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Tetrahydrocarboline analogs as MCH-1 antagonists

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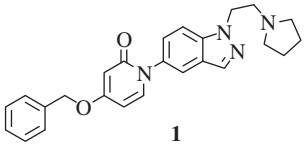
ABSTRACT

A new series of tetrahydrocarbolines with potent MCH-1 antagonist activity were synthesized, using a conformationally constrained design approach towards optimizing pharmacokinetic properties. Two compounds from this series were progressed to a 5-day diet-induced obesity mouse screening model to evaluate their potential as weight loss agents. Both compounds produced a highly significant reduction in weight, which was attributed to their improved pharmacokinetic profile.

Melanin concentrating hormone (MCH) is a peptide originally identified as a color mediating factor in teleost fish.[1] MCH is also found in mammals as a 19 amino acid cyclic neuropeptide.[2] While mammalian MCH has high homology to salmon MCH, the primary function is quite different, with the former being primarily localized in the brain.[3] Rather than mediating changes in pigment, mammalian MCH is highly expressed in the lateral hypothalamus of rodent brains, prompting investigations into the role of the neuropeptide on food intake and body weight regulation.[4] Indeed, a variety of small molecule MCH-1 receptor antagonists have been shown to effectively reduce body weight in rodent models of obesity.[5] As rat and human MCH is identical in structure,[6] MCH-1 antagonists represent an exciting target for controlling food intake and body weight in the overweight and obese population.

Previously, we have disclosed the 5-(pyridinin-1-yl)indazole **1** as a potent MCH-1 antagonist that demonstrated significant efficacy in a diet-induced obesity (DIO) mouse screening model.[7]



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Compound **1** showed a statistically significant reduction in body weight (2.8%) at a 60 mg/kg (qd) dose. However, this weight loss was significantly increased (6.0%) when switching to a twice-daily 30 mg/kg dosing regime, suggesting there were opportunities to improve upon the pharmacokinetic profile of this compound. Thus, a strategy was employed to improve upon the suboptimal pharmacokinetic properties of compound **1**, providing compounds with a profile consistent with once-daily dosing. Our plan was to introduce a conformational constraint between the indazole ring and basic amine, which would hopefully improve the oral exposure, increase the brain concentration and hence improve upon the efficacy seen for the once-daily dosing of compound **1**. It was believed that such a constrained mimic of the indazole could be obtained via the γ-tetrahydrocarboline scaffold **3** and the β-tetrahydrocarboline scaffold **4**, both of which were anticipated to have a basic nitrogen with a similar pK_a to that of scaffold **2** (Fig. 1).

Analogs relating to core scaffold **3** were prepared using a Fisher-indole cyclization as the key step (Scheme 1). 3-Bromophenylhydrazine and 4-*N*-Boc-piperidone were reacted under acidic conditions, and the resultant tetrahydrocarboline was re-protected to provide carbamate **5**. Alkylation of the indole was achieved using sodium hydride and methyl iodide providing **6**. Copper-catalyzed coupling with 4-benzyloxypyridone provided **7**, which could be easily deprotected using methanolic HCl to provide the desired compound **8**. Diverse analogs could be synthesized by introducing alkyl substituents onto the basic amine (via reductive alkylation). Additionally, the pyridone functionality could be varied via preparation of suitably substituted pyridones, which themselves could be prepared from 4-chloropyridine.[8]

Analogs of core scaffold **4** were prepared in an analogous manner to that of scaffold **3**, except a Fisher-indole cyclization between

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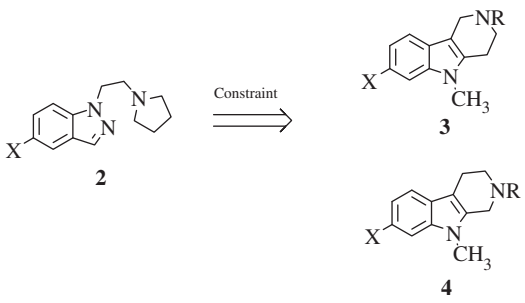
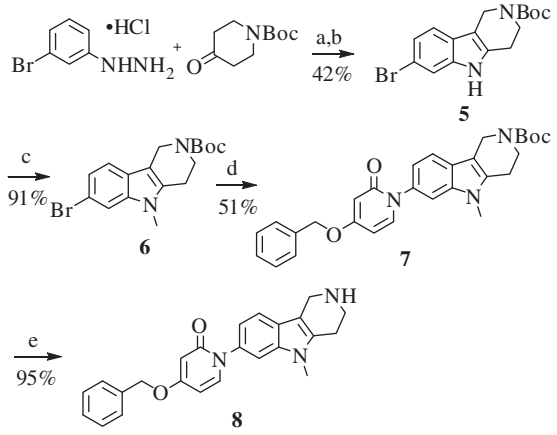


