

## Identification of aminopiperidine benzamides as MCHr1 antagonists

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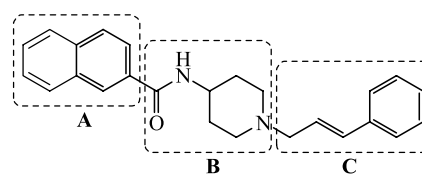
**Abstract**—The identification of a novel series of benzamide-containing MCHr1 antagonists is described. Compound **22** displayed moderate efficacy in a diet induced obesity mice model.

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Melanin concentrating hormone (MCH) is a cyclic neuropeptide that regulates feeding behavior and energy balance in mammals.<sup>1</sup> MCH is expressed throughout the brain with the highest levels in the lateral hypothalamus and zona incerta areas.<sup>2</sup> The hypothalamic MCH mRNA level is up-regulated in leptin-deficient (ob/ob) mice, and further increased by fasting.<sup>3</sup> MCH deficient mice are hypophagic and hypermetabolic with decreased body weight and increased leanness,<sup>4</sup> while over-expression of MCH peptide in mice leads to obesity and insulin resistance.<sup>5</sup> The evidence for MCH regulating food intake and energy homeostasis has been reviewed comprehensively.<sup>6</sup>

We have previously reported on our efforts towards the optimization of a 2-amino-8-alkoxyquinoline-containing high throughput screening hit.<sup>7</sup> In this report, we describe our continued quest for an orally active MCHr1 antagonist as an effective treatment for obesity. The naphthamide A-703362 was identified as a chemical lead with weak affinity for MCHr1 in a competitive radiometric binding assay using receptor obtained from human neuronal IMR-32 cells.<sup>7</sup> Further characterization in an assay designed to measure functional antagonism of MCH-mediated Ca<sup>2+</sup> release using a

fluorometric imaging plate reader (FLIPR<sup>TM</sup>)<sup>7</sup> showed approximately a 20-fold decrease in potency compared with binding IC<sub>50</sub>. The structural simplicity of this lead allowed rapid and comprehensive investigation to delineate structural features essential for potent MCHr1 inhibition and functional activity.



A-703362

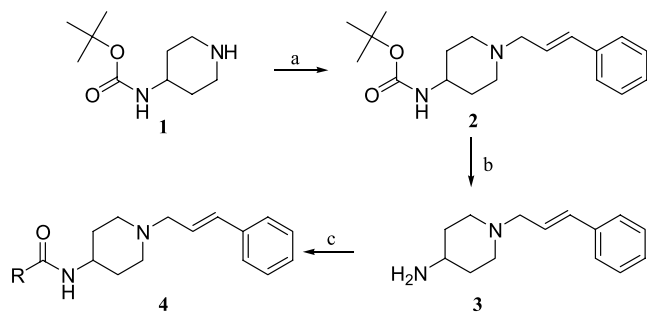
MCHr1 IC<sub>50</sub> = 1.84 μM

Initial SAR investigations were aimed at developing an understanding of region A of the lead molecule. The synthesis of these compounds was accomplished as shown in Scheme 1.

Table 1 shows the MCHr1 binding affinity of these cinnamyl substituted analogs. Compounds which demonstrated MCHr1 binding of less than 100 nM were evaluated for functional antagonism of MCH-mediated Ca<sup>2+</sup> release in a FLIPR<sup>TM</sup> based assay.<sup>7</sup> Replacement of the 2-naphthyl moiety with a phenyl group (**5**) resulted in decreased MCHr1 inhibition, as did

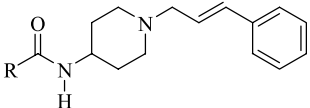
**Keywords:** Melanin concentrating hormone; Benzamides; Obesity.

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**Scheme 1.** Reagents and conditions: (a) cinnamyl chloride,  $K_2CO_3$ , DMF, 50 °C, 76%; (b) 4 N HCl/dioxane, 25 °C, 4 h, quantitative; (c)  $RCOOH$ , PS-DCC,  $^8$  HOBt, DMF, 25 °C.

