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# Design and Syntheses of Melanocortin Subtype-4 Receptor Agonists: Evolution of the Pyridazinone Archetype

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**Abstract**—The discovery and optimization of a new class of non-peptidyl, pyridazinone derived melanocortin subtype-4 receptor agonists is disclosed.


The melanocortin receptors constitute a family of five known seven-transmembrane $G\alpha_s$ G-protein-coupled receptor subtypes (MC1R–MC5R). These receptors and their endogenous ligands, namely the melanocortins and corticotropins, form peripheral and central signaling networks which mediate a diverse number of physiological functions, including skin pigmentation, steroidogenesis, energy homeostasis, sexual function and exocrine secretion.[1] The association between melanocortins and feeding behavior was suggested in 1995, when it was determined that the agouti protein acted as a melanocortin subtype-4 receptor (MC4R) antagonist.[2] Taken in conjunction with an earlier observation that agouti was overexpressed in the central nervous system of the $A^y$ *agouti* mice which were hyperphagic and obese,[3] these observations implied a link between central MC4 receptors and feeding control. This link was further fortified by the discovery of the *Mc4r*$^{-/-}$ mice which developed morbid obesity.[4] Moreover, the *Mc4r*$^{+/-}$ heterozygous mice displayed a proportionately milder obesity suggesting that the extent of MC4 receptor signaling was important in sustaining normal body weight. The most compelling evidence indicating a role for the MC4R in obesity was obtained in humans in which both nonsense and frameshift mutations in *MC4R* led to dominantly inherited obesity.[5] These observations in part, have fueled the recent surge of efforts in drug discovery to identify novel MC4R agonists for the potential treatment of obesity.

As part of our obesity research program, the design and pharmacology of a novel tetrahydroisoquinoline derived (**1**) MC4R agonist was recently described (Fig. 1).[6] Herein, we disclose the discovery and optimization of pyridazinone **2**,[7] which led to a new class of non-peptidyl, functionally selective (versus hMC3R and hMC5R) agonists of the MC4R.

Pyridazinones **9**, **22**, and **23** were prepared according to the general method described below (Scheme 1). Deprotonation of **3**[8] and subsequent exposure to allyl bromide, gave alkene **4** in excellent yield. Oxidative cleavage of the terminal olefin via the Lemieux–Johnson method,[9] followed by reduction of the resultant aldehyde **5**, afforded primary alcohol **6** in >85% overall yield. Functional group interconversion to the azide using the Mitsunobu-type protocol,[10] followed by palladium catalyzed hydrogenation and subsequent acylation, furnished pyridazinone **9** as a 1:1 mixture of *trans* diastereoisomers.

The general synthetic strategy to compounds **12** and **24**–**26** is presented in Scheme 2. Reaction of aldehyde **5** with methyl magnesium bromide provided the requisite

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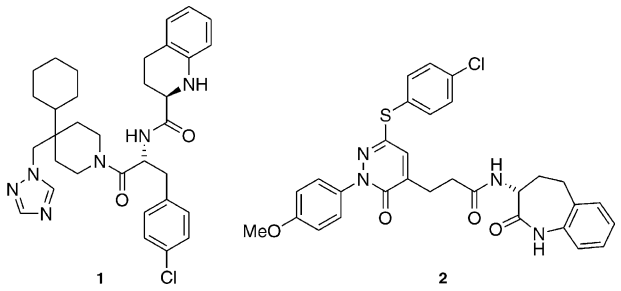
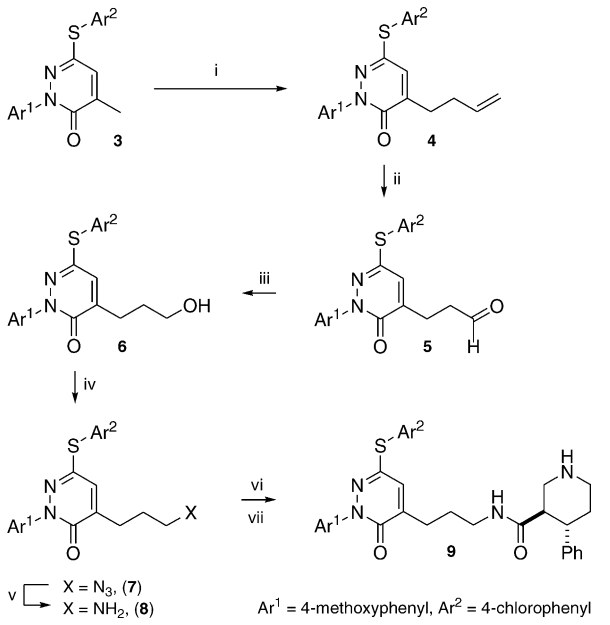
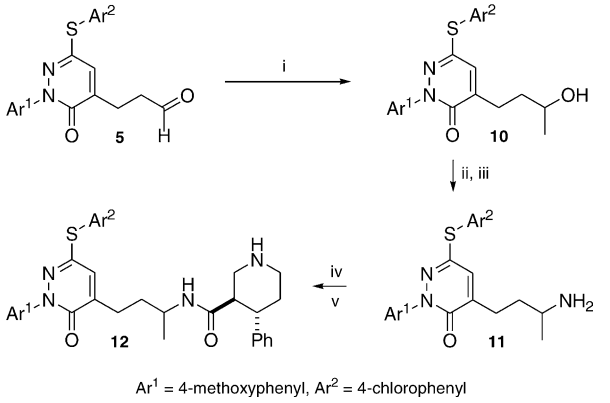


