Discovery of a Tetrazole-Based Growth Hormone Secretagogue: 4-(Hydroxybutyl)carbamic Acid 2-{5-[1-(2-Amino-2-methylpropionylamino)-2-benzyloxyethyl]tetrazol-1-yl}ethyl Ester (BMS-317180)

Jun Li,*,† Stephanie Y. Chen,† James J. Li,† Haixia Wang,† Andres S. Hernandez,† Shiwei Tao,† Christa M. Musial,† Fucheng Qu,† Stephen Swartz,† Sam T. Chao,† Neil Flynn,‡ Brian J. Murphy,‡ Dorothy A. Slusarchyk,‡ Ramakrishna Seethala,‡ Mujing Yan,‡ Paul Sleph,‡ Gary Grover,‡ Mark A. Smith,‡ Blake Beehler,‡ Leah Giupponi,‡ Kenneth E. Dickinson,‡ Hongjian Zhang,§ William G. Humphreys,§ Bharat P. Patel,|| Mark Schwinden,|| Terry Stouch,† Peter T. W. Cheng,† Scott A. Biller,† William R. Ewing,† David Gordon,‡ Jeffrey A. Robl,† and Joseph A. Tino*,†

Departments of Discovery Chemistry, Metabolic Diease, Metabolism and Pharmacokinetics, and Process Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Building 13, P.O. Box 5400, Princeton New Jersey 08543

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Abstract: A tetrazole-based peptidomimetic **2** (BMS-317180) was discovered as a human growth hormone secretagogue (GHS). Compound **2** is a potent, novel, orally effective GHS that shows an excellent safety profile in preclinical studies. The compound was advanced into clinical development.

The rapidly growing global population of the elderly will place a large strain on the health care system as more individuals acquire severe functional disabilities. One of the driving factors in functional decline is loss of muscle mass, eventually leading to the inability to perform basic activities of daily living (driving, shopping, cooking). The inevitable decline eventually leads to dependence on family members and/or institutional caregivers. One potential cause for functional decline is a collapse of the growth hormone (GH)[a] and insulin-like growth factor-1 (IGF-1) axis. Growth hormone is a single polypeptide chain of 191 amino acids with a molecular mass of 22 kDa. The release of growth hormone from somatotrophs in the pituitary gland is known to be regulated by growth-hormone-releasing hormone, which stimulates growth hormone release, and by somatostatin, which inhibits growth hormone release. GH drives increases in IGF-1, as well as the related IGF binding proteins. Human growth hormone has been used clinically for the treatment of GH-deficient children since the 1950s; however, treatment of short-stature GH-deficient children and adolescents, as well as other therapeutic applications, was not widely studied until the availability of recombinant human GH (rhGH) in the mid-1980s. The ready availability of rhGH has made it possible to investigate additional applications for GH replacement therapy. Some recent clinical studies suggest the benefits of using GH for the treatment of frailty in the elderly, osteoporosis, obesity, perisurgical or parenteral nutrition, and in a variety of catabolic states.[1] However, a major drawback of treatment is the inconvenience of intravenous (iv) injections (3−5 times per week). Multiple adverse side-effects such as edema, arthralgia, and carpel tunnel syndrome are also associated with dosing. Furthermore, the cost of the GH replacement therapy is high.

In the 1980s, Bowers and co-workers reported a novel mechanism for releasing endogenous GH from the pituitary.[2] Several small growth-hormone-releasing peptides (GHRPs), including the potent GHRP-6 and hexarelin, were identified in Bowers's lab.[3] The GHRPs demonstrated the production of a GH response from the pituitary in a physiologically pulsatile pattern in animals and humans.[4] These results led the pharmaceutical industry to develop orally active peptidomimetics to replicate the action of GHRPs. Subsequently, the discovery of a GHS receptor which binds the GHRPs was reported.[5] This advance resulted in the discovery of several promising small-molecule GHS agonists, including **1a** (L-692429)[6a] and **1b** (MK-0677),[6b] the latter being the first potent GH secretagogue with excellent oral bioavailability in dogs. Subsequently, additional GHS agonists have been reported by pharmaceutical companies.[7] Recently, the Kojima group reported a major advance with the discovery of ghrelin, a 28-amino acid peptide, which is the natural ligand for the GHS receptor.[8] The discovery of ghrelin led to further interest in this field,[9] and the potential application of ghrelin and GHSs for cancer cachexia, as well as wasting syndrome, has been reported.[10] Encouraged by these discoveries and developments in the field, we initiated a GHS research program and herein report efforts leading to the discovery of tetrazole-based GH secretagogues.

