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Structure–activity relationships of the oxindole growth hormone secretagogues

Teruhisa Tokunaga, W. Ewan Hume, Jun Nagamine, Tetsuya Kawamura, Mutsuo Taiji and Ryu Nagata*

Research Division, Sumitomo Pharmaceuticals Co., Ltd, 1-98 Kasugade Naka 3-chome, Konohana-ku, Osaka 554-0022, Japan

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Abstract—A series of substituted oxindole derivatives of SM-130686 was synthesized and evaluated as ghrelin receptor agonists. Modification of the substituents on the C3-aromatic part of the oxindole led to compounds with subnanomolar binding affinities. Compound **4i** (IC_{50} = 0.02 nM) was orally active at low doses and showed in vivo activity when orally administered, 2 mg/kg twice a day for 4 days, as evidenced by significant body weight gain.

Ghrelin, a 28-amino acid gastric hormone, was discovered in human and rat stomach tissues in 1999 and identified as the endogenous ligand for the Growth hormone secretagogue receptor (GHS-R).[1] Ghrelin has an unprecedentedly unique structure in which the hydroxyl group of the Ser3 residue is acylated by *n*-octanoic acid. This acylation is essential for the biological activity and is supposed to be critical for the transport of the ghrelin molecule into the CNS area across the blood–brain barrier, where the targeted GHS receptors are located.[2] Ghrelin exhibits a wide range of biological activities. In humans as well as in rodents, ghrelin not only stimulates pituitary growth hormone (GH) secretion[3] but also increases food intake and body weight gain and regulates energy balance.[4,5] Prior to the discovery of ghrelin, much effort has been made to identify peptidomimetic and nonpeptidic small molecular GH secretagogues (GHSs) as an alternative to GH replacement therapy. Such synthetic GHSs have been reported to stimulate the secretion of GH through the GHS-R, with a mechanism distinct from growth hormone releasing hormone (GHRH).[6] Among the GHSs reported so far,[7] MK-677,[8] NN703,[9] and CP-424391[10] are noted as extensively studied GHSs and indeed proceeded into clinical trials.

In a previous paper, we demonstrated that certain oxindole derivatives exemplified by the structurally distinct SM-130686 had potent GH releasing activity (Fig. 1).[11–13] We also showed SM-130686 was orally active with a good in vivo profile. To clarify the structure–activity relationships (SARs) and understand the pharmacology of the oxindole class of GHSs, further

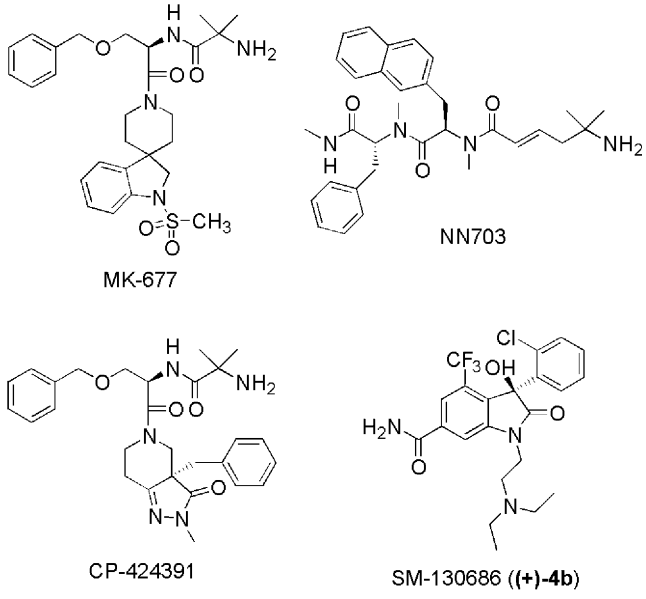

Figure 1. Structures of small molecule growth hormone secretagogues.

Keywords: Ghrelin; Growth hormone secretagogue; SM-130686.
* Corresponding author. Tel.: +81 6 6466 5193; fax: +81 6 6466 5483; e-mail: rnagata@sumitomopharm.co.jp

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studies have been conducted. Herein, we describe the SAR in the C3-aromatic part of the oxindole GHS, and the discovery of highly potent compounds in this series.

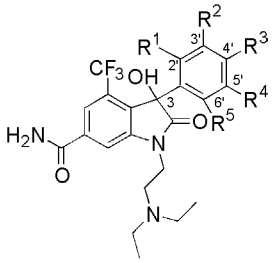
The compounds were synthesized as shown in Scheme 1. Addition of aryl magnesium bromides or aryl lithiums to key intermediary isatin **1**[12,14] afforded oxindoles **2a**–**k** in 32–71% yields. Compounds **2a**–**k** were treated with zinc cyanide in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium in DMF at 80 °C to produce compounds **3a**–**k** in 48–95% yields. Conversion of the nitrile functionality of **3a**–**k** to a carboxamide was accomplished by treatment with potassium hydroxide in anhydrous *tert*-butyl alcohol at 50 °C to give compounds **4a**–**k** in 61–100% yields.