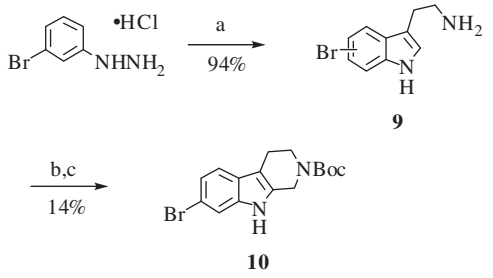
Figure 1. Strategy for conformationally constraining the indazole and basic amine of scaffold **2**.



Scheme 1. Reagents and conditions: (a) EtOH, HCl, reflux; (b) Boc_2O, Et_3N, DMAP, CH_2Cl_2, room temperature; (c) NaH, MeI, DMF, room temperature; (d) 4-benzyloxypyridone, CuI, 8-hydroxyquinoline, DMSO, 130 °C; (e) HCl, MeOH, room temperature.

commercially available 3-bromophenylhydrazine and 4,4-diethoxybutan-1-amine[9] was followed by a Pictet–Spengler reaction using glyoxylic acid[10] to generate the tricyclic ring system (Scheme 2).

The parent compound prepared around the γ-tetrahydrocarboline scaffold (**8**) showed very encouraging MCH-1 potency (Table 1), having similar activity to the indazole (**1**). SAR development was conducted around this scaffold with methylation of the basic nitrogen (**11a**) retaining excellent potency. Further alkyl substitution was also tolerated with the hydroxyethylated analog (**11b**) having similar affinity. However, functionalization of this nitrogen leading to a loss of basicity (as shown by acetamide **11c**) caused a dramatic reduction in potency, highlighting the need for a basic nitrogen within the structures. Furthermore, functionalization of the phenyl ring (pertaining to the benzyloxy motif) with lipophilic 4-substituents (**11d**–**11e**) led to retention of potency whilst offering



Scheme 2. Reagents and conditions: (a) 4,4-diethoxybutan-1-amine, $ZnCl_2$, 180 °C; (b) (1) glyoxylic acid, KOH, H_2O; (2) HCl, H_2O, reflux; (c) Boc_2O, Et_3N, DMAP, CH_2Cl_2, room temperature.

the potential for tuning the physicochemical properties. Increased lipophilicity was not, however, a determinant of improved potency (for example, compound **8** had a *c* Log *P* of 3.89 yet was twice as potent as compound **11d** with a *c* Log *P* of 4.77).

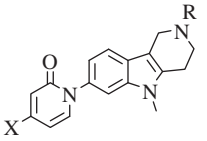
Modification of the pyridone substituent, from a benzyloxy motif to a directly-linked phenyl motif, also provided potent MCH-1 ligands. Guided by SAR from previously published work,[11] where 4-substitution and 2,4-disubstitution was found to be optimal, a number of analogs were prepared (Table 1). The parent phenyl compound (**11f**) showed promising activity, albeit slightly weaker than that seen for the parent compound in the benzyloxy series **(8)**. However, introduction of a lipophilic group into the 4-position significantly improved the MCH-1 binding activity, returning to potency levels seen within the benzyloxy series (**11g–11i**). Interestingly, the more polar methoxy (**11j**) and thiomethyl (**11k**) groups were not only tolerated, but delivered two of the most potent compounds seen within the series. 2,4-Disubstituted phenyl appendages were tolerated (**11l–11n**), although the potency was generally slightly weaker than observed for the monosubstituted counterparts.

