# Identification of 2-(4-Benzyloxyphenyl)-*N*-[1-(2-pyrrolidin-1-yl-ethyl)-1*H*-indazol-6-yl]acetamide, an Orally Efficacious Melanin-Concentrating Hormone Receptor 1 Antagonist for the Treatment of Obesity

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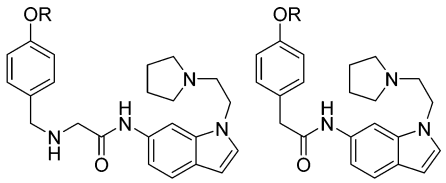
**Abstract:** Optimization of a high-throughput screening hit against melanin-concentrating hormone receptor 1 (MCHr1) led to the discovery of 2-(4-benzyloxy-phenyl)-*N*-[1-(2-pyrrolidin-1-yl-ethyl)-1*H*-indazol-6-yl]acetamide (**7a**). This compound was found to be a high-affinity ligand for MCHr1 and a potent inhibitor of MCH-mediated $Ca^{2+}$ release, showed good plasma and CNS exposure upon oral dosing in diet-induced obese mice, and is the first reported MCHr1 antagonist that is efficacious upon oral dosing in a chronic model of weight loss.[1]

Melanin-concentrating hormone[2] (MCH) is a cyclic 19-amino-acid peptide that is synthesized in cell bodies in the lateral hypothalamus and zona incerta of the central nervous system (CNS). The MCH peptide is understood to play a major role in body weight regulation in rodents[2,3] as a single injection of MCH into the CNS stimulates food intake in rats[4] and chronic administration leads to increased body weight.[5] Transgenic mice overexpressing the MCH gene are susceptible to insulin resistance and obesity,[6] while mice lacking the gene encoding MCH are hypophagic, lean, and maintain elevated metabolic rates.[7] Consistent with this phenotype, genetically altered mice that lack the gene encoding the MCH receptor maintain elevated metabolic rates and thus remain lean despite hyperphagia on a normal diet.[8,9] These data indicate that successful antagonism of the MCHr1 system could lead to weight loss, and the observation that chronic intraperitoneal administration of a small-molecule antagonist leads to the reduction of food intake and body weight[10] provides further validation of MCHr1 blockade as a novel target for antiobesity pharmacotherapy.[11]

High-throughput screening of the Abbott compound collection against MCHr1 resulted in the identification of the 6-substituted 2-pyrolidinylethylindole **1** (Figure 1). This compound displayed moderate affinity in a receptor-binding assay using MCHr1 obtained from human neuronal IMR-32 cells and showed low-micromolar activity in an assay designed to measure functional antagonism of MCH-mediated $Ca^{2+}$ release. When dosed orally at 10 mg/kg in DIO mice, **1** demonstrated significant drug levels in plasma and brain.




| | 1: R = Ph | 2: R = Bn |
|---|---|---|
| MCHr1 $IC_{50}$ (μM)[a] | 0.299 | 0.0220 |
| $Ca^{2+}$ release $IC_{50}$ (μM)[a] | 1.81 | 0.470 |
| Plasma AUC (μg ·hr/mL)[b] | 1.16 | 1.37 |
| Brain AUC (μg ·hr/g)[b] | 0.674 | 0.348 |

**Figure 1.** Indole-based MCHr1 antagonists. (a) Values represent an average of at least two determinations with a deviation of ±45% of the mean value shown. (b) Results are for 10 mg/kg, po in DIO mice, and interanimal variability was less than 25% for all values.

Initial medicinal chemistry efforts to improve upon the binding affinity of **1** by modifying the 6-positional substituent were largely unsuccessful as the incorporation of several linear and branched glycine-based side chains resulted in compounds of comparable or weaker activity. Since deletion of the tertiary amine from the 1-(2-pyrrolidin-1-ylethyl) substituent was also deleterious to activity, we focused on the removal of the 6-positional glycine-based side chain in favor of nonbasic amides. The structure−activity relationship (SAR) of the resultant batch of analogues was similarly unforgiving, affording primarily inactive compounds. One exception was the 6-benzyloxyphenylacetic amide **2**, which was 10-fold more active in the binding assay and showed improved functional antagonism relative to lead **1**. However, indole **2** demonstrated less efficient CNS penetration when evaluated in DIO mice (10 mg/kg, po). The difficulty in obtaining positive SAR trends with respect to the amide side chain along with the disappointing pharmacokinetic profile of **2** prompted the investigation of benzimidazole and indazole surrogates of the indole core.

