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2-Aminoalkyl nicotinamide derivatives as pure inverse agonists of the ghrelin receptor

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ABSTRACT

New inverse agonists of the ghrelin receptor (ghrelinR) were obtained through high-throughput screening and subsequent structural modification of 2-aminoalkyl nicotinamide derivatives. The key structural feature to improve in vitro activity was the introduction of a diazabicyclo ring at the 5-position of the pyridine ring. The final product showed potent inverse agonist activity and, despite its low brain permeability, reduced food intake in both normal and obese mice. These results implied that peripheral ghrelinR activity is important for appetite control and that a peripheral ghrelinR inverse agonist could be an anti-obesity drug with reduced risk of central nervous system (CNS)-related side effects.

Ghrelin is a growth hormone-releasing peptide identified as an endogenous ligand for growth hormone secretagogue receptor 1a (GHS-R 1a), currently known as the ghrelin receptor (ghrelinR).[1] Subsequent studies have revealed that ghrelin regulates energy metabolism by stimulating appetite and gastric motility and by suppressing energy expenditure.[2] Based on these activities, ghrelin has been developed as a potential drug candidate for the treatment of anorexia nervosa[3] and chronic obstructive pulmonary disease cachexia.[4] Furthermore, several small-molecule ghrelinR agonists are in development as potential drug candidates for the treatment of cancer-related anorexia and cachexia syndrome.[5] Conversely, ghrelinR blockage could ameliorate diabetes and obesity, and many ghrelinR antagonists have also been reported by pharmaceutical companies and academic researchers.[6–12] However, ghrelinR inverse agonists are relatively scarce, even though human ghrelinR is constitutively active, and this constitutive signal can correspond to almost the same extent as the ghrelin induced activity.[14] Here, we present new and potent ghrelinR inverse agonists that effectively suppress the constitutive activity of this receptor.

The first small molecule ghrelinR inverse agonists were reported by Merck[13] in 2009, and other groups subsequently reported several inverse agonists with different structures (see Fig. 1).[15–17] We performed high-throughput screening (HTS) using the ^{125}I-ghrelin substitution assay system to obtain ghrelinR binders and the hits obtained were evaluated using an inverse agonist assay employing a reporter-gene system. This system was based on the fact that ghrelinR-derived $G\alpha_q$ signaling results in activation of the nuclear factor of activated T-cells (NFAT) and activator protein-1 (AP-1) response element, stimulating gene expression.[23] Briefly, rat ghrelinR and NFAT response element luciferase vector were transfected into HEK293 cells and constitutive activity was detected as luciferase activity. Limited structure–activity relationship (SAR) information about the hit compounds was obtained in this assay system, which enabled selection of compound **4** as a seed compound in this research (Fig. 2). Interestingly, this 2-aminoalkyl nicotinamide structure showed no functional switching toward neutral antagonism or partial agonism (or even partial inverse agonism[24]) in the entire SAR study, in contrast to the functional switching to partial agonism reported for many ghrelinR antagonists and inverse agonists with small structural modifications.[13,15,17] The concentration–response relationships of inverse agonists in the reporter-gene assay are depicted in Figure 3. A known peptidic ghrelinR inverse agonist, named substance P analog[14] ([D-Arg[1], D-Phe[5], D-Trp[7,9], Leu[11]]-substance P), almost completely suppressed the constitutive activity of this receptor, although its potency (IC_{50} = 250 nM) was lower than the previously reported one for an inositol phosphate turnover assay[25] (IC_{50} = 5.2 nM). High concentration of compound **4** also suppressed the constitutive activity almost completely and the same was true for all compounds described in this article (the concentration–response relationship of compound **12** is depicted as an example). False positive results can be led by cytotoxicity and non-specific inhibition and we therefore tested compound cytotoxicity using

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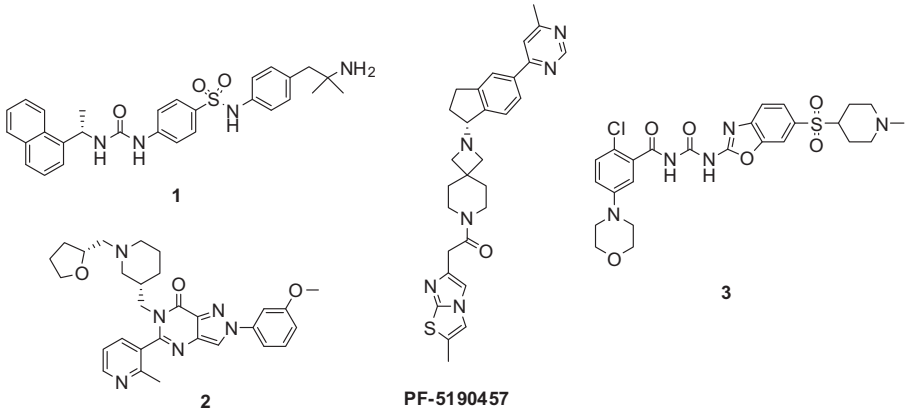

Figure 1. GhrelinR ligands reported as inverse agonists.

