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Discovery of a spiroindane based compound as a potent, selective, orally bioavailable melanocortin subtype-4 receptor agonist

Shuwen He [a,*], Zhixiong Ye [a], Peter H. Dobbelaar [a], Iyassu K. Sebhat [a], Liangqin Guo [a], Jian Liu [a], Tianying Jian [a], Yingjie Lai [a], Christopher L. Franklin [a], Raman K. Bakshi [a], James P. Dellureficio [a], Qingmei Hong [a], Nancy N. Tsou [a], Richard G. Ball [a], Doreen E. Cashen [b], William J. Martin [b], David H. Weinberg [c], Tanya MacNeil [c], Rui Tang [c], Constantin Tamvakopoulos [d], Qianping Peng [d], Randy R. Miller [d], Ralph A. Stearns [d], Howard Y. Chen [c], Airu S. Chen [c], Alison M. Strack [b], Tung M. Fong [c], D. Euan MacIntyre [b], Matthew J. Wyvratt Jr. [a], Ravi P. Nargund [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065-0900, USA
[b] Department of Pharmacology, Merck Research Laboratories, Rahway, NJ 07065-0900, USA
[c] Department of Obesity Research, Merck Research Laboratories, Rahway, NJ 07065-0900, USA
[d] Department of Drug Metabolism and Pharmacokinetics, Merck Research Laboratories, Rahway, NJ 07065-0900, USA

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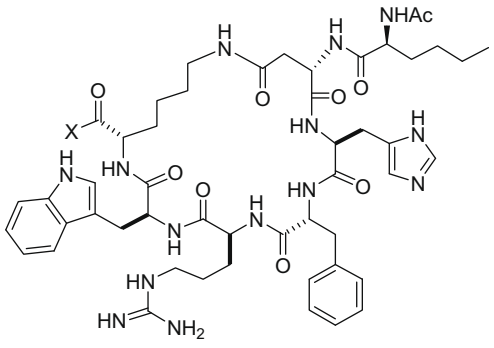
ABSTRACT

We report the design, synthesis and properties of spiroindane based compound **1**, a potent, selective, orally bioavailable, non-peptide melanocortin subtype-4 receptor agonist. Compound **1** shows excellent erectogenic activity in the rodent models.

The melanocortin receptors, a family of seven-transmembrane G-protein coupled receptors (GPCR's), consist of five sub-types (MC1R-MC5R) identified to date. Interactions of the melanocortin receptors with the endogenous ligands, namely the melanocortins and corticotropins, elicit a wide range of biological effects, including feeding behavior and body-weight homeostasis, skin pigmentation, steroid production, sexual behaviors, and exocrine gland secretion.[1]

The melanocortin subtype-4 receptor (MC4R), primarily expressed in the brain, has been established as a crucial molecular component of the complex homeostatic regulation in mammals. Furthermore, MC4R has been implicated in male and female sexual dysfunction.[2] There have been intensive research efforts on identifying small molecule ligands as well as peptide ligands for MC4R as potential therapeutics for obesity and sexual dysfunction.[3]

Strong validation utilizing MC4R agonism as a potential mode of action for the treatment of sexual dysfunction comes from the data generating around MT-II and bremelanotide (formerly known as PT-141) (Fig. 1).[4] MT-II and bremelanotide are cyclic peptidic pan-melanocortin receptor agonists, with activity on MC1R,



X= NH_2: MT-II

X= OH: Bremelanotide (PT-141)

Figure 1. MT-II and bremelanotide.

* Corresponding author. Tel.: +1 732 594 0881; fax: +1 732 594 3007.
E-mail address: shuwen_he@merck.com (S. He).

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MC3R and MC4R. Bremelanotide is a metabolite of MT-II, which was originally studied as a potential tanning drug targeting MC1R. Unexpectedly, MT-II demonstrates strong erectile responses with accompanying skin tanning in men. Recently, preliminary clinical trials show efficacy for bremelanotide as a treatment for both male and female sexual dysfunction. However, bremelanotide is not expected to have good exposure when administered orally due to its peptide structure. Furthermore, it is a pan-melanocortin receptor agonist, with significant activity on MC1R, which can cause skin tanning effect.

Previously, we disclosed THIQ and MB243, selective MC4R agonists, displaying proerectile activity as well as food-intake reduction effect in rodent models.[5] In this Letter, we describe our continued efforts culminating in the discovery of **1**, a potent, selective, orally bioavailable MC4R agonist, with excellent erectile activity augmentation in rodent models (Fig. 2).

