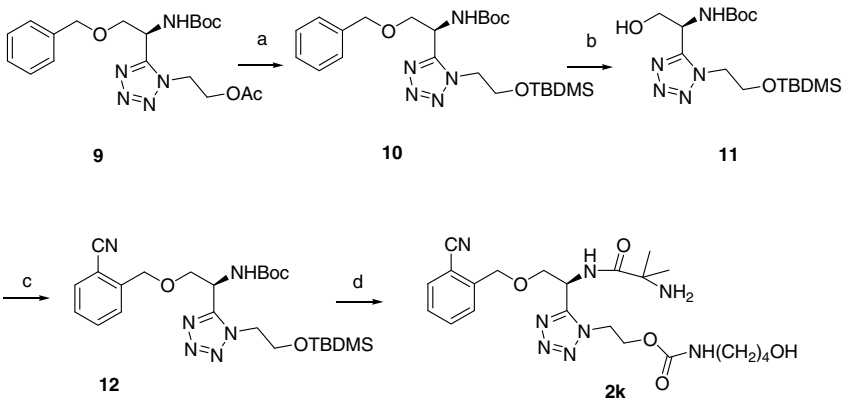
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.02.021

Scheme 1. Direct alkylation of Boc-D-serine by 2,4-difluro-benzyl bromide gave the desired benzylated serine **4**. The chirality of **4** was retained by ensuring no excess NaH during the reaction. The completion of the synthesis of **2a** followed our previously reported procedures.[5] Coupling of **4** with aminoethanol, followed by acylation of the crude alcohol gave the desired acetate **5**. The formation of tetrazole was carried out by treatment of **5** with two equivalents of triphenylphosphine, diethyl azodicarboxylate, and azidotrimethylsilane.[6] After stirring at room temperature for 48 h, the tetrazole **6** was isolated in 55% yield. A three-step sequence of Boc-deprotection of **6**, followed by amino-acid coupling of amine with *N*-Boc-methylalanine, and subsequent hydrolysis of the acetate by lithium hydroxide in THF yielded the alcohol **7**. Alcohol **7** was then treated with 4-nitrophenyl chloroformate in the presence of pyridine to give the carbonate **8** in 50% yield from **6**. Carbonate **8** was reacted with 4-amino-butanol to give the Boc-protected carbamate in 90% yield. Deprotection of the Boc group using 4 N HCl in dioxane gave the HCl salt of **2a**. The overall yield ranged between 5% and 9% starting from Boc-D-serine.

An alternative route was developed for multiple analog synthesis as shown in Scheme 2. Hydrolysis of acetate **9** with lithium hydroxide followed by protection with *tert*-butyldimethylsilyl resulted in the silyl ether **10**. Hydrogenation of **10** afforded a key intermediate, alcohol **11**. Alkylation of **11** with a substituted benzyl bromide or chloride generated **12** without loss of chirality by using no more than two equivalents of sodium hydride. Further elaboration to the final compounds proceeded as described in Scheme 1.

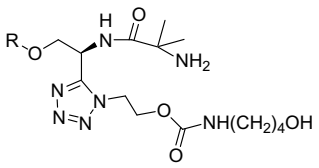


Scheme 1. Reagents and conditions: (a) i—2,4-difluoro-benzyl bromide, NaH, DMF; (b) i—NMM, iso-butyl-chloroformate, −40 °C, ii—2-amino-ethanol, −40 °C, iii—Ac_2O, pyridine; (c) $TMSN_3$, DEAD, Ph_3P, rt, 48 h; (d) i—HCl/dioxane, rt, ii—HOAT, EDAC, *N*-Boc-methylalanine, CH_2Cl_2, overnight, iii—1 N LiOH; (e) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2; (f) i—4-amino-butanol, ii—HCl/dioxane.



Scheme 2. Reagents: (a) i—4 N LiOH, THF, ii—TBDMSCl, imidazole, DMF; (b) 5% $Pt(OH)_2$/C (cat), AcOH (cat), H_2 (60 psi), MeOH; (c) 2-cyno-benzyl bromide, NaH, DMF; (d) See Scheme 1, step c–d.