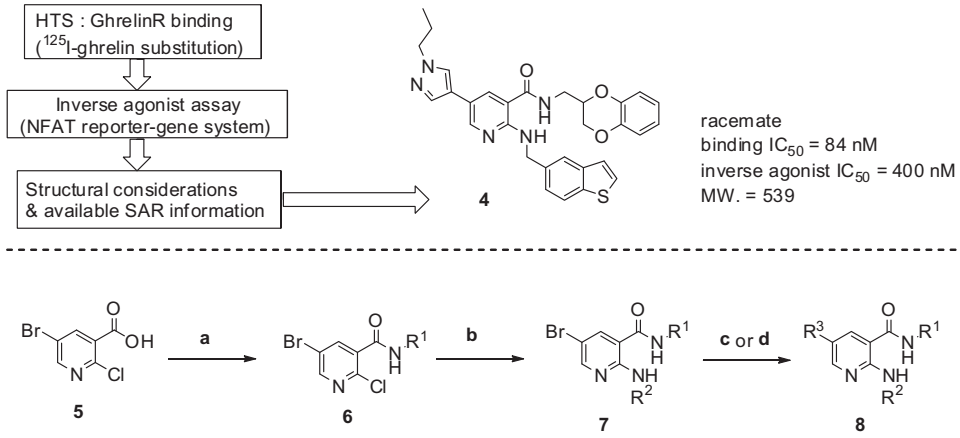

Figure 2. Structure of the lead compound and synthetic scheme of its analogs. Reagents and conditions: (a) R^1-NH_2, BOP reagent, DIPEA, DMF (66–98%); (b) R^2-NH_2, neat or EtOH, 80–100 °C (58–90%); (c) R^3-boronic acid or boronic ester, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane, water, microwave irradiation 100 °C (79–95%); (d) secondary amines, $Pd_2(dba)_3$, 2-(di-*tert*-butylphosphino)biphenyl, NaO*t*Bu, toluene, 100 °C (66–88%).

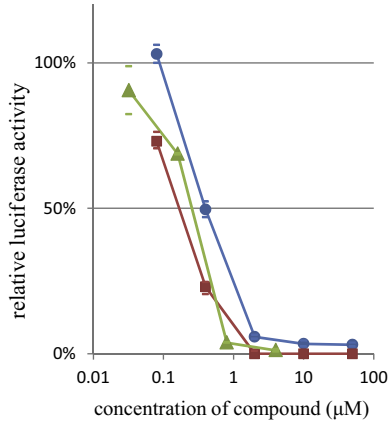

Figure 3. Concentration–response relationship of inverse agonist activity in NFAT-RE-Luc reporter system following the addition of the substance P analog (triangle), compound 4 (circle) and compound 12 (square). The rat ghrelinR and NFAR reporter were co-transfected in HEK 293 cells and luciferase activity with vehicle (0.5% DMSO) treatment was indicated as 100% and luciferase activity without ghrelinR transfection was indicated as 0%. Experiment was duplicated and mean value was used for the caluculation of IC_{50} value (both data of duplicated experiment are indicated as short horizontal bars with the same color). Cell viability at high concentration of compound was tested by the Alamar Blue staining. Stained cells with 2 μM and 50 μM treatment of compound 4 were 101% and 92%, respectively, and that of compound **12** were 85% and 69%, respectively.

the Alamar Blue staining method;[26] ghrelinR specificity was also investigated by testing the compounds in the same reporter-gene system with transfection of neurotensin receptor 2 (NTSR2), instead of ghrelinR. Our compounds were not considered to be cytotoxic enough to compromise the assay system because at least 69% of the cells were viable, even at compound concentration of 50 μM, well above their IC_{50} values. Moreover, these compounds did not suppress the constitutive activity of NTSR2 (data not shown). Therefore, the effects of the compound in this assay were considered to reflect their inverse agonist activity at the ghrelinR.

The synthetic schemes for preparation of compound **4** and its derivatives are depicted in the lower part of Figure 2. Commercially available 5-bromo-2-chloro-nicotinic acid **5** was converted to amide **6** using BOP reagent, and the introduction of an alkylamine at the 2-position of the pyridine ring gave **7**. The solvent and temperature were optimized, depending on amine reactivity. Finally, the bromo group at the 5-position was functionalized by the Suzuki coupling for the aryl and allyl groups, or by the Buchwald–Hartwig reaction for secondary amines, to obtain the final product **8**. The yield of each reaction (>58%) was acceptable for small scale synthesis.

Starting from compound **4**, we mainly modified the 2-aminoalkyl group. The SAR for the 2-aminoalkyl group, with the 5-position fixed with the *N*-propyl pyrazole ring, and calculated log*P* values are listed in Table 1. Binding affinity and inverse agonist activity seemed to correlate well and both activities were