Although a majority of the previously reported GHS agonists were derived from modified dipeptide backbones, we chose to utilize a heterocycle as the core linker in our structural design. Heterocycles are known amide surrogates in peptide mimetic design.[11] In addition to imparting structural novelty as a GHS agent, a heterocyclic linker will fix the geometry by conferring conformational rigidity. The heterocyclic core also imparts interesting physical properties to a GHS agent, such as a unique electron charge density, decreased lipophilicity, and improved water solubility. Peptidomimetics with a heterocyclic linker may also have dramatically different chemical and enzymatic stability in addition to unique SAR at the GHS receptor.[11a] The application of heterocycles as amide surrogates in the design of therapeutic agents has received increasing attention due to the above-described potential benefits. Many five- and six-membered ring heterocycles have been incorporated in the synthesis of peptidomimetics.[11] However, at the time of our work, the tetrazole moiety, a five-membered heterocyclic ring frequently used as a carboxylic acid bioisostere in drug design, had received relatively little attention as an amide surrogate. Some limitations of the tetrazole group as an amide mimic include the lack of a hydrogen bond donor when disubstituted, the restriction to disubstitution, and the length of the synthetic routes. On the other hand, a 1,5-disubstituted tetrazole provides an excellent conformational mimic of a *cis*-amide bond, as that found with proline and *N*-alkylamides, which exists in a wide variety of biologically important peptides.[12] Since the reported small-molecule GHSs (Figure 1a−e) all contain *N*-alkylamide bonds,[7] we hypothesized that a 1,5-disubstituted tetrazole may

* To whom correspondence should be addressed. Phone: 609-818-7123. Fax: 609-818-3450. E-mail: jun.li@bms.com (J.L.); E-mail: joseph.tino@bms.com (J.A.T.).
† Discovery Chemistry.
‡ Department of Metabolic Diease.
§ Department of Metabolism and Pharmacokinetics.
|| Department of Process Chemistry.

[a] Abbreviations: GHS, growth hormone secretagogue; GH, growth hormone; IGF-1, insulin-like growth factor-1; rhGH, recombinant human growth hormone; GHRPs, growth-hormone-releasing peptides; FLIPR, fluorescence imaging plate reader.

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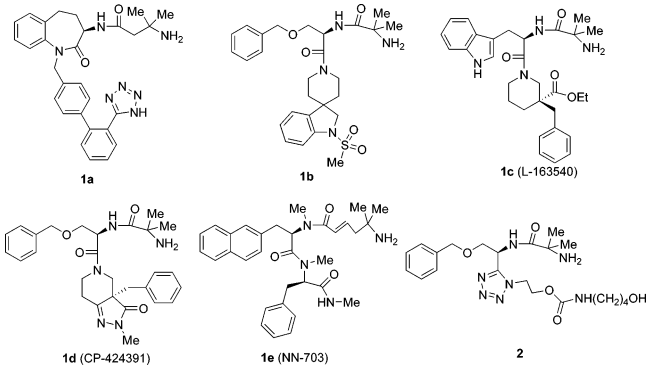
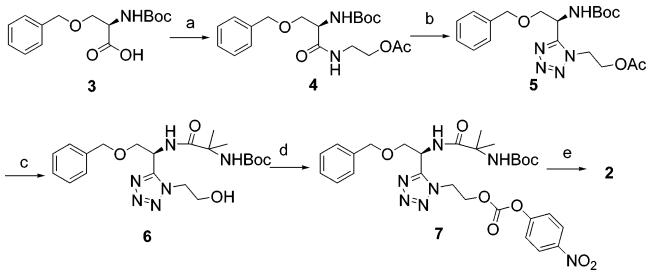


Figure 1. Small-molecule GHS agonists.

Scheme 1. Synthesis of Compound **2**[a]



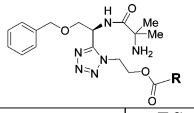
[a] Reagents and conditions: (a) i: NMM, isobutylchloroformate, −40 °C; (ii) 2-aminoethanol, −40 °C; (iii) Ac_2O, pyridine. (b) $TMSN_3$, DEAD, Ph_3P, rt, 48h. (c) (i) HCl/dioxane, rt; (ii) HOAT, EDAC, *N*-Boc-methylalanine, CH_2Cl_2, overnight; (iii)1 N LiOH. (d) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2. (e) (i) 4-aminobutanol; (ii) HCl/dioxane.

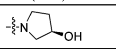
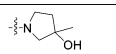
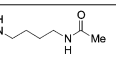
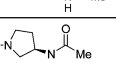
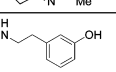
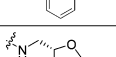
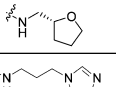
be a suitable surrogate and provide an avenue toward the generation of conformationally restricted, novel, peptidomimetic GH secretagogues.

Herein, we report the tetrazole-based compound **2** (BMS-317180) as a potent, highly water soluble GHS agent with acceptable oral bioavailability in rats, dogs, and monkeys.

