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Aminopiperidine indazoles as orally efficacious melanin concentrating hormone receptor-1 antagonists

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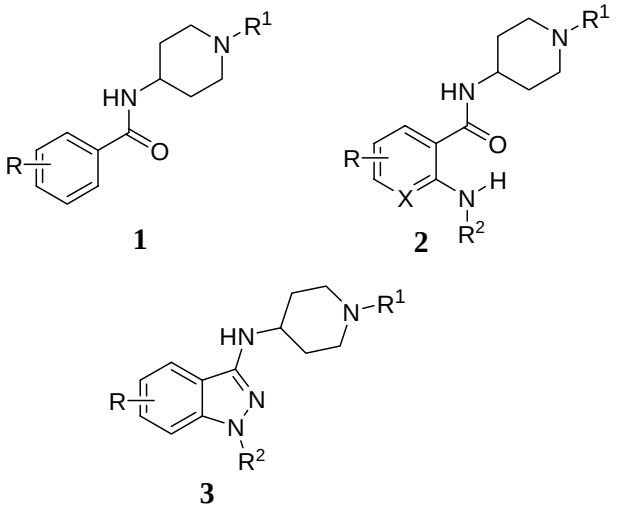
Abstract—The synthesis and biological evaluation of novel 3-amino indazole melanin concentrating hormone receptor-1 antagonists are reported, several of which demonstrated functional activity of less than 100 nM. Compounds **19** and **28**, two of the more potent compounds identified in this study, were characterized by high exposure in the brain and demonstrated robust efficacy when dosed in diet-induced obese mice.

Melanin concentrating hormone (MCH) is an orexigenic neuropeptide, that is produced predominantly by neurons in the lateral hypothalamus and zona incerta.[1] Central MCH administration stimulates food intake while fasting results in an increase in MCH expression.[2] Mice lacking the MCH encoding gene are lean, hypophagic, and maintain elevated metabolic rates,[3] whereas mice over-expressing the MCH gene are susceptible to obesity and insulin resistance.[4] The prospect of MCHr1 as an attractive target for anti-obesity therapy has been reviewed extensively.[5]

We have previously reported the identification of MCHr1 antagonists based on a benzamide scaffold, **1**.[6] Subsequent optimization led to the identification of *ortho* amino benzamides **2**.[7] In this Letter, we describe our continuing efforts aimed at exploring multiple chemotypes for potential MCHr1 inhibition, wherein the *ortho* amino benzamide scaffold is replaced by an indazole moiety to afford compounds such as **3**.



The synthesis of the target compounds was accomplished as described in Scheme 1 or 2. Refluxing a solution of the suitably substituted *ortho* fluoro benzonitrile **4** with hydrazine hydrate in butanol afforded the 3-amino indazole nucleus **5** in moderate to excellent yields, depending on the nature of the R-group. Reductive amination with 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester, followed by deprotection and reductive amination with a variety of monocyclic and bicyclic

Keywords: Melanin concentrating hormone; Obesity; Aminoindazoles; Palladium amination.
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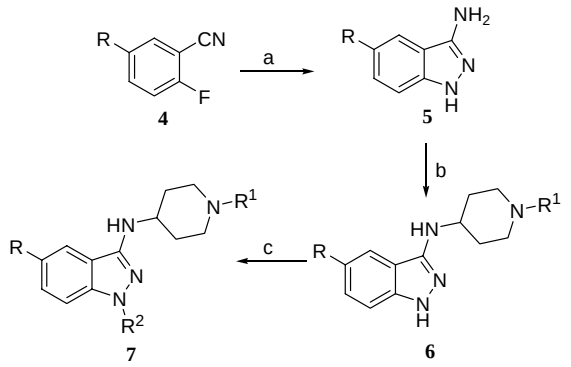
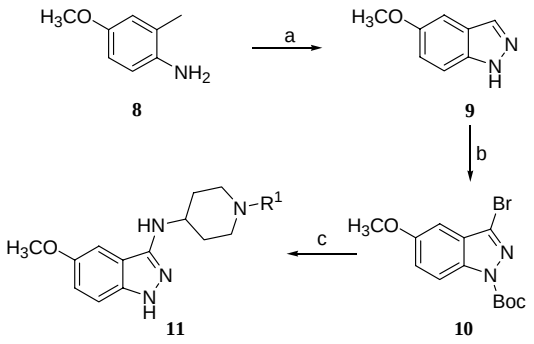


Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, *n*-BuOH, reflux, 4 h; (b) 1. 4-Oxo-piperidine-1-carboxylic acid *tert*-butyl ester, $NaCNBH_3$, AcOH, 50 °C, 12 h; 2. 4 N HCl/dioxane; 3. ArCHO, $NaCNBH_3$, CH_3OH, AcOH (cat.); (c) R^2COOH, PS-DCC, HOBt, *N*,*N*-DMF or NaH, R^2Br, *N*,*N*-DMF.