**Figure 1.** MC4R agonists **1** and **2**.



**Scheme 1.** Reagents and conditions: (i) LHMDS, allyl bromide, THF, −78 °C, 89%; (ii) (a) $OsO_4$, NMO, acetone, $H_2O$; (b) $NaIO_4$, THF, $H_2O$; (iii) $NaBH_4$, MeOH, 0 °C to rt, 86% (two steps); (iv) $Zn(N_3)_2{\cdot}2Py$, $PPh_3$, imidazole, DEAD, $CH_2Cl_2$, 0 °C to rt, 98%; (v) $H_2$ (50 psi), Pd/C, EtOH, rt, 85%; (vi) *trans*-1-(*tert*-butoxycarbonyl)-4-phenylpiperidine-3-carboxylic acid, EDC, HOBT, $CH_2Cl_2$, *N*-methylmorpholine, rt; (vii) HCl, EtOAc, rt, 80% (two steps).



**Scheme 2.** Reagents and conditions: (i) MeMgBr, THF, −78 °C, 64%; (ii) $Zn(N_3)_2{\cdot}2Py$, $PPh_3$, Imidazole, DEAD, $CH_2Cl_2$, 0 °C to rt, 99%; (iii) $H_2$ (50 psi), Pd/C, EtOH, rt, 72%; (iv) *trans*-1-(*tert*-butoxycarbonyl)-4-phenylpiperidine-3-carboxylic acid, EDC, HOBT, $CH_2Cl_2$, *N*-methylmorpholine, rt, 80%; (v) HCl, EtOAc, rt, 90%.

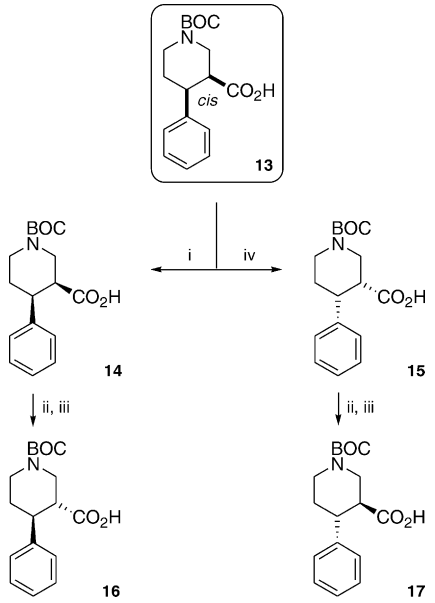
secondary alcohol **10**. Subsequent elaboration to the target amides was conducted according to the synthetic strategy described in Scheme 1.

To access compounds **9** and **12** in enantiomerically pure form, asymmetric routes to carboxylic acids **16** and **17** and amines **20** and **21** were developed (Schemes 3 and 4). Enantiomeric resolution of *N*-BOC-4-phenyl-3-carboxypiperidine **13** was performed according to the known method to afford the *cis* enantiomers **14** and **15** (Scheme 3).[11] Esterification of either **14** or **15**, followed by epimerization and subsequent base catalyzed hydrolysis, furnished the *trans* enantiomers **16** and **17**, respectively.