SAR around the β-tetrahydrocarbolines (**12a–12l**) followed a similar trend to that seen for the γ-tetrahydrocarbolines, demonstrating a level of flexibility around the location of the basic nitrogen (Table 1). These analogs retained very similar affinity for the MCH-1 receptor when compared with their γ-tetrahydrocarboline counterparts. Furthermore, a lack of tolerance towards heterocyclic phenyl replacements was demonstrated within this series, with pyridyl analogs (**12m** and **12n**) having weak MCH-1 binding affinity. This suggests either increased polarity or the presence of a weakly basic center may not be tolerated within this region of this particular scaffold. Measurement of the pK_a of compound **12a** was also undertaken and compared with that of compound **1** to determine whether both scaffolds did indeed contain similarly basic amines. Compound **12a** had a measured pK_a of 8.81 which was in good agreement to that of compound **1** (pK_a = 8.63).

A select set of analogs was chosen for further in vitro evaluation, and compared to compound **1**. Four compounds (**8**, **11a**, **12a** and **12b**) were tested for CYP inhibition, metabolic stability and solubility (Table 2). All four compounds had an attractive profile, showing >1000-fold selectivity for MCH-1 over CYP inhibition, very good aqueous solubility and excellent metabolic stability (with the exception of **12b** in mouse microsomes). Compounds **11a** and **12a** were identified as being of particular interest and were selected for additional in vitro and in vivo studies.

Compound **11a** was found to be a highly selective MCH-1 antagonist, having no significant off-target activity (<55% inhibition @ 1 μM) in a panel of 30 GPCR's and transporters. Similarly, compound **12a** showed remarkable selectivity in a panel of 88 GPCR's and transporters, having no significant off-target affinity. Furthermore, **12a** was identified as an MCH-1 antagonist in a functional assay (IC_{50} = 14.4 nM).[13] Both compounds were then administered to DIO mice to assess their pharmacokinetic profile. Gratifyingly, the strategy employed to constrain the basic center in an effort to improve oral exposure proved to be a good one, as both **11a** and **12a** had a significantly improved pharmacokinetic profile over compound **1**. Plasma levels (after 6 h) were substantially greater than those of **1** at the same dose, and the brain levels for **12a** mirrored this trend (almost three times higher than those for compound **1**). The AUC values for compounds **11a** and **12a** were also significantly higher than that for compound **1**, further demonstrating improved oral exposure. Although **12a** had significantly higher levels in the brain than **1**, this was attributed to an improvement in oral exposure more than brain penetration, as the b/p ratios for both compounds were fairly similar. All three compounds had >90% plasma protein binding in mouse (Table 3).

Table 1
SAR of γ- and β-tetrahydrocarbolines



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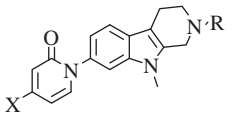
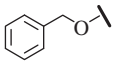
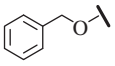
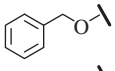
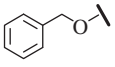
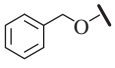
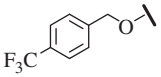
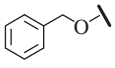
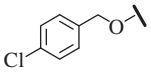
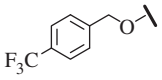
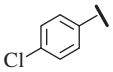
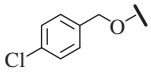
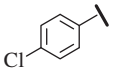
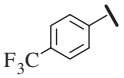
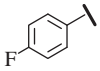
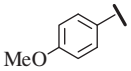
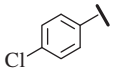
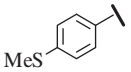
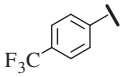
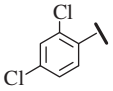
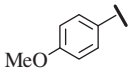
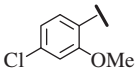
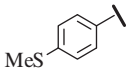
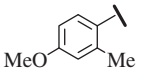
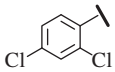
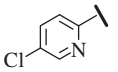
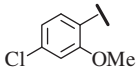
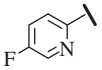
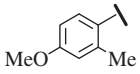


12

Compound	X	R	MCH-1 binding[a,b] K_i (nM)	Compound	X	R	MCH-1 binding[a,b] K_i (nM)
1	N/A	N/A	2.6				
8		H	3.8	**12a**		H	4.9
11a		Me	4.5	**12b**		Me	5.2
11b		$-CH_2CH_2OH$	3.6	**12c**		Me	25
11c		$-COCH_3$	168	**12d**		Me	7.6
11d		H	6.7	**12e**		H	11
11e		H	4.2	**12f**		Me	5.6
11f		H	15	**12g**		H	11
11g		Me	7.8	**12h**		H	14
11h		H	2.7	**12i**		H	8.4
11i		H	4.2	**12j**		Me	14
11j		H	2.6	**12k**		H	17
11k		H	2.4	**12l**		H	14
11l		H	7.2	**12m**		H	128
11m		H	7.0	**12n**		Me	708
11n		H	7.6				

