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Potent achiral agonists of the ghrelin (growth hormone secretagogue) receptor. Part I: Lead identification

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Abstract—High throughput screening combined with efficient datamining and parallel synthesis led to the discovery of a novel series of indolines showing potent in vitro ghrelin receptor agonist activity and acceleration of gastric emptying in rats.

Ghrelin, a 28-amino acid gastric hormone, exhibits a wide range of biological activities via its cognate G-protein coupled receptor, previously known as the growth hormone secretagogue receptor. In humans as well as in rodents, ghrelin stimulates pituitary growth hormone secretion[1] and in addition increases food intake and body weight gain and regulates energy balance.[2,3] Ghrelin exerts powerful effects on the gastrointestinal tract, increasing gastric emptying[4] and defecation,[5] as well as having the potential to reduce emesis.[6] Hence, agents which mimic the actions of ghrelin have potential in disorders requiring increased nutritional intake, such as cancer-associated cachexia and post-operative ileus, and requiring increased gastric emptying, such as functional dyspepsia.[7] A number of first generation ghrelin receptor agonists have undergone extensive clinical evaluation in humans. To date these have shown disappointing results in the treatment of diseases such as age-related frailty[8] but the potential for ghrelin receptor agonists in other indications remains to be studied.

The discovery and development of ghrelin receptor agonists has focussed on peptide-derived privileged fragment approaches, which have led to relatively high molecular weight, synthetically complex molecules such as MK-0677,[9] and CP-424391.[10] More recently a series of partial agonists represented by oxindole SM-130686 has been discovered using diversity screening.[11] Here, we report the discovery of a novel series of low molecular weight achiral ghrelin receptor agonists, employing high throughput screening using a recombinant functional assay, and early optimization by parallel synthesis.

A high throughput screen of the GSK compound collection using FLIPR technology delivered a moderately potent and tractable hit series represented by the indoline amide **1** (Table 1), originally prepared as 5-HT_{1B} receptor antagonists.[12] Analogues of **1** were selected for further screening with the goal of generating divergent SAR data, taking into account the previous observation that arylpiperazines bearing an arylsulfonamide

Keywords: Ghrelin receptor agonist; Growth hormone secretagogue.
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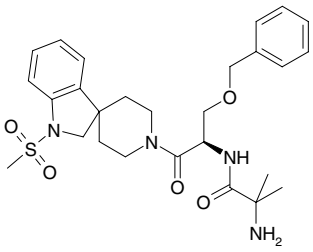
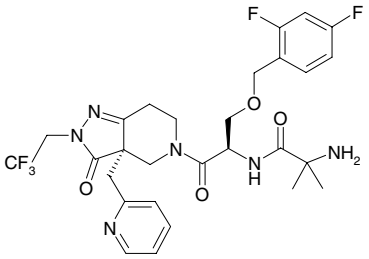
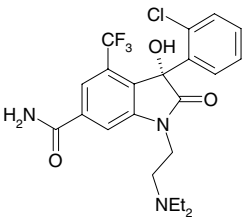


MK-0677

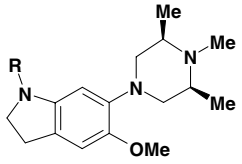


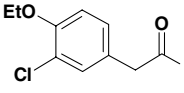
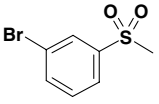
CP-424391



SM-130686

Table 1. Potency and selectivity of HTS hits[14]



Compound	R	GHSR pEC_{50} (IA)[a]-FLIPR	$5HT_{1B}$ pK_i-binding
1		6.8 (0.7)	7.9
2		6.9 (0.7)	5.7

[a] IA refers to intrinsic activity compared to human ghrelin.

moiety in a meta-orientation show reduced 5-HT_1 receptor affinity.[13] This led to the discovery of the sulfonyl indoline **2** which retains the promising activity of the amide and shows a 100-fold reduction in affinity for 5-HT_{1B} receptors (Table 1).

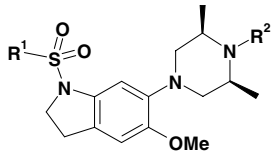
Synthetic studies were initiated to explore SAR in the sulfonamide series. Replacement of the 3-bromophenyl group in **2** with a 2-naphthyl group to give **3** led to a moderate increase in potency and efficacy (Table 2). The 2,6-dimethylpiperazinyl analogue **4** of this compound showed subnanomolar potency and full efficacy. As a result, the 2,6-dimethylpiperazinyl indoline was used for subsequent sulfonyl group modifications.