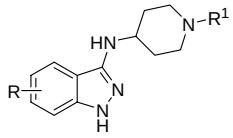


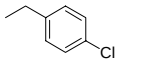
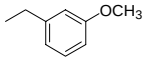
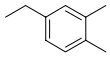
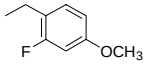
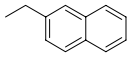
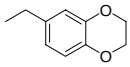
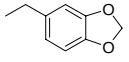
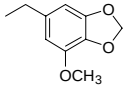
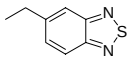
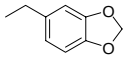
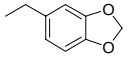
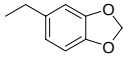
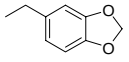
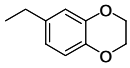
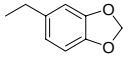
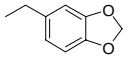
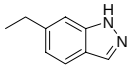
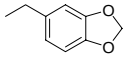
Scheme 2. Reagents and conditions: (a) 1. $NaNO_2$, AcOH; 2. Et_3N. (b) 1. KOH, NBS; 2. $(Boc)_2O$, THF. (c) 1. Functionalized 4-amino piperidine, Pd_2dba_3, Xantphos, Cs_2CO_3, dioxane; 2. HCl/*i*PrOH.

aldehydes and ketones, afforded the target compounds. An alternate synthesis shown in Scheme 2 was devised to access target compounds, where R was an electron-donating group, such as a methoxy moiety. Diazotization and cyclization of 4-methoxy-2-methyl aniline afforded 5-methoxy indazole **9**, which was brominated using *N*-bromosuccinimide. Palladium coupling of the boc-protected intermediate **10** with a suitably functionalized aminopiperidine using Xantphos[8] as the ligand afforded the desired target compounds.

All the compounds synthesized in this study were evaluated for their MCHr1 inhibition in a competitive radiometric-binding assay using receptor obtained from human neuronal IMR-32 cells.[9] Further characterization was performed in an assay designed to measure functional antagonism of MCH-mediated Ca^{2+} release using a fluorometric imaging plate reader ($FLIPR^{TM}$).[9] A cursory analysis of the binding data shown in Table 1 indicates that in general, compounds with bicyclic substituents (as R^1) afforded more potent MCHr1 inhibition compared to those with monocyclic substituents. Comparison of the activities of compound **12** with **13**–**16** indicates that the addition of hydrophobic subtituents to **12** improved MCHr1 inhibition as well as functional potency. Replacement of the phenyl substituent in **12** with a 2-naphthyl moiety (**17**) resulted in a 20-fold

Table 1. MCHr1-binding affinity (IC_{50}) and functional activity (IC_{50}) of indazole analogs



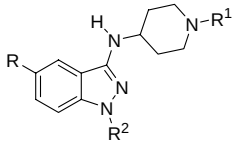
Compound	R	R^1	IMR32 binding IC_{50} (μM)	IMR32 $FLIPR^{TM}$ IC_{50} (μM)
12	5-Cl		0.85	>10
13	5-Cl		0.20	6.2
14	5-Cl		0.15	4.5
15	5-Cl		0.10	1.38
16	5-Cl		0.24	5.2
17	5-Cl		0.04	1.82
18	5-Cl		0.07	1.89
19	5-Cl		0.02	0.26
20	5-Cl		0.28	>10
21	5-Cl		0.05	4.91
22	5-H		0.44	>10
23	5-CF_3		>2	>10
24	5-CH_3		0.11	1.06
25	5-NO_2		0.006	0.07
26	5-NO_2		0.03	0.52
27	5-CN		0.22	3.4
28	5-OCH_3		0.008	0.06
29	5-OCH_3		0.05	0.38
30	4-OCH_3		0.33	>10

improvement in MCHr1 inhibition. Introduction of bicyclic heterocycles such as the 1,4-benzodioxan-6-yl (**18**), piperonyl (**19**), and benzothiadiazolyl (**21**) resulted