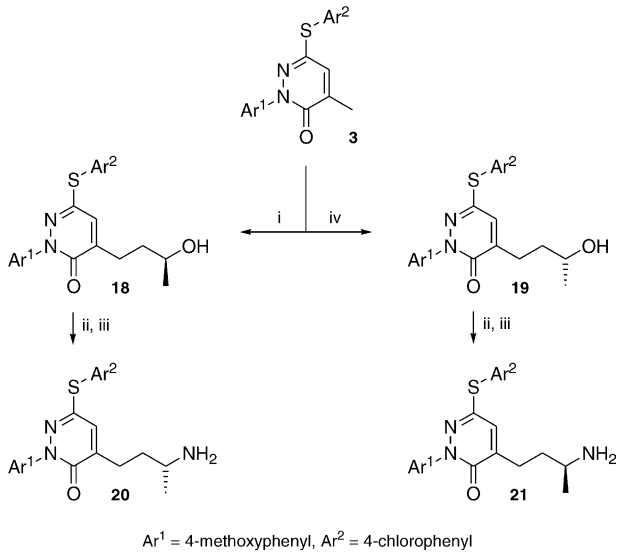
Asymmetric syntheses of amines **20** and **21** were accomplished as shown in Scheme 4. Allylic deprotonation of **3** and subsequent treatment with either *S*- or *R*-propylene oxide gave enantiomerically pure alcohols **18** and **19**, respectively. Functional group interconversion followed by hydrogenation afforded optically pure amines **20** and **21**, respectively.

Pyridazinone **2**, which was discovered through an earlier structure–activity relationship (SAR) effort,[7] showed good binding affinity and modest functional activity for the MC4R (Table 1).

Deletion of the phenyl portion of the benzolactam residue in **2**, resulted in a dramatic loss of binding and functional activity.[13] Also, comparison of **2** with **1** and other compounds of that class, suggested that **2** lacked a key basic site found in other MC4R agonists. We therefore reasoned that incorporation of an appropriately positioned basic residue could improve the relatively modest functional activity observed for **2**. These observations guided us to a series of compounds



**Scheme 3.** Reagents and conditions: (i) (a) (*S*)-(−)-α-methyl-benzylamine, MeOH; (b) fractional crystallization; (c) 1 N HCl; (ii) diazomethane, $Et_2O$, MeOH; (iii) (a) Na, MeOH, 65 °C; (b) 5 N NaOH, 65 °C; (iv) (*R*)-(+)-α-methylbenzylamine, MeOH; (b) fractional crystallization; (c) 1 N HCl.




**Scheme 4.** Reagents and conditions: (i) LHMDS, (*S*)-(−)-propylene oxide, THF, −78 °C; (ii) $Zn(N_3)_2$·2Py, $PPh_3$, imidazole, DEAD, $CH_2Cl_2$, 0 °C to rt; (iii) $H_2$ (50 psi), Pd/C, THF, EtOH, rt, 37% (three steps); (iv) LHMDS, (*R*)-(+)-propylene oxide, THF, −78 °C.

**Table 1.** Binding affinity and functional activity of **1** and **2** at the human MC4R[12]

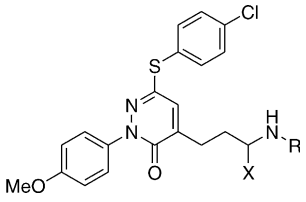
| Compd | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) (%max) |
|---|---|---|
| **1** | 1.2 | 2.1 (97%) |
| **2** | 144 | 3060 (34%) |

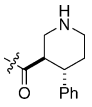
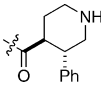
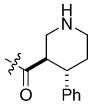
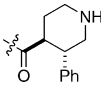
typified by **9**, **22**, and **23**, which contains both the desired phenyl substituent and a basic contact (Table 2). The in vitro data showed a modest improvement in functional activity for **9** and **23** relative to **2**, but this was accompanied by ≥10-fold erosion of binding affinity for the MC4R. We envisioned that binding and functional activity might be enhanced by restricting the conformational freedom in the tether adjoining the pyridazinone nucleus to the aryl-piperidine/pyrrolidine portion. Accordingly, a methyl group was installed proximal to the amide to limit the number of low energy bond rotamers in this region. This resulted in an overall improvement in binding and functional activity for compounds **12**, **24** and **25** relative to their corresponding des-methyl counterparts. In the case of **24**, the increased functional activity was at least an order of magnitude greater than **22**. Unexpectedly, activation of the MC4R was also increased in all cases.