The synthesis of compound **2** and its analogues is depicted in Scheme 1. Amino acid coupling of commercially available *N*-Boc-*O*-benzyl-D-serine (**3**) with aminoethanol, followed by acylation of the crude amide, gave the crude acetate **4** in adequate purity for the subsequent reaction. Alternatively, the amide **3** and acetate **4** could each be purified by a short silica gel pad filtration. The optimal conditions for tetrazole formation were sequential treatment of acetate **4** with one equivalent of triphenylphosphine, diethyl azodicarboxylate, and azidotrimethylsilane, followed by additional equivalents of each after 24 and 48 h.[13] After a total of 72 h, the solid tetrazole **5** was isolated in 85% yield after silica gel column chromatography. The three-step sequence of Boc deprotection of **5**, amino acid coupling with *N*-Boc-methylalanine (or Boc-aminoisobutyric acid) and subsequent lithium hydroxide hydrolysis of the acetate to give alcohol **6**, was conducted without purification of the intermediates due to partial loss of the acetate protecting group during the Boc deprotection step. The alcohol **6** is a low melting solid, isolated by silica gel column chromatography in 71% overall yield from acetate **5**. Treatment of alcohol **6** with 4-nitrophenyl chloroformate gave the carbonate **7** in 83% yield after silica gel column chromatography. Subsequent reaction with 4-aminobutanol gave the Boc-protected carbamate in high yield after aqueous workup. Deprotection of the Boc group using 4 N HCl in dioxane gave the HCl salt of **2** as an oily foam, which could be neutralized during aqueous workup to obtain the pure free base as an oil. The hydrochloride salt could be regenerated by addition of a 2 M HCl solution in ether to the free base, followed by ether trituration of the resulting solid. The resulting salt is a hygroscopic solid that can be weighed quickly in the air without appreciable moisture uptake. Compound **7** also served as a useful intermediate in the generation of additional analogues in this series. Reaction of **7** with various amines, followed by Boc deprotection, afforded the related carbamates **8**−**18**.

Table 1: In Vitro Activity of **2** and Its Analogues



Entry	R	EC_{50} (nM)*
ghrelin		1.4
2	-$NH(CH_2)_4OH$	1.9
8	-$NH(CH_2)_2OH$	3.0
9	-$NH(CH_2)_3OH$	5.5
10	-$NH(CH_2)_5OH$	14.6
11	-$NMe(CH_2)_4OH$	3.8
12		1.0
13		10.1
14		82.5
15		3.3
16		7.9
17		9.5
18		45.3

*Full intrinsic activity (relative to ghrelin) was observed for all tested compounds.

One commonly used method for in vitro evaluation of GHS activity has been the measurement of GH release from isolated rat pituitary cells.[7c] Since this method was not amenable for high-throughput screening, a higher throughput cell-based assay was required. Utilizing the GHS receptor's natural signaling through a calcium phospholipase C mediated pathway, agonists could be tested for their ability to stimulate intracellular calcium mobilization using a calcium-sensitive fluorescent probe in an automated fluorescence imaging plate reader (FLIPR).[14] Accordingly, a cell-based functional FLIPR assay was developed from H4 glioma cells in which expression of the endogenous human GHS receptor was enhanced by RAGE-activation.[15] The EC_{50} was measured by determining the intracellular calcium concentration in a FLIPR assay, with ghrelin serving as the full agonist reference standard. All of the compounds were determined to be full agonist of the receptor, and their in vitro EC_{50} values are summarized in Table 1.

The secondary carbamates **2**, **8**, and **9**, bearing a primary hydroxyl group, all showed good to excellent potency despite variation of the chain length. However, compounds with more than four carbon atoms in the chain, such as alcohol **10**, were less potent. The aromatic phenol **16** also exhibited moderate potency, with an EC_{50} of 7.9 nM. Attempts to replace the primary hydroxyl group by an acetamide (e.g., **14**) or other hydroxyl isosteres, such as ureas or sulfonamides, led to a 5−20 fold drop in potency. Heterocyclic replacements such as tetrahydrofuran (**17**) maintained moderate potency with an EC_{50} of 10 nM, while the imidazole was clearly less active at 45 nM. The results suggested that an oxygen group may play an important role in GHS receptor activation. To investigate the role of the carbamate N−H group, the N-methylated tertiary carbamate analogue **11** was prepared, resulting in only a small drop in potency compared to that of the parent **2**. This result