in a 12-, 42-, and 17-fold improvement in MCHr1 inhibition, respectively, compared to **12**. Of these three heterocycles, incorporation of the piperonyl substituent (**19**) afforded the best combination of MCHr1 inhibition and functional potency, though substituents on the phenyl ring resulted (**20**) in a diminution of both parameters. The substituent at the 5-position of the indazole was also found to be a crucial determinant of activity. Removal of the 5-chloro moiety (**22**) resulted in a 22-fold loss in MCHr1-binding affinity, whereas the 5-trifluoromethyl (**23**) and methyl (**24**) substituted analogs were inactive and 5-fold less active than **19**, respectively. The 5-nitro substitution resulted in a very potent MCHr1 antagonist (**25**) with an IC_{50} of 6 nM and functional activity of 70 nM. Replacement of the piperonal moiety in **25** with a benzodioxan-6-yl substituent (**26**) resulted in a 5-fold loss in MCHr1 inhibition and a 7-fold loss in functional potency. The 5-cyano substituted compound **27** was a moderately potent MCHr1 antagonist, though the functional potency was compromised significantly.[10] A methoxy substituent at the 5-position (**28**) afforded comparable MCHr1 inhibition and functional activity as the 5-nitro group (8 and 60 nM, respectively). Other bicyclic heterocycles such as the indazole moiety were also investigated, though the MCHr1 inhibition was compromised to some extent. Moving the methoxy substituent to the 4-position resulted in a significant drop in MCHr1 inhibition and functional potency.

Prior studies on the *ortho* amino benzamide scaffold (**2**)[7] had indicated that substitution off the *ortho* amino group was well tolerated by the MCH1 receptor and provided a suitable handle for modulation of pharmacokinetic parameters. Alkylation and acylation of the indazole nitrogen was performed as shown in Scheme 1, by using the requisite alkyl bromide or carboxylic acid, respectively, and selected data are shown in Table 2. In general, a wide range of substituents and linkers on the *N*-1 nitrogen were well tolerated by the MCH1 receptor, though the functional potency of these analogs was compromised. Introduction of a methyl group resulted in a 4-fold decrease in MCHr1 inhibition (**28** → **31**), whereas introduction of a phenyl group resulted in a 2-fold decrease in MCHr1 inhibition (**19** → **32**). The benzyl substituted analogs **33** and **34** were comparable in potency to the parent analogs (**25** and **19**, respectively), whereas introduction of a pyridylmethyl substituent resulted in a two to 6-fold

Table 2. MCHr1-binding affinity (IC_{50}, μM) and functional activity (IC_{50}, μM) of indazole analogs



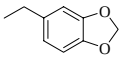
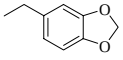
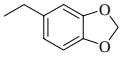
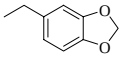
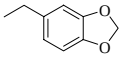
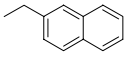
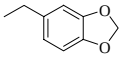
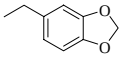
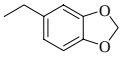
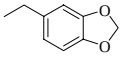
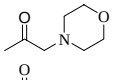
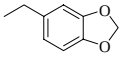
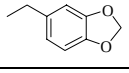
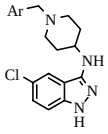
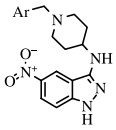
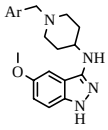
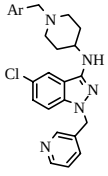
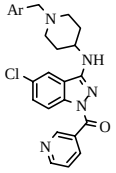
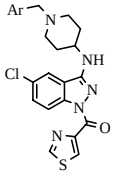
Compound	R	R^1	R^2	IMR32 binding	IMR32 FLIPR™
31	OCH_3		CH_3	0.03	0.19
32	Cl			0.05	>10
33	NO_2			0.005	0.35
34	Cl			0.04	5.23
35	Cl			0.009	0.55
36	Cl			0.02	0.64
37	Cl			0.02	0.29
38	Cl			0.007	0.56
39	Cl			0.04	>10
40	Cl			0.02	1.14
41	Cl			0.02	0.93
42	Cl			0.02	2.54

Table 3. Plasma and brain exposure of **19**, **25**, **28**, **37**, **41**, and **42** in DIO mice after 10 mg/kg oral dose

Compound		Plasma AUC (ng h/ml)	Brain AUC (ng h/ml)	Plasma $T_{1/2}$ (h)	Brain $T_{1/2}$ (h)	Plasma C_{max} (ng/ml)	Brain C_{max} (ng/ml)
19		8762	59057	3.1	2.9	1883	12,140
25		14448	22824	3.3	4.0	1524	3076
28		7626	28461	3.9	4.9	1407	6035
37		11931	5342	4.7	6.0	1692	758
41		164	—	2.3	—	63	—
42		400	—	1.0	—	271	—

Ar = 3,4-methylenedioxyphenyl.

improvement in MCHr1 inhibition, depending on the pyridine regioisomer. Acylation of the *N*-1 nitrogen of **19** with various hydrophobic, heterocyclic, and basic moieties resulted in retention of MCHr1 inhibition though the functional potency was compromised.