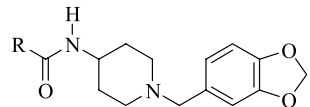
**Table 1.** MCHr1 binding affinity and functional activity of analogs<sup>a</sup>

|  |   |                                        |                                                    |
|-----------------------------------------------------------------------------------|---|----------------------------------------|----------------------------------------------------|
| Compound                                                                          | R | IMR32 binding<br>$IC_{50}$ ( $\mu M$ ) | IMR32 FLIPR <sup>TM</sup><br>$IC_{50}$ ( $\mu M$ ) |
| 5                                                                                 |   | $6.50 \pm 0.81$                        | —                                                  |
| 6                                                                                 |   | $5.19 \pm 0.92$                        | —                                                  |
| 7                                                                                 |   | $0.05 \pm 0.03$                        | $0.56 \pm 0.1$                                     |
| 8                                                                                 |   | $0.17 \pm 0.05$                        | —                                                  |
| 9                                                                                 |   | $0.11 \pm 0.08$                        | —                                                  |
| 10                                                                                |   | >10                                    | —                                                  |
| 11                                                                                |   | $0.30 \pm 0.16$                        | —                                                  |

replacement with several heterocycles (only one example is shown, **6**). Introduction of a methoxy or chloro substituent at the meta position of **5** resulted in a 130- and 59-fold increase in MCHr1 binding affinity, as demonstrated by **7** and **9**, respectively. However, the functional potency of **7** was 10-fold lower than its binding affinity. The benzyl analog **10** was devoid of MCHr1 affinity, whereas introduction of a bromo moiety at the meta-position of **10** resulted in a significant boost in binding affinity (**11**). Having established that a significant improvement in MCHr1 binding affinity could be accomplished with a substituent at the meta position, we sought to explore this feature in more detail.

Prior to refining the SAR of region A, we explored several changes in region C and identified the piperonyl moiety as a suitable replacement for the cinnamyl moiety. Further SAR investigations of regions A and B were performed with this heterocycle. Table 2 outlines further exploration of region A. The meta-methoxy and meta-chloro substituted compounds, **12** and **13**, were comparable in potency with **7** and **9**, though the functional antagonism of **12** was approximately 4-fold

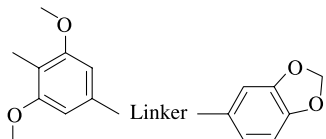
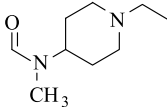
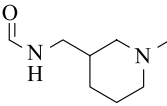
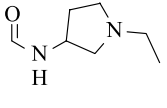
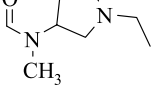
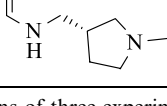
**Table 2.** MCHr1 binding affinity and functional activity of analogs<sup>a</sup>

|  |                |                                        |                                                    |
|------------------------------------------------------------------------------------|----------------|----------------------------------------|----------------------------------------------------|
| Compound                                                                           | R <sup>1</sup> | IMR32 binding<br>$IC_{50}$ ( $\mu M$ ) | IMR32 FLIPR <sup>TM</sup><br>$IC_{50}$ ( $\mu M$ ) |
| 12                                                                                 |                | $0.05 \pm 0.03$                        | $0.13 \pm 0.01$                                    |
| 13                                                                                 |                | $0.11 \pm 0.09$                        | —                                                  |
| 14                                                                                 |                | $0.09 \pm 0.02$                        | $0.54 \pm 0.01$                                    |
| 15                                                                                 |                | $0.33 \pm 0.05$                        | —                                                  |
| 16                                                                                 |                | $0.32 \pm 0.20$                        | —                                                  |
| 17                                                                                 |                | $1.56 \pm 0.73$                        | —                                                  |
| 18                                                                                 |                | $0.12 \pm 0.10$                        | —                                                  |
| 19                                                                                 |                | $0.01 \pm 0.002$                       | $0.12 \pm 0.04$                                    |
| 20                                                                                 |                | $4.30 \pm 0.25$                        | —                                                  |
| 21                                                                                 |                | $0.03 \pm 0.01$                        | $0.53 \pm 0.07$                                    |
| 22                                                                                 |                | $0.003 \pm 0.001$                      | $0.09 \pm 0.002$                                   |

<sup>a</sup> Values are means of three experiments.