All the compounds described in this study were evaluated in a competitive binding assay on a human recombinant ghrelin receptor membrane preparation using ^{125}I–ghrelin as a radioligand (Table 1).[15] In order to examine the effect of a substituent at the C3-aromatic ring of SM-130686 on the activity, we prepared unsubstituted phenyl, various monochloro and monomethoxy substituted phenyl analogues. Removal of the C2′ chloro substituent in racemic SM-130686 resulted in a 29-fold loss in binding affinity. Moving the chloro substituent to the C3′ and C4′ positions also resulted in a decrease in activity. These results indicated that existence of a substituent at the C2′ position was important for the activity. We assumed that the substituent at the C2′ position might help the C3-phenyl ring to adopt a preferable conformation. Monomethoxy derivatives **4e**,**f** and **4g** displayed weaker activity than the corresponding monochloro analogues, suggesting that a hydrophobic substituent at the C3 aromatic ring was favourable.

Next, we assessed more hydrophobic 3-dichlorophenyl analogues. Gratifyingly, while compound **4k** had only retained activity, compounds **4h**,**i** and **4j** showed 2.5, 200 and 17 times enhanced activities, respectively, compared to **4b**. In particular, the IC_{50} value of 2′,4′-dichlorophenyl compound **4i** was about ten times superior to that of ghrelin itself. It was noteworthy that the introduction of only one substituent on the phenyl ring influenced the activity to such a great extent.

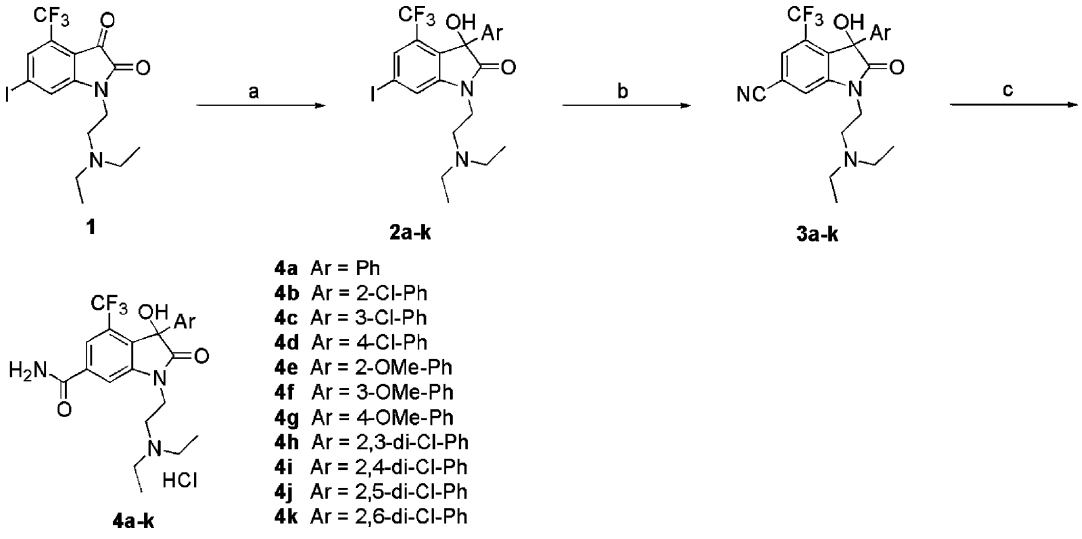
Compound **4i** was evaluated further in vitro and in vivo assay. In a fluorescent calcium indicator assay (FLIPR) measuring [Ca^{2+}] accumulation induced by a compound

Table 1. Structure–activity relationships of 3-aromatic part of oxindole ring



Compd	R^1	R^2	R^3	R^4	R^5	Binding IC_{50} (nM)[a]
4a	H	H	H	H	H	116
4b	Cl	H	H	H	H	4.0
(+)-**4b**[b]	Cl	H	H	H	H	2.1
4c	H	Cl	H	H	H	15
4d	H	H	Cl	H	H	83
4e	OMe	H	H	H	H	160
4f	H	OMe	H	H	H	77
4g	H	H	OMe	H	H	106
4h	Cl	Cl	H	H	H	1.6
4i	Cl	H	Cl	H	H	0.02
4j	Cl	H	H	Cl	H	0.24
4k	Cl	H	H	H	Cl	4.2
Ghrelin						0.14

[a] See Ref. 15.
[b] SM-130686.



