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Orally active ghrelin receptor inverse agonists and their actions on a rat obesity model

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ABSTRACT

A series of 2-alkylamino nicotinamide analogs was prepared as orally active ghrelin receptor (ghrelinR) inverse agonists. Starting from compound **1**, oral bioavailability was improved by modifying metabolically unstable sites and reducing molecular weight. Brain-permeable compound **33** and compound **24** with low brain permeability were tested in rat models of obesity; 30 mg/kg of compound **33** suppressed weight gain. PK/PD analysis revealed that the anti-obesity effect of ghrelinR inverse agonists depends on their brain concentrations.

1. Introduction

Ghrelin is a gut-derived 28-amino-acid peptide that has an important role in food intake and energy homeostasis and is the endogenous ligand for growth hormone secretagogue receptor 1a (GHS-R 1a),[1] currently known as the ghrelin receptor (ghrelinR). It has been suggested that ghrelin acts as a feeding signal as the plasma ghrelin level increases before meals and declines after eating,[2] and ghrelin is the only peripheral orexigenic hormone identified to date.[3] Besides orexigenic action, ghrelin is known to regulate glucose homeostasis,[4] gastric motility[5] and addictive behavior.[6] Therefore, disruption of ghrelin signal is an attractive target for anti-obesity, anti-diabetes and anti-alcohol addiction therapeutics.[7]

In determining target profiles of small molecules that suppress ghrelinR signaling, we considered two things: antagonists or inverse agonists and peripheral or central. GhrelinR exhibits high constitutive activity, and the constitutive signal reaches almost half that of ghrelin-induced activity,[8] which implies that ghrelinR inverse agonists might suppress the signal more effectively than ghrelinR antagonists. Nevertheless, ghrelinR inverse agonists are relatively scarce and have been reported only recently by Merck,[14] AstraZeneca[15] and Pfizer.[16] Therefore, we decided to search for inverse agonists. It is said that orexigenic action of ghrelin is caused mainly via three routes: the vagal afferent nerves, the pituitary gland, and the central nervous system (CNS). Although how much each route works remains under dispute, some data showed that the vagal afferent nerves route is dominant[17,18] whereas other data showed that the CNS effect is dominant.[19–21] While almost all anorectic drugs act through CNS targets, from the viewpoint of drug discovery, a non-CNS anti-obesity drug is attractive because of the lower risk of CNS-related adverse effects. During the target validation, our in-house data reproduced the result that ghrelin-induced food intake was canceled in vagotomized rats,[17] which encouraged us to seek peripheral ghrelinR ligands.

Our first effort led to a non-CNS penetrable ghrelinR inverse agonist (**1**)[22] whose brain/plasma ratio (B/P ratio) was 0.005 (Fig. 1). This compound suppressed ghrelin-induced growth hormone (GH) release in rats, ghrelin-induced gastric mobility in mice, ghrelin-induced food intake in mice and cumulative food intake for two weeks in high-fat diet-induced obesity (HFDIO) mice, which further supports the idea that peripheral ghrelinR inhibition can be sufficient for reducing food intake. Since compound **1** is not orally available, we tried to optimize the structure to improve oral bioavailability.

2. Results and discussion

2.1. Chemistry

The 5-diazabicyclo pyridine analogs were prepared by the method depicted in Scheme 1. Optically pure (*S*)-2-aminomethyl-

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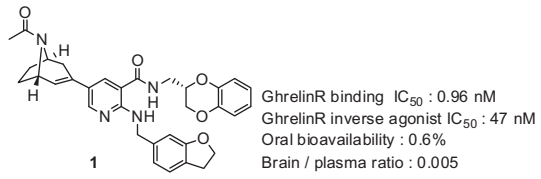
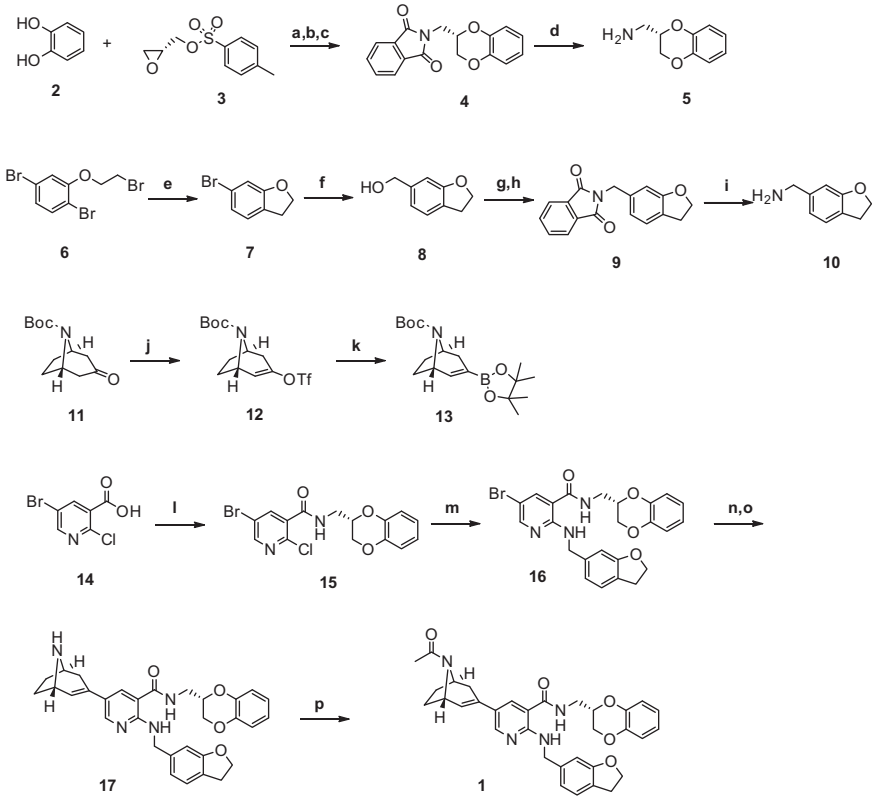