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**Table 4.** MCHr1 binding affinity and functional activity of analogs<sup>a</sup>

|  |                                                                                     |                                                     |                                                                 |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------------------|
| Compound                                                                          | Linker                                                                              | IMR32 binding<br>IC <sub>50</sub> (μM) <sup>a</sup> | IMR32 FLIPR <sup>TM</sup><br>IC <sub>50</sub> (μM) <sup>a</sup> |
| 36                                                                                |    | >2                                                  | —                                                               |
| 37                                                                                |    | >2                                                  | —                                                               |
| 38                                                                                |    | >2                                                  | —                                                               |
| 39                                                                                |    | >2                                                  | —                                                               |
| 40                                                                                |  | 0.28 ± 0.05                                         | —                                                               |

<sup>a</sup> Values are means of three experiments.**Table 5.** Selected PK parameters<sup>a</sup> of **22** in DIO mice (10 mg/kg po)

|        | C <sub>max</sub> (ng/ml or ng/g) | T <sub>1/2</sub> (h) | AUC (ng h/ml or ng h/g) |
|--------|----------------------------------|----------------------|-------------------------|
| Plasma | 2297                             | 2.8                  | 9801                    |
| Brain  | 5083                             | 3.7                  | 24,926                  |

<sup>a</sup> Values are means of three experiments.

better than **7**. Introduction of bulky substituents such as a phenoxy group at the meta position of the phenyl ring (**15**) resulted in decreased MCHr1 binding affinity as did replacement of the phenyl ring with a 2-naphthyl moiety (**16**). A dramatic decrease in MCHr1 affinity was observed by introduction of a methoxy group at the *ortho* position of **12** to give **17**. A similar effect was observed with *ortho*-chloro and methyl groups (data not shown). However, introduction of a *meta*-methoxy group to afford **19** resulted in a small improvement in MCHr1 affinity compared to **12**,

though the potency in MCH functional assays was not improved. Replacement of the methoxy groups of **19** with chloro groups (**21**) resulted in a 2-fold decrease in MCHr1 binding affinity, whereas the dihydroxy compound **20** demonstrated very weak MCHr1 binding activity. Introduction of a *para*-methyl group to **19** afforded **22**, which was a 3 nM inhibitor of MCHr1 and demonstrated functional antagonism, with an IC<sub>50</sub> of 90 nM.

Replacement of the amide with a sulfonamide or carbamate resulted in at least a 40-fold decrease in MCHr1 binding affinity (Table 3), whereas a urea moiety resulted in a 25-fold loss in potency. Homologation of the urea linker to provide **27–29** resulted in comparable MCHr1 affinity as **26**.

Homologated versions of the benzamide chemotype provided interesting results. Whereas homologation of **19** to afford **30** resulted in an 80-fold decrease in MCHr1 binding affinity, introduction of phenyl, methoxyphenyl and cyanophenyl moieties at the *para* position afforded analogs **31–35** with significantly improved MCHr1 affinity. However, the functional potency of these homologated compounds was not improved compared to the benzamide analogues described in Table 2.

Continued SAR investigations of region B prompted the search for an optimal diamine linker, a few of which are shown in Table 4. *N*-methylation of **22** resulted in abolishment of MCHr1 activity as did incorporation of a 3-aminopyrrolidine, 3-aminomethylpiperidine, and 1,3-diaminopropyl (data not shown) linkers. The (*R*)-3-aminomethylpyrrolidine substituted compound **40** was the only linker amongst all the spacers investigated which provided for sub-micromolar MCHr1 binding affinity.

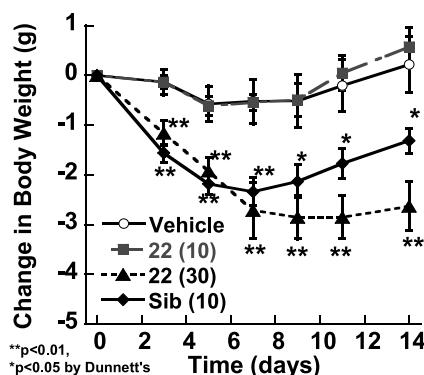
Since the largest concentration of MCHr1 is located in the lateral hypothalamus, it is likely that potential drug candidates acting through this receptor will be required to penetrate the blood–brain barrier. In order to evaluate this hypothesis, compound **22**, which demonstrated the best combination of MCHr1 binding and functional potency, was dosed orally at 10 mg/kg in diet induced obese (DIO) mice.<sup>7</sup> As shown in Table 5, **22** preferentially partitioned into the brain suggesting that efficacy could potentially be achieved using this compound.