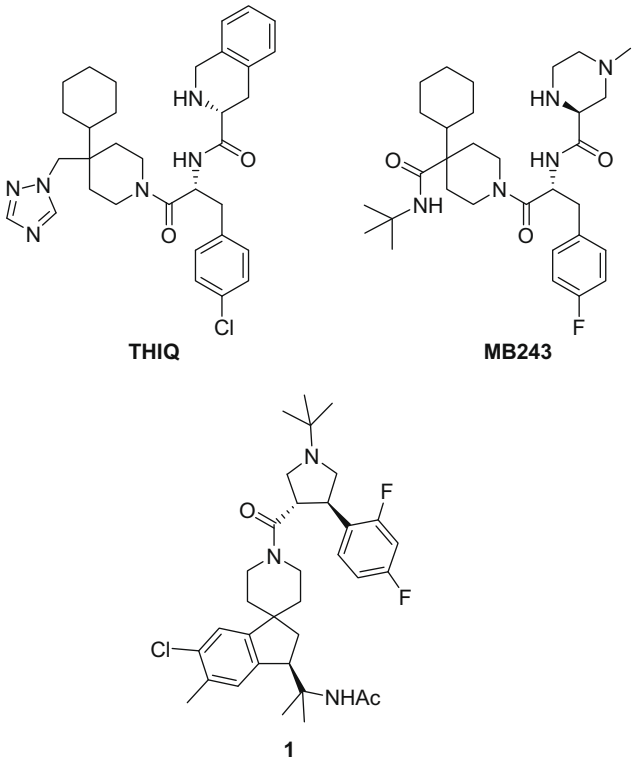


Figure 2. MC4R selective agonists.

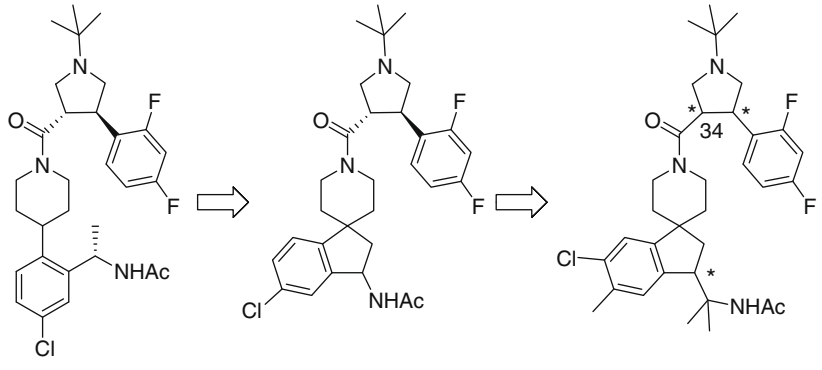
The design of compound **1** starts with compound **5**, following our previous SAR studies in this series (Fig. 3).[6] We envision a cyclization to form **6** containing a spiroindane privileged structure, which has been shown to improve pharmacokinetic properties during the work on a growth hormone secretagogue program.[7] The substitution pattern on the phenyl ring in the spiroindane moiety comes from a SAR study of a related series.[8] The appendage is extended to introduce the gem-dimethyl substituents to satisfy the presumed steric requirement.[9]

Synthetic effort of compounds **1**–**4** focused on the preparation of the spiroindane privileged structures in two stages. Stage 1 constructed the spiroindanone core structure. Stage 2 concentrated on converting the ketone functionality to the appending acetamide moiety.

Construction of the spiroindanone core structure began with a Knoevenagel condensation of Boc protected 4-piperidone with ethyl cyanoacetate (Scheme 1). Cuprate addition on unsaturated cyanoester **7** set up the quarternary carbon center in the core of the structure. Decarboethoxylation followed by hydrolysis of the nitrile and protection of the piperidine nitrogen gave acid **9**. Intramolecular Friedel–Crafts reaction afforded the spiroindanone **10**.