Table 1. In-vitro potency of *O*-benzyl serine side chain analogs[8]



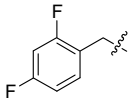
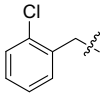
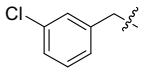
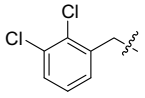
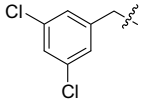
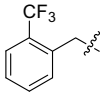
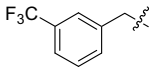
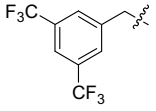
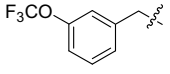
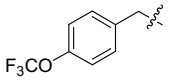
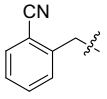
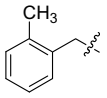
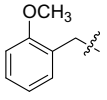
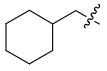
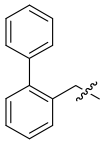
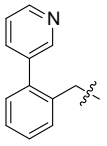
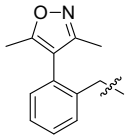
Compound	R	EC_{50}[a] (nM)
Ghrelin		1.41
2	Bn	1.92
2a		0.42
2b		0.45
2c		1.6
2d		0.61
2e		6.75
2f		0.72
2g		1.79
2h		34
2i		3.1
2j		33.2
2k		1.26
2l		0.51
2m		1.11

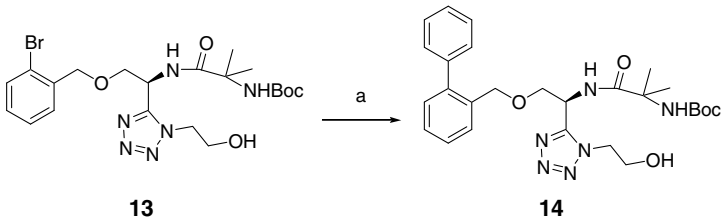
Table 1 (*continued*)

Compound	R	EC_{50}[a] (nM)
2n		1.66
2o		0.34
2p		1.18
2q		1.04

[a] Values are means of three experiments.

The major objective of this work was to study the substituent effects on the benzyl ring. Halogen-related substituents were initially chosen to study the positional effect of benzyl substitution as shown in Table 1. Small substituents such as fluorine at both *para* and *ortho* positions (**2a**) improved the potency over compound **2**. The *ortho*-chlorine or *ortho*-trifluoromethyl-substituted benzyl analogs (**2b**, **2f**) were also found to improve functional in-vitro activity 3- to 4-fold when compared to **2**. The same substituents at the *meta* position (**2c**, **2g**, and **2i**) had little effect on the potency. In contrast, a *para*-substituted trifluoromethyloxy (**2j**) resulted in a significant loss in potency when compare to the *meta*-substituted analog **2i**. The potency was also significantly reduced when both *meta* positions of the benzyl were occupied by either chlorine or trifluoromethyl groups (**2e**, **2h**). However, the 2,3-di-chloro-substituted phenyl (**2d**) still maintained the excellent functional activity. These initial results suggested that the ortho-position may be a favorable site for further SAR study of the substituent effects on the benzyl ring. For this purpose, several non-halogen functional groups with various stereo and electronic properties were introduced. All groups including both electron-withdrawing group such as cyano (**2k**), and electron-donating groups such as methyl (**2l**) or methoxy (**2m**), gave similar results with good to excellent in vitro potency. This result indicated that the electronic effect of the substituents on the benzyl ring had minimal or negligible consequences on potency. Furthermore, removing the aromaticity by using cyclohexane as the replacement for the phenyl ring resulted in **2n** showed in-vitro potency similar to the parent compound **2**. This result suggested that the aromatic ring may not be essential to maintain the potency.

We next turned our attention to aromatic substitutions at the *ortho*-position (Scheme 3). Addition of the second aromatic ring was achieved via Suzuki coupling of aryl



Scheme 3. Reagent and condition: (a) PhB(OH)2, Pd (0) (cat), toluene, 100 °C.

Table 2. In vivo activity of the compounds in the acute anesthetized rat model at 1.74 μmol/kg

Compound	Increase GH[a] at 1.74 μmol/kg (% of vehicle)	Responders[b]
2	750 (±112)	5/5
2a	1081 (±130)	5/5
2k	920 (±257)	5/5
2l	1441 (±332)	5/5
2n	1654 (±117)	5/5
2o	836 (±192)	5/5
2q	5237 (±1233)	5/5

[a] Values are means of five experiments, standard deviation is given in parentheses.