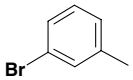
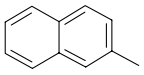
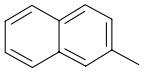
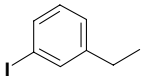
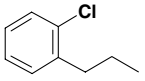
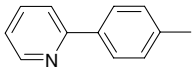
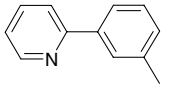
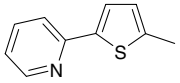
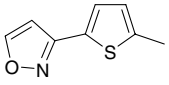
Replacement of the 2-naphthyl group with more flexible substituted benzyl or phenethyl groups led to a moderate drop in potency (compounds **5** and **6**, respectively). Extension to a biaryl system uncovered more marked SAR: the 4-linked 2-pyridylphenyl sulfonamide **7** shows over 100-fold higher potency compared to the 3-linked isomer **8**. In the biaryl system, the sulfonyl-bearing phenyl group could be replaced by a thiophene with little effect on potency and efficacy (compounds **9** and **10**).

Retaining the highly potent 2-pyridylthienyl sulfonyl group of compound **9**, systematic removal of the methyl groups in the dimethylpiperazine moiety led to incremental loss in potency (**11**–**13**, Table 3). No significant preference for either of the 2-methylpiperazine enantiomers **11** or **12** was observed; however, after introduction of a third methyl substituent (compounds **14** and **15**), a preference for the S-configuration at the 6-position of the piperazine was observed. The geminal dimethylpiperazine **16** shows somewhat reduced potency compared to either of the monomethyl enantiomers **11** or **12**.

The thienyl analogue **9** was further characterized in a number of in vitro pharmacology assays. The

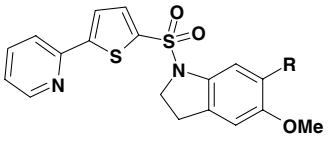
Table 2. GHSR potency and efficacy for sulfonamide and piperazine analogues[14]

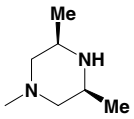
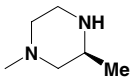
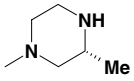
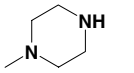
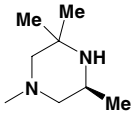
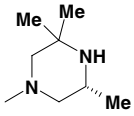
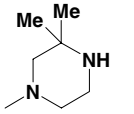


Compound	R^1	R^2	GHSR pEC_{50} (IA)[a]
2		Me	6.9 (0.7)
3		Me	7.4 (0.8)
4		H	9.3 (1.0)
5		H	7.5 (0.8)
6		H	8.3 (0.9)
7		H	9.7 (1.1)
8		H	7.7 (0.9)
9		H	9.8 (0.9)
10		H	8.6 (0.9)

[a] IA refers to intrinsic activity compared to human ghrelin.

Table 3. GHSR potency and efficacy for piperazine analogues[14]



Compound	R	GHSR pEC_{50} (IA)[a]
9		9.8 (0.9)
11		8.5 (1.0)
12		8.4 (1.0)
13		7.2 (0.8)
14		9.1 (1.0)
15		8.0 (0.8)
16		7.8 (0.9)

[a] IA refers to intrinsic activity compared to human ghrelin.

Table 4. In vivo DMPK parameters after 1 mg/kg iv dose

Cmax/μM	$t_{1/2}$	Clb	Vss (L/Kg)
0.25	2.3	64	8.8

compound is inactive at the closest homologue, the motilin receptor (GPR38), and showed >1000-fold selectivity against over a hundred diverse receptor and enzyme selectivity targets.

Compound **9** possesses promising physicochemical properties, with a $\log D_{7.4}$ of 1.8, aqueous solubility from solid of 168 μg/ml at pH 7.4, and a molecular weight of 484 Da. Its metabolic stability in liver microsomes is moderate (rat 2.6, human 4.5 ml/min/g liver). After intravenous dosing in rats the compound shows a promising half-life and relatively high volume of distribution (Table 4); however, after oral dosing exposure is low resulting in negligible oral bioavailability.

Compound **9** shows significant acceleration of gastric emptying after subcutaneous dosing in rats (Fig. 1) with an ED_{50} of 0.1 mg/kg.

The synthesis of 1,2,6-trimethylpiperazinyl indoline amides analogous to **1** has been described previously.[12] Sulfonamides **2** and **3** were prepared from 1,2,6-trimethylpiperazinyl indoline **17** using standard conditions (Scheme 1).

The 2,6-dimethylpiperazinyl analogue **4** was prepared similarly by sulfonylation of the 2,6-dimethylpiperazinyl indoline **18** (Scheme 2).[12] In order to allow efficient array synthesis of sulfonamide analogues, conditions using polystyryl-*N*-methylmorpholine were chosen. This allowed simple parallel extraction of reaction products using solid phase cation exchange cartridges, thereby removing any minor byproducts arising from sulfonylation of the more hindered piperazine nitrogen. In this way two 24-member SAR arrays were prepared.