The position of the methyl group was critical with respect to potency, and attempts to relocate this moiety β- or γ- to the amide bond (**27** and **28**, respectively) resulted in a profound loss of activity (Table 3).

The size of the α-amido substituent was investigated briefly. While ethyl substitution could be tolerated with impunity, the sterically larger isopropyl group resulted in diminished binding and functional activity.[14] Having established the correct position and size for the α-amido substituent, we focused our attention on determining

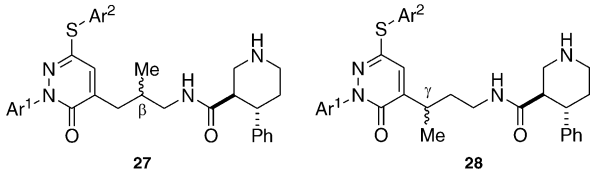
**Table 2.** Binding affinity and functional activity of compounds at the human MC4R[12]



| Compd[a] | X | R[b] | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) (%max) |
|---|---|---|---|---|
| **9** | H |  | 1970 | 500 (20%) |
| **22** | H |  | 2755 | >5000 (28%) |
| **23** | H |  | 1245 | 820 (22%) |
| **12** | *R*/*S*-Me |  | 41 | 227 (82%) |
| **24** | *R*/*S*-Me |  | 339 | 260 (79%) |
| **25** | *R*-Me |  | 380 | 250 (97%) |
| **26** | *S*-Me |  | 975 | 5800 (67%) |

[a]All compounds are racemic, >95% pure (HPLC) and were tested as either TFA or HCl salts.
[b]Relative stereochemistry.

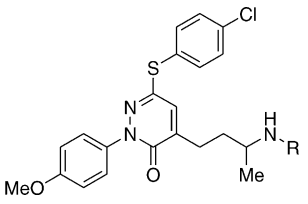
**Table 3.** Binding affinity and functional activity of **27** and **28** at the human MC4R[12]

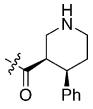
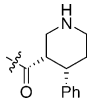
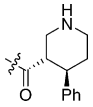
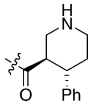
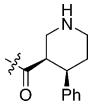
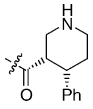
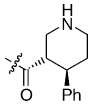
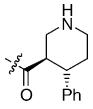
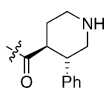
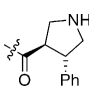



| Compd[a] | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) (%max)[b] |
|---|---|---|
| **27** | 2170 | >5000 (35%) |
| **28** | 3930 | >5000 (31%) |

[a]All compounds are racemic, >95% pure (HPLC) and were tested as HCl salts.
[b]Relative stereochemistry.

**Table 4.** Binding affinity and functional activity of compounds at the human MC4R[12]




| Compd[a] | Stereo (Me) | R[b] | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) (%max) |
|---|---|---|---|---|
| **29** | *S* |  | 9020 | 13% @ 10 μM |
| **30** | *S* |  | 82% @ 20 μM | 12% @ 10 μM |
| **31** | *S* |  | 2990 | 5% @ 10 μM |
| **32** | *S* |  | 1197 | 1733 (65%) |
| **33** | *R* |  | 2240 | 4050 (54%) |
| **34** | *R* |  | 95% @ 20 μM | >5000 (38%) |
| **35** | *R* |  | 2055 | >5000 (32%) |
| **36** | *R* |  | 33 | 177 (77%) |
| **37** | *R* |  | 130 | 237 (85%) |
| **38** | *R* |  | 190 | 405 (103%) |

[a]All compounds are single enantiomers, >95% pure (HPLC) and were tested as HCl salts.
[b]Absolute stereochemistry.