The general synthetic approach to the 2-pyrrolidin-1-ylethylindazole amides is outlined in Scheme 1. The 4-nitroindazole was synthesized from the corresponding 3-nitro-*o*-tolylamine, while the 5- and 6-nitroindazoles were obtained from commercial sources. Alkylaton of the nitroindazole cores (**4**) with 2-chloroethylpyrrolidine hydrochloride afforded a mixture of N1 and N2-alkylated isomers in ratios that varied by the position of the nitro substituent (see Supporting Information). After chromatographic separation of the isomers, iron-mediated reduction afforded the individual anilines **5** and amide bond formation furnished the final products **7a**−**c** and **8a**−**c**. A similar strategy was taken for the benzimidazole analogues **6a**,**b**, starting with the alkylation of 5-nitrobenzimidazole followed by separation of the isomers before reduction and amide coupling.

Table 1 summarizes the SAR of the MCHr1 binding and functional activities of the heterocyclic analogues. The 6-substituted benzimidazole analogue **6a** demon-

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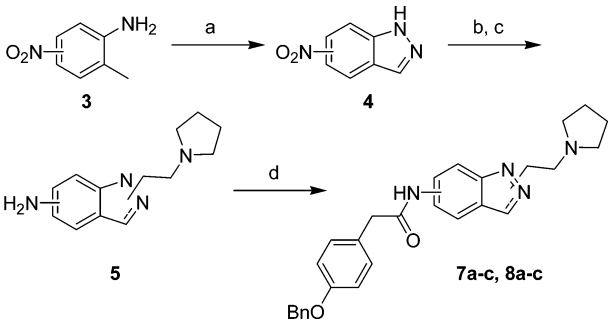
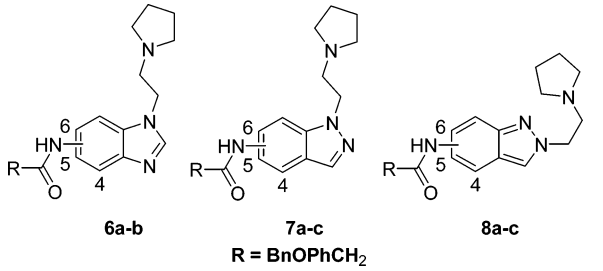
**Scheme 1**[a]




[a] Reagents and conditions: (a) $NaNO_2$, AcOH, room temp, 95%; (b) chloroethylpyrolidine hydrochloride, $K_2CO_3$, DMF, 60 °C, then $SiO_2$ chromatography, 27−67%; (c) Fe, $NH_4Cl$, 65 °C; (d) 4-benzyloxy-4-phenylacetic acid, EDCI, HOBt, DMF, NMM, 56−89%.

**Table 1.** SAR of Heterocylic Analogues of **2**[a]




| compd | position | MCHr1 binding $IC_{50}$ (nM)[b,d] | $Ca^{2+}$ release $IC_{50}$ ($\mu$M)[c,d] |
|---|---|---|---|
| **6a** | 6 | 161 ± 91 | >10 |
| **6b** | 5 | 772 ± 317 | >10 |
| **7a** | 6 | 1.40 ± 0.40 | 0.011 ± 0.002 |
| **7b** | 5 | 558 ± 79 | >10 |
| **7c** | 4 | 24.5 ± 10.4 | 0.071 ± 0.035 |
| **8a** | 6 | 146 ± 59 | 0.874 ± 0.157 |
| **8b** | 5 | 1985 ± 346 | >10 |
| **8c** | 4 | 41.4 ± 14.1 | 0.287 ± 0.148 |

[a] All compounds were >95% pure by HPLC and characterized by $^1H$ NMR and HRMS. [b] Displacement of [$^{125}$I]-MCH from MCHr1 expressed in IMR-32 (I3.4.2) cells (MCH binding $K_d = 0.66 \pm 0.25$ nM, $B_{max} = 0.40 \pm 0.08$ pmol/mg). [c] Inhibition of MCH-mediated $Ca^{2+}$ release in whole IMR-32 cells (MCH $EC_{50} = 62.0 \pm 3.6$ nM). [d] All values are mean values ± SEM and are derived from at least three independent experiments (all duplicates).

strated moderate MCHr1 affinity but showed only weak functional antagonism at the assayed concentration. A similar result was observed for the 5-amino isomer **6b**. In contrast, incorporation of the 6-aminoindazole core imparted significant improvements in binding affinity and functional potency relative to indole **2** as analogue **7a** showed low-nanomolar inhibition of MCH binding and MCH-mediated $Ca^{2+}$ release. Transposition of the amide side chain to the 5-position (**7b**) of the indazole core imparted an over 500-fold decrease in binding affinity. However, binding activity was rescued to a significant degree when the amide side chain was moved to the 4-position to afford **7c**, which was also a potent inhibitor of $Ca^{2+}$ release.