Synthesis of the alternatively substituted piperazine templates present in **11**–**16** was carried out using Pd-catalysed amination of the corresponding 6-bromoindoline, as described previously for the trimethylpiperazine.[12]

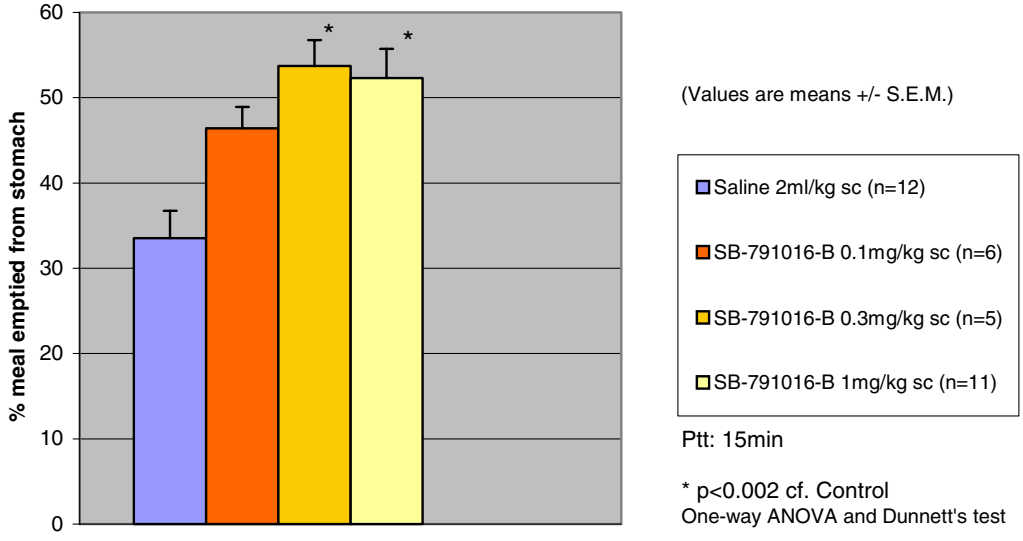
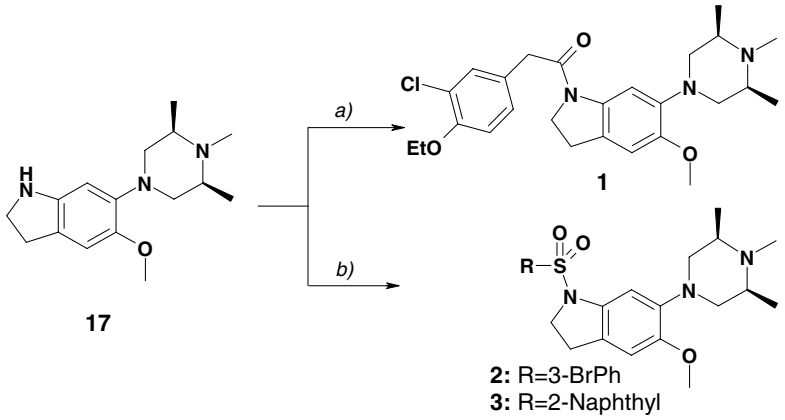
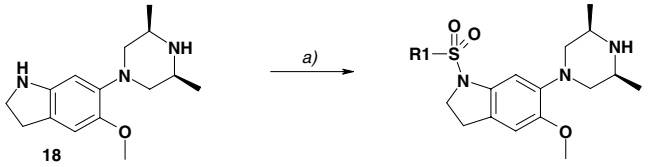


Figure 1. Effect of compound **9** on gastric emptying in the conscious rat (subcutaneous dosing, 60 min test meal).[15]



Scheme 1. Reagents: (a) RCO_2H, EDC, HOBt, CH_2Cl_2; (b) RSO_2Cl, Et_3N, CH_2Cl_2.



Scheme 2. Reagents: (a) $R1SO_2Cl$, polystyryl-*N*-methylmorpholine, DCM; SCX cartridge.

Conclusions. High throughput screening combined with efficient parallel synthesis led to the discovery of a novel achiral series of ghrelin receptor agonists with potential as gastroprokinetic agents for the treatment of disorders such as functional dyspepsia. Further optimization of this series will be described in due course.

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14. All novel compounds were characterized at 95% purity or greater by ^1H NMR and LC/MS. Ghrelin receptor agonist BACMAM FLIPR assay was used to determine the potency and efficacy of the test compounds. Media were aspirated from cell plates using a cell washer (leaving 10 μl of media). Cells are immediately loaded with loading buffer (Tyrodes (Elga water + 145 mM NaCl + 5 mM KCl + 20 mM Hepes + 10 mM glucose + 1 mM $MgCl_2$) + 1.5 mM $CaCl_2$ + 0.714 mg/ml Probenicid (predissolved in 1 M NaOH) + 0.5 mM brilliant black + 2.5 μM Fluo 4 dye, and incubated at 37.5 °C for 1 h. Ten microliter from compound plates is then added immediately to cell plates using a FLIPR 3 calcium imaging instrument. Fluorescence measurements are then taken. Values given are means of at least three test replicates.
15. Gastric emptying protocol based on procedure described in Droppleman et al., *J. Pharmacol. Methods*, **1980**, *4*, 227.