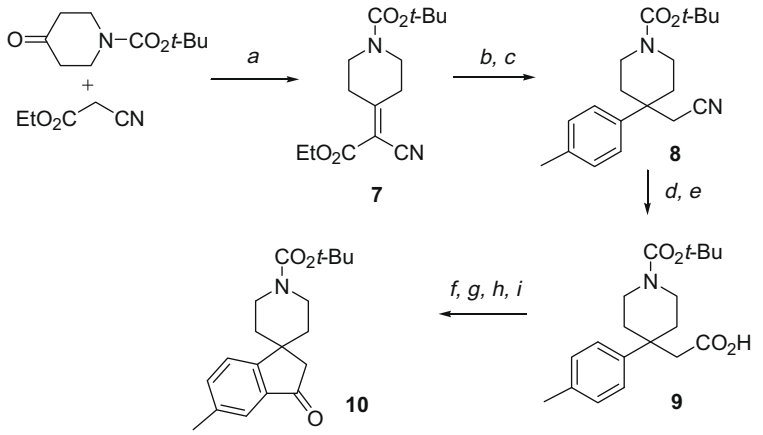
With spiroindanone **10** in place, the rest of the synthesis installed the acetamide appendage (Scheme 2). Horner–Emmons reaction on the ketone **10** gave the unsaturated nitrile as a mixture of geometric isomers. Reduction with magnesium in MeOH furnished the saturated nitrile **11** as a diastereomeric mixture.[10] Methylation gave the gem-dimethyl nitrile, which underwent hydrolysis to form the acid **12**. Curtis rearrangement on acid **12** gave the isocyanate intermediate, which was trapped with benzyl alcohol to give the Cbz protected amine **13**. Hydrogenolysis followed by acetylation of the amine provided the acetamide **14**. Chlorination on the phenyl ring with *N*-chlorosuccinimide gave the privileged structure **15**, with chlorination occurring primarily at the required position.[11] The racemic privileged structure was resolved on chiral HPLC to give separated enantiomers (**15a** and **15b**). The absolute steric configuration (*S*) of **15b** was established by single-crystal X-ray diffraction analysis (Fig. 4).[11] Coupling of Boc de-protected **15a** and **15b** with the enantiomers of the pyrrolidine acids (**16a** and **16b**), respectively, provided compounds **1**–**4**.

Compounds **1**–**4** were initially evaluated in two in vitro assays: MCR binding assays and MCR functional assays. Binding assay was performed to assess the competitive binding of test compounds versus [^{129}I]-NDP-α-MSH. The functional assay was conducted by



1: *R*, *trans*(3*S*, 4*R*)
2: *S*, *trans*(3*S*, 4*R*)
3: *R*, *trans*(3*R*, 4*S*)
4: *S*, *trans*(3*R*, 4*S*)

Figure 3. Design of MC4R agonists.



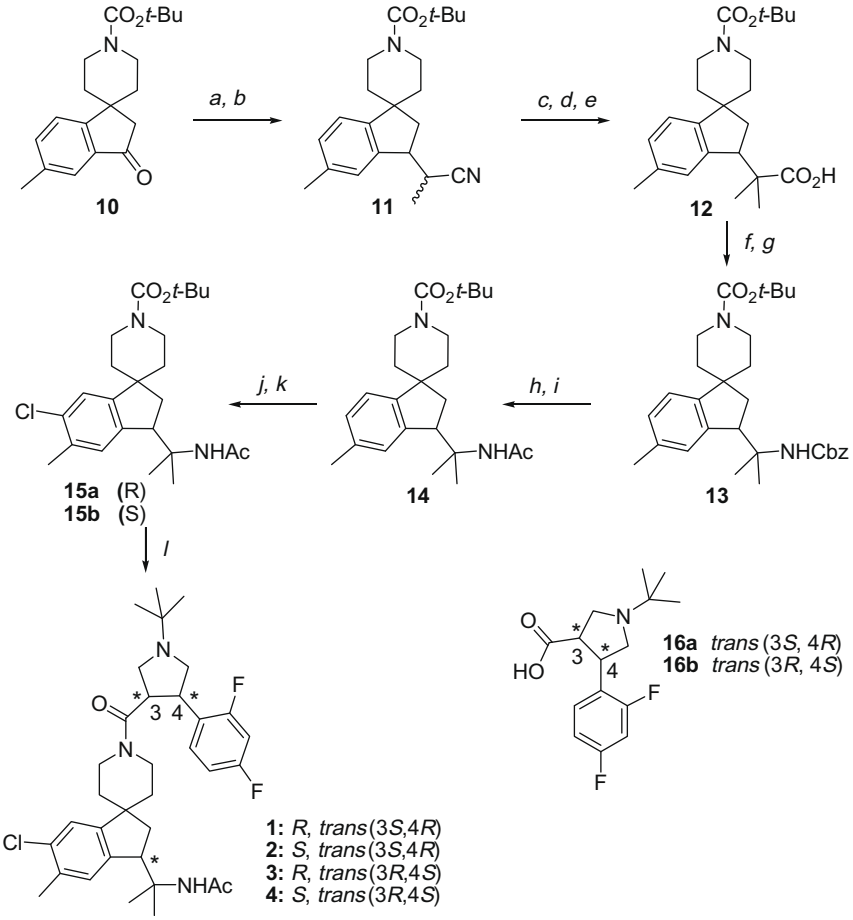
Scheme 1. Reagents and conditions: (a) NH_4Cl, AcOH, C_6H_6; (b) *p*-tolylmagnesium bromide, CuCN, THF; (c) LiCl, H_2O, DMSO; (d) concd HCl; (e) $(Boc)_2O$, NaOH (aq.), dioxane; (f) $(COCl)_2$, CH_2Cl_2, DMF (cat.); (g) HCl, dioxane; (h) $AlCl_3$; (i) $(Boc)_2O/Et_3N/CH_2Cl_2$.