Figure 1. A previously reported ghrelinR inverse agonistic 2-aminoalkyl nicotinamide derivative.

1,4-benzodioxane (**5**) was prepared using a chiral glycidol derivative.[9] Catechol and *p*-toluenesulfonic acid (2*R*)-(−)-glycidyl ester were coupled to yield (*S*)-2-hydroxymethyl-1,4-benzodioxane and the hydroxyl group was converted to an amino group via a phthalimide intermediate (**4**). The dihydrobenzofuran ring of compound **7** was constructed by intramolecular C–C bond formation,[10] and the bromo group of compound **7** was converted to a hydroxymethyl group by lithiation, followed by paraformaldehyde treatment. The same procedure for compound **5** was adopted for the conversion of the hydroxymethyl group to the aminomethyl group to yield **10**. The diazabicyclo boronate intermediate (**13**) was prepared by the method described in the literature[11] with slight modification. Commercially available *N*-Boc-nortropine was converted to vinyl triflate **12** and the triflate was coupled with bis(pinacolate)diboron to yield the boronate. Finally, these intermediates (**5**, **10** and **13**) were coupled with the nicotinic acid scaffold (**14**). The amidation of compound **14** and compound **5** was performed using BOP reagent and the amination at the 2-position of the pyridine ring was accomplished by simply heating the mixture of reactants. Suzuki coupling of the aryl bromide (**16**) and the borate (**13**) proceeded smoothly, and following deprotection and acetylation yielded the final product **1**. The yield of each reaction was moderate to good, and the whole scheme was applicable to 10 g scale synthesis without difficulty.

By analyzing the ^{1}H NMR spectrum of compound **1**, we found that protons at the pyridine ring and the diazabicyclo moiety were split into two peaks, and the integral ratio of the peaks was approximately 0.45:0.55. Because compound **1** is a mixture of diastereomers, each diastereomer was separated through a chiral column and analyzed by ^{1}H NMR spectroscopy. Interestingly, NMR spectra of both isomers were almost identical, and the splits of the peaks were also observed. We, therefore, concluded that the splits indicate *cis*- and *trans*-rotamers of the amide bond on the bridge-head nitrogen.

Preparation of the alkynyl pyridine analogs is described in Scheme 2. The key intermediate **20** was prepared from commercially available 2-hydroxy nicotinic acid **18** through 5-iodination using *N*-iodosuccinimide, chlorination using thionyl chloride, and esterification. Compound **20** was coupled with 3,4-(methylenedioxy)benzylamine by heating without solvent, and an alkynyl moiety was introduced by a Sonogashira reaction. No protection of 2-methyl-3-butyl-2-amine was necessary in the reaction, and compound **22** was obtained in good yield (90%). Acetylation, ester hydrolysis, and amide coupling with 3-chloro-4-methoxybenzylamine yielded compound **24**. The amide part of compound **33** was prepared from compound **25** through di-fluorination using *N*,*N*-diethylaminosulphur trifluoride (DAST), cuprate coupling to introduce a cyano group, and reduction of the cyano group. Starting from the 5-bromo intermediate **29**, compound **33** was prepared by applying the same procedure for compound **24**. All the



Scheme 1. Reagents and conditions: (a) K_2CO_3 DMF, 60 °C; (b) TsCl, pyridine, rt; (c) phthalimide potassium, DMF, 90 °C, 57% (3 steps); (d) hydrazine hydrate, MeOH, reflux, 88%; (e) *n*-BuLi, THF, paraformaldehyde, −74 °C, 74%; (f) *t*-BuLi, THF, paraformaldehyde, −74 °C, 74%; (g) $SOCl_2$ CH_2Cl_2, rt; (h) phthalimide potassium, DMF, 90 °C, 69% (2 steps); (i) hydrazine hydrate, MeOH, 77 °C, 80%; (j) LDA, THF, −78 °C, then, $PhNTf_2$ rt, 46%; (k) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, dioxane, 80 °C, 67%; (l) **5**, BOP reagent, DIPEA, DMF, 0 °C–rt, 93%; (m) **10**, 160 °C, 74%; (n) **13** $Pd(pph_3)_4$, K_3PO_4, dioxane, H_2O, 100° C; (o) TFA, CH_2Cl_2, rt, 61% (2 steps); (p) AcCl, Et_3N, CH_2Cl_2, rt, 72%.

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