**Table 5.** MCR activity profile of **36**[12]

| Receptor | Binding $IC_{50}$ (nM) | cAMP $EC_{50}$ (nM) | Activation at 10 μM (%) |
|---|---|---|---|
| MC3R | 2172 | — | 8 |
| MC4R | 33 | 177 | 77 |
| MC5R | 1410 | — | 5 |

the activities of the individual components in **12**, since this mixture of four compounds displayed the best combination of binding and functional activity. Compounds **31** and **32** which feature an *S*-methyl group and a *trans* disposition of the carboxamide and aryl groups (piperidine ring), displayed significantly reduced binding and functional potency at the MC4R relative to **12** (Table 4). The corresponding *cis* substituted piperidine variants **29** and **30**, which were readily accessible from **14** and **15**, resulted in an inactive design. Compounds **33**–**36** in which the methyl group exists in a *R*-configuration experienced a modest to dramatic right-shift in potency. Remarkably, the lack of activity observed for the *cis* diastereoisomers **29** and **30** could be partially rescued in the presence of a *R*-methyl substituent. Compound **36** showed the most pronounced improvement in binding and functional activity, and was ~10-fold more active than the methyl epimer **32**. Therefore the most active constituent of **12** was **36** and both were judged equipotent in the context of assay reproducibility. Relaying the preferred stereochemical pattern found in **36** to compounds **24** and **25** gave **37** and **38**, respectively. The activity differences between **24** and **37** and between **25** and **38** were again inconsequential, and these data suggested that the stereochemical relationships found in **36**, **37** and **38** were optimum in all three cases, and moreover, independent of ring size or location of the ring nitrogen atom.

A more complete MCR activity profile for pyridazinone **36** is shown in Table 5. These data indicate that **36** is a subtype selective (versus hMC3R and hMC5R) agonist of the MC4R.

In conclusion, we have described the design and asymmetric synthesis of a new class of non-peptidyl, MC4R agonists derived from a pyridazinone architecture. This preliminary SAR study has transformed the moderately potent partial MC4R agonist **2** to the potent and functionally selective (versus hMC3R and hMC5R) agonist **36**.

## References and Notes

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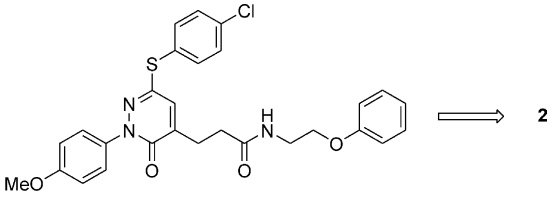


Screening Hit
MC4R Binding $IC_{50}$ = 5,598 nM
MC4R cAMP $EC_{50}$ = 15% @ 20 μM

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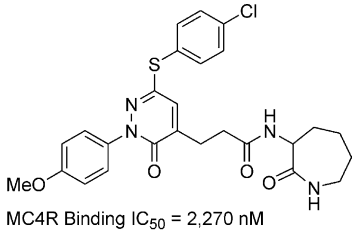
12. (a) All data represent mean values for at least two separate experiments. MC4R binding $IC_{50}$ is defined as the concentration of compound which can inhibit binding of [$^{125}$I]-NDP-α-MSH by 50% from membranes prepared from CHO cells expressing human MC4R. MC4R cAMP $EC_{50}$ is defined as the inflection point of the cAMP dose–response curve for any given compound or the cAMP accumulation (relative to α-MSH) observed at the highest compound concentration tested. %Max is the percentage of cAMP accumulation at 10 μM of compound relative to α-MSH. For a detailed description of the assay protocols see: Bednarek, M. A.; MacNeil, T.; Kalyani, R. N.; Tang, R.; van der Ploeg, L. H. T.; Weinberg, D. H. *J. Med. Chem.* **2001**, *44*, 3665. (b) Bednarek, M. A.; Silva, M. V.; Arison, B.; MacNeil, T.; Kalyani, R. N.; Huang, R.-R. C.; Weinberg, D. H. *Peptides* **1999**, *20*, 401.
13.




MC4R Binding $IC_{50}$ = 2,270 nM
MC4R cAMP $EC_{50}$ = 0% @ 10 μM

14. Ujjainwalla, F.; Warner, D., unpublished results.