measuring the accumulated cAMP in CHO cells expressing the human receptors.[12]

Compounds **1** and **2** were significantly more potent than compounds **3** and **4** in both binding and functional assays on MC4R (Tables 1 and 2). In functional assay, compound **1** had better MC4R selectivity against MC1R than compound **2** (Table 2). Therefore, compound **1** was selected for further evaluation.

Compound **1** was further evaluated on human, rat, and mouse melanocortin receptors for in vitro binding and functional assays. It had excellent selectivity against other sub-types in human receptors. It also has single digit nanomolar potency on rat and human MC4R with slight loss of potency in mouse MC4R (Table 3).

Compound **1** was then tested in pharmacokinetics study. It showed an outstanding pharmacokinetic profile with excellent



Scheme 2. Reagents and conditions: (a) $(EtO)_2POCH(CH_3)CN$, NaH, THF, reflux; (b) Mg, MeOH; (c) LDA, MeI, HMPA, THF; (d) concd HCl, reflux; (e) $(Boc)_2O$, NaOH, dioxane; (f) DPPA, Et_3N, toluene, reflux; (g) BnOH, reflux; (h) H_2, Pd/C, EtOH; (i) AcCl, Et_3N, CH_2Cl_2; (j) *N*-chlorosuccinimide, DMF, 50 °C; (k) Chiral AD column, EtOH–Heptane; (l) **16a** or **16b**, HATU, HOAt, NMM, CH_2Cl_2.

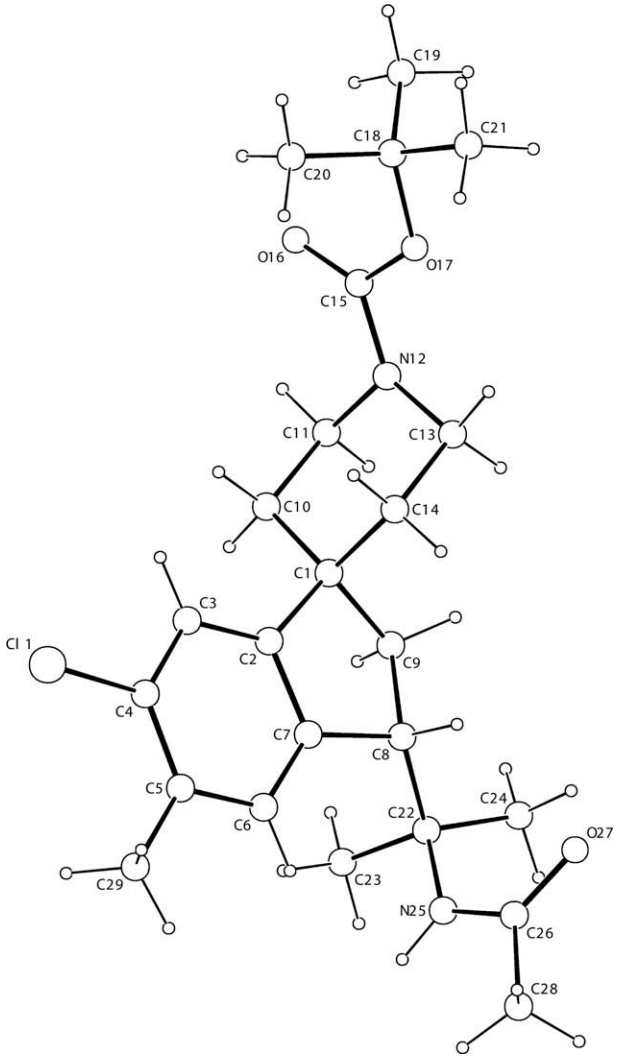


Figure 4. Structure of **15b** by single-crystal X-ray diffraction.

Table 1
Binding affinity and selectivity of compounds **1**–**4** for the human melanocortin subtype-4 receptor

Compounds	MC4R IC_{50}[a] (nM)
1	13.0
2	4.3
3	89.0
4	39.7

[a] Displacement of [^{125}I]-NDP-α-MSH from human receptors expressed in CHO cells.