[a] Displacement of [^{3}H]Compound **1** from MCH-1 expressed in CHO-K1 cells (K_d = 1.42 ± 0.08 nM and B_{max} = 13.3 ± 0.7 pmol/mg protein; mean ± SEM n = 4).[12]
[b] Values are means of at least two determinations where each determination is within ±40% of the mean value shown.

In order to assess whether the improved PK of compounds **11a** and **12a** would translate to improved efficacy through once-daily dosing, both compounds were progressed to a DIO mouse screening study for analysis of their effects on body weight lowering. Each compound was dosed over a 5 day period, with the effect on body weight monitored daily. Compound **11a** showed a highly significant reduction in body weight (7.2%) at a once-daily 30 mg/kg dose as shown in Figure 2. This compared favorably with compound **1**, which showed a modest reduction in body weight (2.8%) at double the dose (60 mg/kg) when administered once-daily.[7]

Compound **12a** induced a slightly greater effect, producing a 9.1% reduction in body weight after dosing at 30 mg/kg (Fig. 3). This reduction in body weight represented a greater than threefold improvement upon the efficacy seen for compound **1**, at half the dose.

In conclusion, a conformational constraint approach was used to identify tetrahydrocarbolines as potent MCH-1 antagonists. Select compounds from this class had an excellent in vitro profile which led to superior pharmacokinetics over the initial lead compound from our MCH-1 program. These modifications translated to greatly improved efficacy in a DIO mouse model of obesity.

Table 2
Additional in vitro data for selected compounds

Compound	CYP 3A4[a] IC_{50} (μM)	Half-life (min)		Aqueous solubility[d] (μM)
		MLM[b]	HLM[c]	
1	10	695	303	492
8	68	98.4%[e]	462	470
11a	67	>1000	770	110
12a	12	482	450	>500
12b	24	63	>1000	93

[a] 1A2, 2B6, 2C9, 2C19, 2D6 and 3A4 isoforms tested. Isoforms not reported have IC_{50}'s greater than 10 μM.
[b] Mouse liver microsomes.
[c] Human liver microsomes.
[d] Measured in PBS at pH 7.4 after a 24 h equilibration period.
[e] Percentage remaining after 1 h.

Table 3
Comparison of PK data for compounds **1**, **11a** and **12a** in DIO mice

PK Profile	**1**	**11a**	**12a**
Dose (mg/kg)	30 (po)	30 (po)	30 (po)
$[Plasma]_{6\ h}$ (ng/mL)[a]	1950	5027	8026
$[Brain]_{6\ h}$ (ng/g)[b]	1377	—	4012
B/P ratio[c]	0.72	0.9[d]	0.51
$AUC_{0-6\ h}$ (h∗ng/mL)[e]	14,760	26,976	41,333
PPB (mouse)[f]	91.4%	98.4%	94.1%

[a] Plasma concentration 6 h post dose.
[b] Brain concentration 6 h post dose.
[c] Ratio of brain concentration to plasma concentration at 6 h.
[d] b/p ratio derived from lean mice after a 10 mg/kg oral dose.
[e] Area under curve determined between 0 h and 6 h.
[f] Plasma protein binding determined by an ultrafiltration method.