Scheme 1. Reagents and conditions: (a) ArMgBr, THF, 0 °C or ArLi, THF, −78 °C, 32–71%; (b) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 48–95%; (c) (1) powdered KOH, *tert*-butyl alcohol, 50 °C, (2) HCl, dioxane, rt, 61–100% (two steps).

in CHO cells over-expressing GHS-R, compound **4i** was demonstrated to possess a partial agonistic activity,[13,16] with 91% of the maximal response obtained by ghrelin and an EC_{50} of 17 nM, while **4b** displayed 65% of the maximal response with an EC_{50} of 11 nM. The reason for the dissociation of the IC_{50} value in the binding assay from the EC_{50} value in the FLIPR assay was unclear. Administration of **4i** orally increased body weight gain in a dose dependent manner after 4 days in rats. When **4i** was administered at doses of 2, 6 and 20 mg/kg twice a day for 4 days, the body weight gains were 11.6, 13.6 and 21.1 g, respectively, whereas the distilled water administered control group showed body weight gain of 5.0 g (Fig. 2). These body weight gains were greater than those of SM-130686 at the corresponding doses.[12] In a rat pharmacokinetic study, C_{max} and bioavailability of **4i** were 491 ng/mL and 28%, respectively, after oral dose of 20 mg/kg. When intravenously administered to rats with 5 mg/kg, Cl, Vd and $t_{1/2}$ were 51 mL/min/kg, 5.4 L/kg and 1.6 h, respectively. This pharmacokinetic profile was quite similar to that of SM-130686.[12] Although serum protein bindings of both compounds were not measured, we assume that the greater in vivo efficacy of **4i** compared to SM-130686 would simply be attributed to the increased potency.

In summary, the structure–activity relationships of the C3-aromatic part of SM-130686 was examined and a series of 3-dichlorophenyl analogues were identified as potent ghrelin agonists. Compound **4i**, the most potent compound in the binding assay, showed excellent in vivo activity and was found to be a ghrelin partial agonist, as evidenced by the FLIPR assay. Recent accumulating evidence of the strong orexigenic and adipogenic effects of ghrelin has raised the possibility that a ghrelin modulator or antagonist could be used in the treatment of obesity.[17] Owing to the high binding affinity and the partial agonistic activity of theses oxindole derivatives such as **4i**, modification of these compounds may lead to the identification of a new class of antiobesity drugs mediated by the modulation of the ghrelin receptor (GHS receptor).

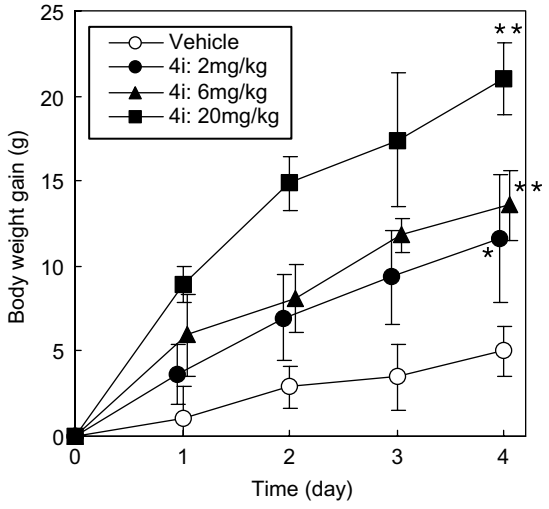


Figure 2. Effect of compound **4i** on the body weight gain. Compound **4i** was orally administered twice a day for 4 days in normal female rats. Data are represented as means ± SD ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, Shirley–Williams test (vs vehicle-treatment-group).

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15. Binding assay: Human recombinant ghrelin receptor membrane preparation was purchased from Euroscreen. These were suspended in binding buffer (25 mM Hepes pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.4% protease free BSA, 1 mM EGTA, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 μg/mL pepstain A) to a concentration of 50 μg protein/mL. These membranes (1 μg protein/tube) were mixed with ^{125}I–ghrelin (30,000 dpm/tube), with or without different concentrations (0.01–10,000 nM) of test compounds and the binding buffer up to a total volume of 100 μL. Nonspecific binding was obtained by adding 500 nM of cold ghrelin. The membranes were incubated at 25 °C for 30 min, and the bound radioligand was separated from free radioligand by washing three times with 3 mL of the binding buffer through a 0.5% polyethylenimine-presoaked GF/B filter (Whatman). The radioactivity on the filters was counted in 1470 Wallac Wizard Gamma Counter (PerkinElmer, MA).
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