Compound **22** was also dosed in dogs<sup>9</sup> to evaluate its pharmacokinetic parameters, and details are shown in Table 6. It was characterized by high plasma concentrations, providing low plasma clearance values (Clp = 0.18 L/h kg) and moderate volume of distribution

**Table 6.** Mean (*n* = 3) plasma concentrations of **22** in dog

| IV dose (2.5 mg/kg)   | <i>t</i> <sub>1/2</sub> (h)     | <i>V</i> <sub>c</sub> (l/kg) | <i>V</i> <sub>B</sub> (l/kg) | AUC <sub>0–∞</sub> (ng h/ml) | Clp (l/h kg) |
|-----------------------|---------------------------------|------------------------------|------------------------------|------------------------------|--------------|
| Mean <sup>a</sup>     | 4.5                             | 0.9                          | 1.2                          | 14,118                       | 0.18         |
| Oral dose (2.5 mg/kg) | <i>C</i> <sub>max</sub> (ng/ml) | <i>T</i> <sub>max</sub> (h)  | <i>t</i> <sub>1/2</sub> (h)  | AUC <sub>0–∞</sub> (ng h/ml) | <i>F</i> (%) |
| Mean <sup>a</sup>     | 930                             | 3.0                          | 6.0                          | 11,885                       | 84.2         |

<sup>a</sup> Harmonic mean.



**Figure 1.** Effect of compound **22** (dosed at 10 and 30 mpk, q.d., po, dosed in 1% Tween 80 in water) on the body weight of DIO mice.

( $V_{ss} = 1.2$  L/kg) with an apparent elimination half-life of 4.5 h. Peak plasma concentration after oral dosing averaged 930 ng/ml, with 84% bioavailability.

Encouraged by these results, we explored the effects of administration of **22** in a study measuring food intake and body weight in DIO mice<sup>10</sup> (see Fig. 1). For a two-week period, DIO mice fed a high-fat diet *ad libitum* were dosed orally with **22** (10 or 30 mpk, q.d.), sibutramine (10 mpk, q.d.), or vehicle. Food intake and body weight were measured at days 1, 4, 7, 11, and 14 for each group. DIO mice receiving a 30 mpk dose of **22** steadily decreased body weight commencing from day 1, though the loss in body weight had stabilized by the end of the study (~6% body weight loss). Mice receiving 10 and 30 mpk of **22** continued to eat comparable amounts of food compared with DIO vehicle-treated controls. In the Irwin behavioral study (data not shown), there was no evidence of hypothermia or gross abnormalities. These results suggest that the weight loss observed upon treatment with MCHr1 antagonist **22** could be the result of an alteration in energy expenditure.

In summary, a series of potent benzamide containing MCHr1 antagonists have been identified. The compound with the best combination of MCHr1 binding affinity and functional activity had good oral bioavailability in dog and was evaluated in a DIO mouse model for efficacy. Compound **22** demonstrated sustained moderate efficacy when dosed at 30 mpk q.d. in this chronic model of weight loss.<sup>11</sup>

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- Efficacy studies were performed according to the following protocol: C57BL/6J mice were group housed in groups of five under conditions of 12 h lights on, 12 h lights off, with food and water available *ad libitum*. At the beginning of the study, mice were administered a purified low fat diet (D12450Bi, 10 kcal% fat, 3.8 kcal/g) or a high fat content diet (D12492i, 60 kcal% fat, 5.2 kcal/g) for approximately 16 weeks. Mice were weighed, individually housed, and food consumption monitoring initiated three weeks prior to commencement of drug administration. Pharmacological treatments were administered twice a day at 08:00 h and 15:00 h. Mice were conditioned to oral gavage and daily vehicle administration for one week prior to drug administration. The vehicle used for conditioning and study was 1% Tween 80. All doses were given in 4 ml/kg body weight volume of vehicle. All compound doses are expressed as base equivalent weights per unit body weight. Food intake and body weight were determined on the first day and periodically thereafter for 28 days. Compound **22** was administered po by gavage, at doses of 10 and 30 mg/kg q.d., and sibutramine at a dose of 10 mg/kg po, q.d. At the end of the study (day 28), statistical analyses of body weight, food intake, plasma analyte, DEXA, dual-energy X-ray absorptiometry and tissue weight data were performed by analysis of variance, followed by Dunnett's post hoc test. All comparisons were made at a 0.05 level of significance. Data are presented as means  $\pm$  SEM.
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