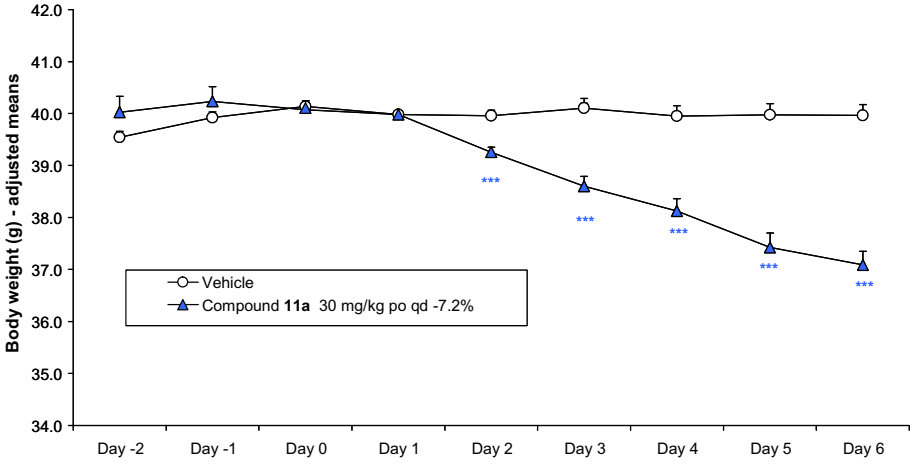


Figure 2. Effect of compound **11a** (dosed at 30 mg/kg, po, qd in 1% methylcellulose in water) on the body weight of male C57BL/6J DIO mice. Data are adjusted means (n = 8–10). SEMs are calculated from the residuals of the statistical model. Data analysed by ANCOVA with body weight on Day 1 as covariate followed by Dunnett's test for adjusted data; $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001. Figures in legend refer to % difference from control on Day 6 (i.e., after 5 days dosing).

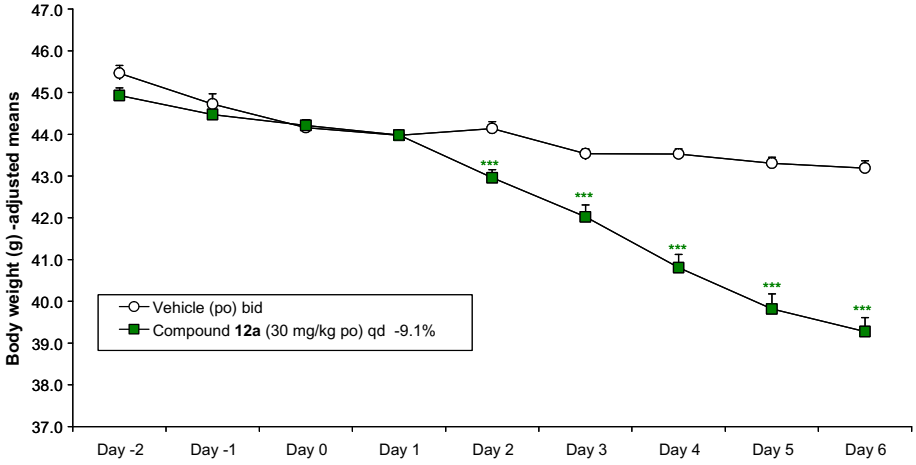


Figure 3. Effect of compound **12a** (dosed at 30 mg/kg, po, qd in 1% methylcellulose in water) on the body weight of male C57BL/6J DIO mice. Data are adjusted means (n = 8–10). SEMs are calculated from the residuals of the statistical model. Data analysed by ANCOVA with body weight on Day 1 as covariate followed by Dunnett's test for adjusted data; $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001. Figures in legend refer to % difference from control on Day 6 (i.e., after 5 days dosing).

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12. MCH-1 binding affinity was measured using 4-(3,4,5-tritritiumbenzyloxy)-1-(1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-indazol-5-yl)pyridin-2(1*H*)-one and membranes prepared from stable CHO-K1 cells expressing the human MCH-1 receptor, obtained from Euroscreen (Batch 1138). Cell membrane homogenates (8.92 μg protein) were incubated for 60 min at 25 °C with 1.4 nM of the [^{3}H]-labeled compound in the absence or presence of the test compound in 50 mM Tris–HCl buffer, pH 7.4. Nonspecific binding was determined in the presence of 50 μM 1-(5-(4-cyanophenyl)bicyclo[3.1.0]hexan-2-yl)-3-(4-fluoro-3-(trifluoromethyl)-phenyl)-1-(3-(4-methylpiperazin-1-yl)propyl)urea.
13. MCH-1 functional response was measured at Euroscreen assessing Ca^{2+} mobilization using an Aequorin cell line expressing the MCH-1 recombinant receptor. Agonist activity was expressed as a percentage of activity of reference agonist (MCH) at its EC_{100} concentration. Antagonist activity was expressed as percentage inhibition of reference agonist activity at its EC_{80} concentration.