Table 2
Functional activity of compounds **1**–**4** at human melanocortin receptors

Compounds	EC_{50}[a] (nM) (% max)[b]	
	MC4R	MC1R
1	4.9 [105]	1020 [18]
2	3.2 [97]	228 [39]
3	94.0 [107]	[15]
4	37.2 [111]	620 [41]

[a] Concentration of compound at 50% maximum cAMP accumulation.
[b] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.

Table 3
Binding affinity and functional activity of **1** at human, rat, and mouse melanocortin receptors

Receptor	Binding[a] IC_{50} (nM)	cAMP[b] EC_{50} (nM)	Activation[c] at 10 μM (%)
hMC1R	1938	1020	18
hMC3R	890	351	71
hMC4R	13.0	4.9	105
hMC5R	83.9		9
rMC3R		997	97
rMC4R	nd[d]	2.0	126
mMC4R		22	197

[a] Displacement of [^{125}I]-NDP-α-MSH receptors expressed in CHO cells.
[b] Concentration of compound at 50% maximum cAMP accumulation.
[c] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.
[d] Data is not available.

bioavailability, low clearance, and high plasma drug exposure, especially in dog and monkey (Table 4).

Compound **1** was evaluated in rodent in vivo obesity models. It showed mechanism based acute food-intake reduction in DIO mice.[13] Dose-dependent food-intake and body-weight reduction were observed in a 14-day food-intake study in DIO rats when compound **1** was administered orally at 10 and 20 mpk.[14]

Finally, in vivo studies were performed to evaluate the erectogenic activity of compound **1** using a conscious rat model in which the occurrence of spontaneous erections was quantified in the absence of an external stimulus.[15] Compound **1** elicited 1.0 ± 0.27–1.5 ± 0.19 erections at doses of 0.001–3.0 mg/kg, iv, compared to vehicle with 0.5 ± 0.16 erections. Even more significantly 75–100% of compound **1** treated rats presented at least one erection during the observation period versus 28% for the vehicle treated rats. The number of erections produced by compound **1** at its maximally effective dose (0.1 mg/kg) compared favorably to the maximal number of erections produced by bremelanotide (PT-141) (1.3 ± 0.42; 1 μg/kg, iv) (Fig. 5).

Table 4
Pharmacokinetic data for **1**

PK parameter	Rat[a]	Dog[b]	Monkey[c]
F (%)	33	71	53
Cl (mL min^{-1} kg^{-1})	12	3.5	9.6
V_{dss} (L kg^{-1})	5.1	3.5	3.5
$t_{1/2}$ (h)	4.5	11.2	3.9
AUCn (μM h/mpk)	0.8	6.1	1.5

[a] Compound dosed in Sprague-Dawley rats as a solution in EtOH/PEG400/water (10:40:50) at 1 mg/kg, iv and 4 mg/kg, po.
[b] Compound dosed in beagles as a solution in EtOH/PEG400/water (10:40:50) at 0.5 mg/kg, iv and in 0.5% methylcellulose in water at 1.0 mg/kg, po.
[c] Compound dosed in rhesus as a solution in EtOH/PEG400/water (10:40:50) at 0.5 mg/kg, iv and in 0.5% methylcellulose in water at 2 mg/kg po.

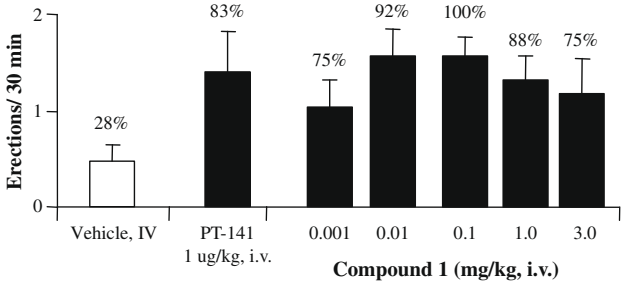


Figure 5. Penile erections in conscious rat observation model following treatment with vehicle (n = 28), PT-141 (n = 6) or compound **1** (n = 8/group). Compound **1** dose-dependently increased the number of penile erections comparable to PT-141. Bar graph represents mean ± S.E.M., values depict percent incidence of responder.

In summary, we report the design, synthesis and evaluation of MC4R agonists containing spiroindane substructure. This study led to the discovery of compound **1**, a potent, selective, bioavailable, non peptidic MC4R agonist. Compound **1** displayed excellent proerectile activity comparable to the effect of bremelanotide (PT-141).

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