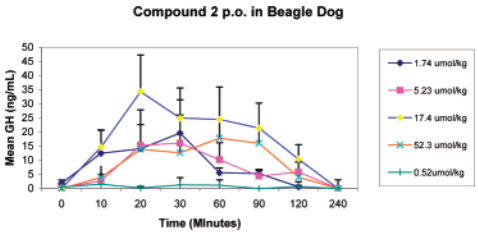


Figure 2. Pharmacodynamic response to a single ascending oral dose of **2** in conscious beagle dogs.

suggested that a secondary or cyclic amine might be suitable as a replacement for the primary amine. Indeed, the pyrrolidine-based secondary alcohol **12** (EC_{50} = 1.0 nM) was identified as one of the most potent compounds in this series. However, the pharmacokinetic profile of compound **12** was inferior to that of compound **2**. Efforts to address the potential liability of the secondary alcohol in **12** by incorporation of the tertiary alcohol **13** (EC_{50}=10.1 nM) resulted in a 10-fold reduction in potency. In contrast to that of the acyclic acetamide **14**, the pyrrolidine acetamide **15** exhibited good in vitro functional potency (EC_{50} = 3.3 nM) as a mixture of diastereomers. Unfortunately, the PK profile of **15** was unacceptable due to its low bioavailability in a rat model (F < 5%). The carbamate moiety was found to be a metabolically stable linker for the chemotype; the overall hydroxybutyl carbamic acid gave excellent potency and PK properties. Other attempts to replace the carbamate in **2** with an amide, urea, or reversed carbamate resulted in similar or less potent analogues; the results are to be reported elsewhere.

The in vivo efficacy of compounds was evaluated in an acute anesthetized intravenously dosed rat model, monitoring plasma GH increases.[16] In this model, **2** showed excellent activity, producing a rapid and robust GH plasma elevation when administered iv to rats (ED_{50} = 0.08 μmol/kg). Figure 2 shows the growth hormone pharmacodynamic response following single oral doses of **2** in conscious beagle dogs. The vehicle (water) treated control animals showed no GH increase over the 4 h time course of the experiment (data not shown). At a dose of 0.52 μmol/kg, only a minimal increase in plasma GH was observed. Significant increases in plasma GH were achieved at the doses of 1.74 and 5.23 μmol/kg, and a further increase in plasma GH was observed at the dose of 17.4 μmol/kg. The response plateaued at 52.3 μmol/kg. The maximum GH concentration occurred between 15 and 60 min after dosing and remained elevated for up to 120 min.

The pharmacokinetic characteristics of compound **2** were evaluated in fasted rats, cynomolgus monkeys, and beagle dogs at single oral doses of 15, 10, and 10 μmol/kg, respectively. The iv dose was 5 μmol/kg for all species. The oral bioavailability of **2** in rats was 9%, with a half-life of 0.7 h. In monkeys, the oral bioavailability was 12%, with a half-life of 1.1 h. Compound **2** demonstrated superior exposure in dogs relative to the other species, with an oral bioavailability of 40% and a 2.3 h half-life. The low oral bioavailability of **2** in rats and monkeys was probably attributable to its high plasma clearance (213 and 37 mL/min/kg in rats and monkeys, respectively). For comparison, the clearance rate of **2** in dogs was 21 mL/min/kg.

Compound **2** was found to have no significant activity (>50% inhibition at 10 μM) in the PanLabs SpectrumScreen in vitro assay panel[17] and was not hepatotoxic up to 250 μg/mL in a human hepatocyte assay. The IC_{50} values for compound **2** were all greater than 100 μM against human CYP enzymes 3A4, 2C9, 2C19, 2D6, and 1A2. Ames testing also showed that compound **2** was not mutagenic to the tester strains TA 98 and TA 100.

In summary, we report a potent, highly water soluble (>100 mg/mL at pH 4), orally active GH secretagogue containing a novel tetrazole linker. The safety profile and acceptable pharmacokinetic properties of **2** were suitable for long-term efficacy evaluation studies for the prevention of frailty and treatment for cancer cachexia as well as wasting syndrome, and as a result, the compound was advanced for clinical evaluation.

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Supporting Information Available: Experimental procedure for the preparation of **2** and ^{1}H NMR, HPLC, and HRMS data for compounds **2**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Fasted male Wistar rats (200−250 g) were anesthetized via intraperitoneal injection with ketamine (30 mg) and xylazine (10 mg) per kilogram of body weight. The drug or vehicle (10% ethanol, 0.09% saline v/v) was administered intravenously at a volume of 10 mL/kg to a group of five rats. After 15 min, a 1.5 mL blood sample was drawn from the abdominal aorta. Plasma samples were then assayed for rat growth hormone by radioimmunoassay using a modification of the kit supplied by the National Pituitary Hormone Center (Dr. A. Parlow, Harbor-UCLA Medical Center, Los Angeles, CA).

(17) The PanLabs Spectrum Screen is a proprietary in vitro screen using a panel of 85 receptors and ion channels.

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