[b] A rat with more than 200% GH increase over vehicle control is considered as a responder.

boronic acids with the *ortho*-bromo-phenyl intermediate **13,** which was prepared similarly according to Scheme 1. The completion of the synthesis from **14** proceeded as described in Scheme 1. The biphenyl analog **2p** was found to be the most potent compound within the series in this in-vitro assay. However, polar aromatic substituents such as pyridine or dimethyl-isoxazole (**2p, 2q**), only slightly improved in-vitro potency over compound **2**. This phenomenon suggested a deeper hydrophobic binding pocket might be located in this region of the GHS receptor.[9]

Several compounds were chosen for evaluation in an acute anesthetized rat model measuring the increased GH levels as the efficacious response. The compounds were administered intravenously at a dose of 1.74 μmol/kg. After 15 min, blood samples were collected and the plasma isolated and analyzed for rat growth hormone (GH) via radioimmunoassay.[12] Data are expressed as the percentage of increase GH release compared with vehicle control animals (Table 2).

In agreement with the in-vitro results, compounds **2a**, **2k** and **2l** all showed excellent in-vivo activity in the acute anesthetized rat model and compared favorably to the parent compound **2**. Interestingly, compound **2n,** with a cyclohexyl group instead of phenyl, is twice as potent as compound **2** in the rat model despite their similar in-vitro potency. However, compound **2o**, with the most potent in-vitro potency in this series, showed less than expected in-vivo activity considering its fivefold improvement in intrinsic potency. Unexpectedly, compound **2q** showed significant in-vivo activity in the rat model. One possible explanation for this greater than expected potency may be more favorable distribution to the target site(s) and/or more favorable pharmacodynamic properties. Several of the analogs in Table 2 were studied in a rat PK screen at a 10 mg/kg oral dose. However, all compounds tested, including the most potent in vitro compound **2o,** and the most active in vivo compound **2q**, gave low plasma exposures. Since the in vitro activity was determined on human cell-based assay, a species difference might be another reason for this unexpected in vivo activity in the rat model. The disconnection of the in vitro and in vivo activities for some compounds in certain animal models was also reported by other research groups.[10]

In summary, the SAR of the benzyl moiety of BMS-317180 (**2**) has been described. The results suggest that the ortho position of the benzyl moiety is the preferred position for substitution. A hydrophobic aromatic group at the ortho position of the phenyl was found to greatly increase the functional activity at the GHS receptor. Some revealed analogs also improved the in vivo activity in the rat model when compared to parent compound **2**. The initial efforts to replace the phenyl ring also resulted in the discovery of the non-aromatic functional group cyclohexane as an alternative for the aromatic benzene ring.

Acknowledgment

We thank Dr. Robert Zahler for his advice and suggestions for the manuscript.

References and notes

1. (a) Bowers, C. Y. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 9–28; For a review on the clinical actions of GHRPs, see: (b) Ghigo, E.; Arvat, E.; Muccioli, G.; Camanni, F. *Eur. J. Endocrinol.* **1997**, *136*, 445, and references therein.
2. (a) Smith, R. G.; Ploeg, L. T. V. D.; Howard, A. D.; Feighner, S. D.; Cheng, K.; Hickey, G. J.; Wywratt, J. J.; Fisher, M. H.; Nargund, R. P.; Patchett, A. A. *Endocrinol. Rev.* **1997**, *18*, 621; (b) Smith, R. G.; Cheng, K.; Pong, S. S.; Hickey, H.; Jacks, T.; Butler, B.; Chan, W.-S.; Chaung, L. Y. P.; Judith, F.; Taylor, J. A.; Wyvratt, M. J.; Fisher, M. H. A. *Science* **1993**, *260*, 1640; (c) Howard, A. D.; Feighner, S. D.; Cully, D. F.; Arena, J. P.; Liberator, P. A.; Rosenblum, C. I.; Hamelin, M.; Hreniuk, D. L.; Palyha, O. C.; Anderson, J.; Paress, P. S.; Diaz, C.; Chou, M.; Liu, K. K.; McKee, K. K.; Pong, S.-S.; Chaung, L.-Y. P.; Elbrecht, A.; Dashkevicz, M.; Heavens, R.; Rigby, M.;

Sirinathsinghji, D. J. S.; Dean, D. C.; Melillo, D. G.; Patchett, A. A.; Nargund, R. P.; Griffin, P. R.; DeMartino, J. A.; Gupta, S. K.; Schaeffer, J. M.; Smith, R. G.; Van der Ploeg, L. H. T. *Science* **1996**, *273*, 974; (d) Smith, R. G. *Endocrine Rev.* **2005**, *16*, 436.