To probe the positional requirements of the 2-pyrrolidin-1-ylethyl substituent, the N2-alkylated nitroindazoles were elaborated to the corresponding final products (Scheme 1, **8a**−**c**). While the binding activity of 6-amino isomer **8a** decreased over 140-fold relative to the N1-alkylated **7a**, it is noteworthy that indazole **8a** retained moderate antagonism of $Ca^{2+}$ release. The combination of 5-amino substitution and N2-alkylation led to a predictable decrease in binding and functional activity as **8b** was the least active of the compounds shown. Interestingly, the N2-alkylated 4-amino isomer **8c** showed similar binding affinity to **7c**, indicating a greater tolerance of the receptor for the 4-amino substituted analogues. However, the preference for N1-alkylation in the functional assay was further demonstrated by **8c**, which dropped over 4-fold in activity relative to **7c**.

**Table 2.** Selected Pharmacokinetic Parameters of Benzimidazole and Indazole Analogues[a]

| compd | plasma AUC ($\mu$g·h/g)[b] | brain AUC ($\mu$g·h/g)[b] | brain $C_{max}$ (ng/g)[b] | brain $C_{12h}$ (ng/g)[c] |
|---|---|---|---|---|
| **6a** | 0.937 | 0.244 | 54.4 ± 0.3 | 0.0 |
| **7a** | 2.12 | 1.33 | 177 ± 16 | 29.0 ± 1.8 |
| **7b** | 8.51 | 10.4 | 4261 ± 656 | 76.0 ± 15.2 |
| **7c** | 0.611 | 1.18 | 464 ± 40 | 8.11 ± 1.58 |
| **8c** | 2.09 | 0.214 | 214 ± 31 | 12.3 ± 3.8 |

[a] All values are mean values ± SEM ($n = 3$ unless specified otherwise). Compounds are dosed in DIO mice at 10 mg/kg, po, in a vehicle containing 1% Tween-80 and water. [b] The three mice with highest plasma and brain concentrations were averaged to provide the peak plasma and brain concentrations $C_{max}$ ± SEM, respectively. The mean plasma or brain concentration data were submitted to multiexponential curve fitting using WinNonlin. The area under the mean concentration−time curve from 0 to $t$ hours (time of the last measurable concentration) after dosing ($AUC_{0-t}$) was calculated using the linear trapezoidal rule for the concentration−time profile. The residual area was extrapolated to infinity, determined as the final measured mean concentration ($C_t$) divided by the terminal elimination rate constant ($\beta$) and was added to $AUC_{0-t}$ to produce the total area under the curve ($AUC_{0-\infty}$). [c] $C_{12h}$ = brain concentration taken at 12 h postdose.

Several of these analogues were dosed orally in DIO mice to investigate the structural requirements for brain and plasma exposure (Table 2). This set included the N1-alkylated analogues **7a**−**c** in order to assess the effect of transposing the indazole amide side chain on the pharmacokinetic profile. Similarly, the most potent representatives from the benzimidazole (**6a**) and N2-alkylated subseries (**8c**) were included for further comparison of the differing heterocyclic cores. The pharmacokinetic profile of the benzimidazole analogue **6a** was characterized by moderate plasma exposure and poor CNS penetration. Indazole analogue **7a** showed improved pharmacokinetic properties relative to **6a** and a nearly 4-fold increase in tissue exposure with respect to lead indole **2**. Although the $brain_{AUC}/plasma_{AUC}$ ratio was less than 1, the compound maintained brain levels that provided coverage of the functional $IC_{50}$ for at least 12 h. Interestingly, each of the N1-substituted indazole isomers **7a**−**c** demonstrated good brain penetration. The 5-substituted indazole **7b** resulted in the highest levels of parent compound in the brain at the maximal concentration ($C_{max}$) and at 12 h ($C_{12h}$). The N2-alkylated indazole **8c** was less efficient in penetrating the CNS and had a $brain_{AUC}/plasma_{AUC}$ ratio of 0.1.