The pharmacokinetic parameters of selected analogs were evaluated in a DIO mouse model using methods described before,[6] and the data are shown in Table 3. Several interesting trends were observed with these compounds. Compounds **19**, **25**, and **28** demonstrated high exposure in the brain with peak concentrations of 12140, 3076, and 6035 ng/g, respectively. These compounds preferentially partitioned into the brain, a desirable feature since the largest concentration of MCHr1 is in the lateral hypothalamus. Alkylation of the *N*-1 nitrogen (**37**) resulted in compounds with moderate brain exposure. However, the preferential partitioning in the brain observed with the non-alkylated analogs was compromised. A drastic change in the brain exposure and half-lives was observed when the *N*-1 nitrogen was acylated. Compound **41**, and to a lesser extent **42**, was rapidly cleared from the plasma, with concentrations declining below the limits of quantitation within four hours after oral dosing. Further, brain concentrations were not detected in any of the time points.

Due to their superior activity in the MCH functional assay and favorable pharmacokinetic profile, compounds **19** and **28**[11] were evaluated in an efficacy study measuring food intake and body weight in DIO mice.[6] Compound **19** was dosed at 3, 10, and 30 mg/kg, p.o., q.d, whereas **28** was dosed at 10 and 30 mpk, p.o. q.d. with body weights and food intake measured at regular intervals. At the 10 and 30 mg/kg dose, **19** caused significant weight loss starting on day 4 (Fig. 1), whereas no significant weight loss was seen with the 3 mpk dose. Compound **28** caused a decrease in body weight with the 10 and 30 (Fig. 2) mpk dose. Further, there was no significant loss of lean body mass seen with any of the treatment groups, indicating that the observed weight loss was due to loss of fat mass.

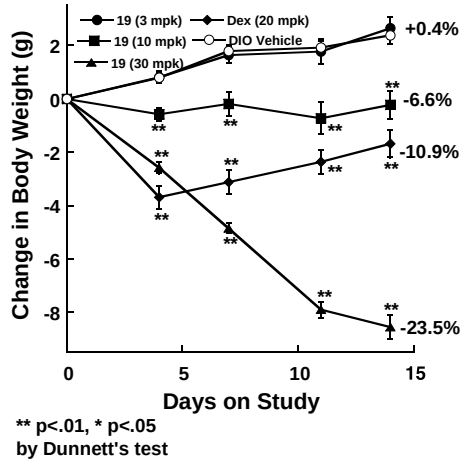


Figure 1. Effect of compound **19** (dosed at 3, 10, and 30 mg/kg, q.d., p.o., in 1% Tween 80 in water) on the body weight of DIO mice.

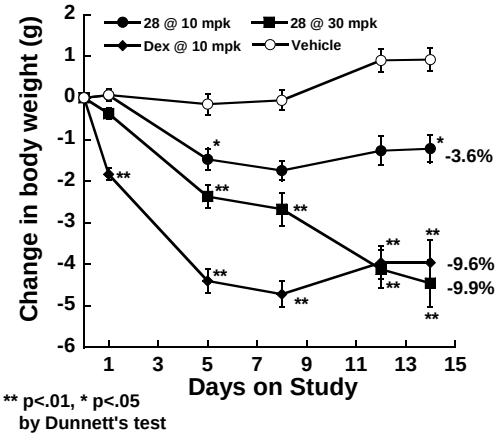


Figure 2. Effect of compound **28** (dosed at 10 and 30 mg/kg, q.d., p.o., in 1% Tween 80 in water) on the body weight of DIO mice.

In conclusion, several indazole containing MCHr1 antagonists have been identified. The pharmacokinetic parameters of this class of MCHr1 antagonists could be modulated depending on substitution at the *N*-1 nitrogen. Compounds demonstrating the most potent MCHr1 inhibition and functional potency demonstrated robust efficacy in a DIO mouse model.

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10. In certain cases, a significant disconnect between MCHr1-binding affinity and activity in the functional assay was observed. Studies are underway to determine the cause of this phenomenon and results will be reported elsewhere.
11. Compound **25** was also evaluated for efficacy in a DIO mouse model and was found to cause robust weight-loss (data not shown).