3. (a) Schoen, W. R.; Pisano, J. M.; Prendergast, K.; Wyvratt, M. J., Jr.; Fisher, M. H.; Cheng, K.; Chan, W. W. S.; Butler, B.; Smith, R. G.; Ball, R. G. *J. Med. Chem.* **1994**, *37*, 897; (b) Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M.-H.; Barkat, K. J.; Johnston, D. B. R.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Hickey, G.; Jacks, T.; Schleim, K.; Pong, S.-S.; Chaung, L.-Y. P.; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S.-H. L.; Smith, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7001; (c) Hansen, T. K.; Ankersen, M.; Hansen, B. S.; Raun, K.; Nielsen, K. K.; Lau, J.; Peschke, B.; Lundt, B. F.; Thgersen, H.; Johansen, N. L.; Madsen, K.; Andersen, P. H. *J. Med. Chem.* **1998**, *41*, 3705; (d) Yang, L.; Morriello, G.; Patchett, A. A.; Leung, K.; Jacks, T.; Cheng, K.; Schleim, K. D.; Feeney, W.; Chan, W. W.-S.; Chiu, S. L.; Smith, R. G. *J. Med. Chem.* **1998**, *41*, 2439; (e) Tokunaga, T.; Hume, W. E.; Umezome, T.; Okazaki, K.; Ueki, Y.; Kumagai, K.; Hourai, S.; Nagamine, J.; Seki, H.; Taiji, M.; Noguchi, H.; Nagata, R. *J. Med. Chem.* **2001**, *44*, 4641; (f) Carpino, P. A.; Lefker, B. A.; Toler, S. M.; Pan, L. C.; Hadcock, J. R.; Murray, M. C.; Cook, E. R.; DiBrino, J. N.; DeNinno, S. L.; Chidsey-Frink, K. L.; Hada, W. A.; Inthavongsay, J.; Lewis, S. K.; Mangano, F. M.; Mullins, M. A.; Nickerson, D. F.; Ng, O.; Pirie, C. M.; Ragan, J. A.; Rose, C. R.; Tess, D. A.; Wright, A. S.; Yu, L.; Zawistoski, M. P.; Pettersen, J. C.; DaSilva-Jardine, P. A.; Wilson, T. C.; Thompson, D. D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3279.
4. Nargund, R. P.; Patchett, A. A.; Bach, M. A.; Murphy, M. G.; Smith, R. G. *J. Med. Chem.* **1998**, *41*, 3103.
5. Li, J.; Chen, S.; Ewing, W.; Hernandez, A.; Li, J. J.; Musial, C.; Qu, F.; Swartz, S.; Tao, S.; Wang, H.; Beehler, B.; Dickinson, K.; Giupponi, L.; Grover, G.; Rehberg, E.; Seethala, R. K.; Sleph, P.; Slusarchyk, D.; Smith, M.; Yan, M.; Stouch, T.; Humphreys, W. G.; Zhang, H.; Harper, T.; Gordon, D.; Biller, S.; Robl, J.; Tino, J. *J. Med. Chem.* **2007**, *50*, 5890.
6. Duncia, J. V.; Pierce, M. E.; Santella, J. B., III *J. Org. Chem.* **1991**, *56*, 2395.
7. Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. *Nature* **1999**, *402*, 656.
8. (a) Cells overexpressing endogenous human growth hormone secretagogue receptor by Random Activation of Gene Expression (RAGE)^{TM} (Athersys, Inc.) technology were used.[13] The EC_{50} was measured by determining intracellular calcium concentration in a FLIPR assay with Ghrelin as having 100% intrinsic functional activity. All EC_{50} values are the averages of multiple measurements; (b) Harrington, J. J.; Sherf, B.; Rundlett, S.; Jackson, P. D.; Perry, R.; Cain, S.; Leventhal, C.; Thornton, M.; Ramachandran, R.; Whittington, J.; Lerner, L.; Costanzo, D.; McElligott, K.; Boozer, S.; Mays, R.; Smith, E.; Veloso, N.; Klika, A.; Hess, J.; Cothren, K.; Lo, K.; Offenbacher, J.; Danzig, J.; Ducar, M. *Nat. Biotechnol.* **2001**, *19*, 440.
9. Ariens, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. In *The Receptors, a Comprehensive Treatise*; O'Brien, R. D., Ed.; Plenum Press: New York, London, 1979; p 33.
10. Hansen, T. K.; Ankersen, M.; Hansen, B. S.; Raun, K.; Hansen, B. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1915.