Compound **7a**, which showed the best combination of binding affinity, functional antagonism, and CNS exposure, was selected for further evaluation. In receptor selectivity panels, **7a** showed weak affinity ($IC_{50}$ > 10 $\mu$M) for other peptide receptors that are structurally related to MCHr1, including MCHr2,[12] somatostatin, and the opioid receptors.[13] We next explored the effects of administration of **7a** in a study measuring food intake and body weight in DIO mice. For a 2-week period, DIO

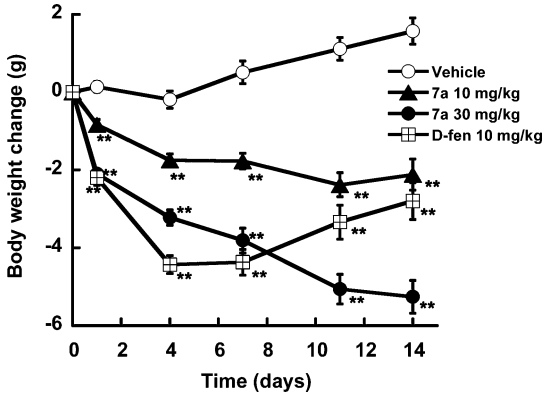



**Figure 2.** Effect of **7a** (dosed at 10 and 30 mg/kg, po, bid in 1% Tween-80 in water) and D-fenfluramine (D-fen, 10 mg/kg, po, qd) on the body weight of DIO mice. Change is registered as the number of grams of body weight difference for each measurement time point relative to day zero. All values are mean values ± SEM for $n = 12$: (**) $p < 0.05$ for comparisons against vehicle group.

**Table 3.** Body Weight (BW) of All Animal Groups at Days 0 and 14 and Percent Change of the Drug-Treatment Groups Relative to Vehicle[a]

| group[b] | $BW_{day0}$ (g) | $BW_{day14}$ (g) | % change from vehicle |
|---|---|---|---|
| lean | 31.7 ± 0.8 | 31.8 ± 0.8 | |
| vehicle | 45.3 ± 0.8 | 46.9 ± 0.8 | |
| D-fen, 10 mg/kg | 43.2 ± 1.5 | 40.4 ± 1.6 | −10.1 ± 1.1 |
| **7a**, 10 mg/kg | 44.7 ± 0.5 | 42.6 ± 0.7 | −8.25 ± 0.92 |
| **7a**, 30 mg/kg | 45.0 ± 0.7 | 39.7 ± 0.8 | −15.2 ± 0.9 |

[a] All values are mean values ± SEM ($n = 12$). [b] All doses were given in 4 mL/kg body weight volume of vehicle (1% Tween-80 in water). Compound **7a** was administered po by gavage at doses of 10 and 30 mg/kg, bid, and D-fenfluramine was administered at a dose of 10 mg/kg, po, qd. Food and body weights were determined on the first day and periodically thereafter for 14 days.

mice fed a high-fat diet ad libitum were dosed orally with **7a** (10 or 30 mg/kg, bid), D-fenfluramine (10 mg/kg, qd), or vehicle. Food intake and body weight were measured at days 1, 4, 7, 11, and 14 for each group. The vehicle group continued to gain weight throughout the study (Figure 2), while D-fenfluramine caused a rapid decrease in body weight followed by a slight rebound starting on day 7.

Compound **7a** caused a dose-dependent decrease in body weight throughout the duration of the treatment. At the end of the study, mice treated with D-fenfluramine weighed 10.1 ± 1.1% less than vehicle-treated mice (Table 3, $p < 0.01$), whereas the mice dosed with indazole **7a** weighed 8.25 ± 0.92% ($p < 0.01$) and 15.2 ± 0.9% ($p < 0.01$) less for the 10 and 30 mg/kg groups, respectively.

Interestingly, the cumulative food intake was not significantly altered in the mice treated with **7a** (Figure 3) at either dose (41.9 ± 3.3 and 44.7 ± 4.8 g, respectively) compared to vehicle-treated controls (41.1 ± 1.3 g). In contrast, treatment with D-fenfluramine caused a significant decrease in food intake over the first 7 days (15.7 ± 1.2 g, $p < 0.05$ vs 20.6 ± 0.8 g for vehicle-treated controls) and lower total food consumption by the end of the 2-week study (36.0 ± 1.7 g). These results suggest that the weight loss observed upon treatment with MCHr1 antagonist **7a** could be the result of an alteration in energy expenditure. Similar findings have been reported in MCHr1 −/− mice, which consumed the same amount of food on a normal diet as the wild-type mice,

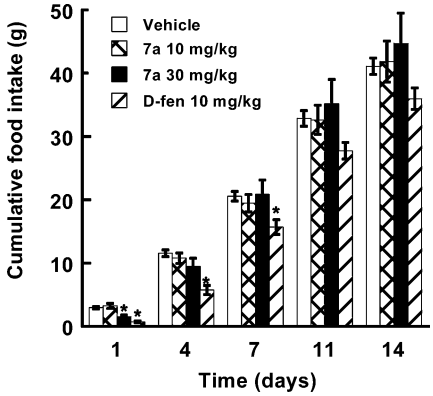



**Figure 3.** Effect of **7a** (10 and 30 mg/kg, po, bid, dosed in 1% Tween-80 in water) and D-fenfluramine (D-fen, 10 mg/kg, po, qd) on daily and cumulative food intake of DIO mice. All values are mean values ± SEM for $n = 12$: (*) $p < 0.05$ for comparisons against vehicle group.

**Table 4.** Exposure Levels of **7a** at 1 h ($C_{max}$) and 17 h ($C_{17h}$) after Final Dose on Day 14

| dose (mg/kg, po, bid) | time (h) | plasma (ng/mL)[a] | brain (ng/g)[a] |
|---|---|---|---|
| 10 | 1 | 168 ± 25.8 | 115 ± 67 |
| 10 | 17 | 0.390 ± 0.220 | 7.02 ± 2.66 |
| 30 | 1 | 824 ± 270 | 233 ± 115 |
| 30 | 17 | 2.23 ± 0.280 | 5.35 ± 1.18 |

[a] Drug concentrations in plasma and in brain were determined 1 or 17 h after the final dose. All values are mean values ± SEM ($n = 3$).

yet were resistant to weight gain as a result of increased energy expenditure.[8,9]

The exposure levels for **7a** at 1 h ($C_{max}$) and 17 h ($C_{17h}$) following the final dose of the chronic study were determined (Table 4). The plasma $C_{max}$ for the 10 mg/kg treatment group is low (therapeutic plasma concentration of 0.37 $\mu$M), which bodes well for the establishment of a therapeutic safety index upon chronic dosing. Additionally, it was apparent that a $C_{max}$ concentration of 115 ± 67 ng/g in the brain was sufficient to deliver significant chronic weight loss in this model.

No significant changes in locomotor activity were observed upon treatment with **7a** at either dose, indicating that treatment did not cause any overt behavioral effects (data not shown). Finally, analysis of body composition following the 2-week treatment was performed with dual-energy X-ray absorptiometry[14] (DEXA) to determine the contributions of changes in fat and lean mass relative to the observed decrease in body weight. Fat mass was significantly reduced by treatment with **7a** for both groups (fat $mass_{day14} = 16.6 \pm 1.7$ and 13.1 ± 0.3 g, $p < 0.05$, for 10 and 30 mg/kg, respectively) relative to vehicle-treated controls (22.1 ± 0.1 g), while no loss of lean mass was apparent. Administration of D-fenfluramine also resulted in a significant decrease in fat mass (fat $mass_{day14} = 12.3 \pm 1.0$ g, $p < 0.05$) with no change in lean mass.

In summary, optimization of the high-throughput screening lead structure **1** led to the identification of **7a**, an MCHr1 antagonist that binds with high affinity to the MCHr1 receptor and potently inhibits $Ca^{2+}$ mobilization in IMR-32 cells. This compound shows good brain and plasma exposure upon oral dosing in DIO mice and is the first MCHr1 antagonist reported to date that is efficacious upon oral dosing in a chronic model

of weight loss.[1] Furthermore, the dose-dependent anorectic effects of **7a** were achieved at low concentrations in the plasma and brain. A contribution from the activation or antagonism of other receptors cannot be ruled out, and further studies are needed to delineate the full actions of treatment with **7a**. However, given the excellent potency and good CNS penetration of this compound, it is likely that MCHr1 antagonism plays a major role in the observed weight loss.

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**Supporting Information Available:** Experimental procedures including characterization data for compounds and procedures for in vitro and in vivo assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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