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Design and syntheses of melanocortin subtype-4 receptor agonists. Part 2: Discovery of the dihydropyridazinone motif

Feroze Ujjainwalla,[a,*] Daniel Warner,[a] Christine Snedden,[a] Ricky D. Grisson,[a] Thomas F. Walsh,[a] Matthew J. Wyvratt,[a] Rubana N. Kalyani,[b] Tanya MacNeil,[b] Rui Tang,[b] David H. Weinberg,[b] Lex Van der Ploeg[c] and Mark T. Goulet[c]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065-0900, USA*
[b]*Department of Obesity and Metabolic Research, Merck Research Laboratories, Rahway, NJ 07065-0900, USA*
[c]*Merck Research Laboratories-Boston, 33 Avenue Louis Pasteur, Boston, MA 02115, USA*

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Abstract—Optimization of the biological activity of a new class of non-peptidyl, pyridazinone derived human melanocortin subtype-4 receptor agonists is disclosed.

The association between the melanocortin subtype-4 receptor (MC4R) pathway and the modulation of feeding has been established through the course of a number of elegant genetic and pharmacological studies, involving both animals and humans.[1,2] These studies, in part, have provided the impetus in drug discovery to identify novel, small molecule agonists of the hMC4R for the potential treatment of human obesity.[3] In a recent report from this laboratory, we described the discovery of a structurally unique pyridazinone derived class of functionally selective (vs hMC3R and hMC5R) hMC4R agonists, exemplified by compounds **1**–**3** (Fig. 1).[4]

In this report, we disclose our endeavors to optimize the biological activity of these compounds, with particular emphasis on improving functional potency and selectivity. This article presents the syntheses and structure–activity relationships (SARs) of both pyridazin-3(2*H*)-ones and 4,5-dihydropyridazin-3(2*H*)-ones as potent and subtype selective hMC4R agonists.

The general synthetic approaches to the pyridazinones presented in this study are illustrated in Schemes 1–4. We envisioned that modification of the aryl ring attached to either the piperidine of **1** and **2** or the pyrrolidine of **3** would be a fertile area in which to investigate

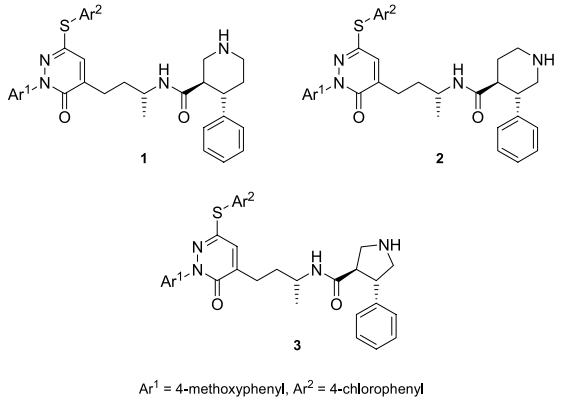

Figure 1. Human MC4R agonist **1**, **2**, and **3**.

potency and selectivity. Assembly of these compounds was executed through a late stage amide bond coupling of the pyridazinone and the aryl piperidine/pyrrolidine halves. The requisite aryl piperidine/pyrrolidine fragments were synthesized using the procedures described in Scheme 1.

Preparation of the 4-aryl piperidine fragment **9** began with commercially available piperidone **4** (Scheme 1). From the outset of the synthesis, we elected to replace the *N*-benzyl protecting group with a *t*-butyl carbamate derivative, since this would accommodate potentially reducible functionality under catalytic hydrogenation

* Corresponding author. Tel.: +1 732 594 1719; fax: +1 732 594 2210; e-mail: f_ujjainwalla@merck.com

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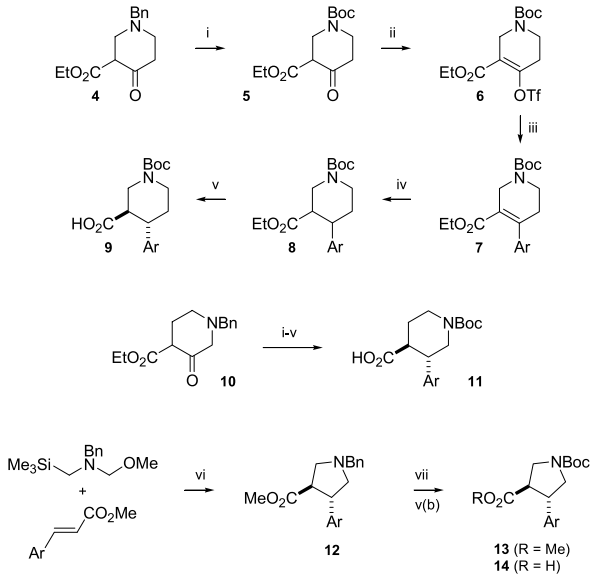
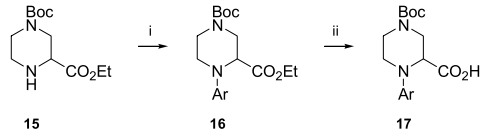
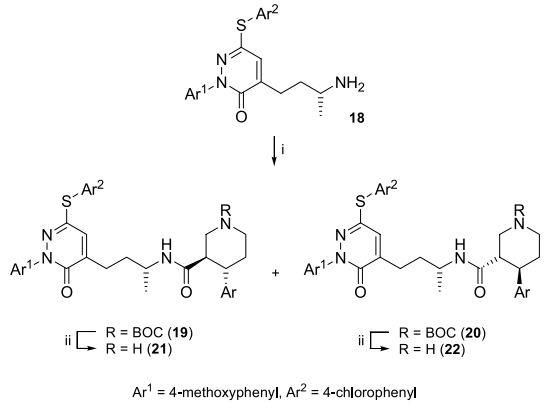


Scheme 1. Reagents and conditions: (i) (a) H_2 (50 psi), Pd/C, EtOH, H_2O, rt; (b) $(Boc)_2O$, $NaHCO_3$, NaCl, $CHCl_3$, H_2O, 60 °C; (ii) Tf_2O, DIPEA,CH_2Cl_2, −78 °C; (iii) arylboronic acid, Pd(dppf)Cl_2, EtOH, toluene, 2 M $NaHCO_3$, 80 °C; (iv) Mg, MeOH, rt; (v) (a) Na, MeOH, 65 °C; (b) 5 N NaOH, MeOH, 65 °C; (vi) cat. TFA, CH_2Cl_2, 0 °C; (vii) (a) HCO_2NH_4, Pd(OH)$_2$/C, MeOH, 65 °C; (b) $(Boc)_2O$, $NaHCO_3$, NaCl, $CHCl_3$, H_2O, 60 °C.



Scheme 2. Reagents and conditions: (i) arylboronic acid, Cu(OAc)$_2$, pyridine, 4 Å sieves, CH_2Cl_2, rt; (ii) 5 N NaOH, MeOH, 65 °C.



Scheme 3. Reagents and conditions: (i) **9**, EDC, HOBT, CH_2Cl_2, *N*-methylmorpholine, rt; (ii) HCl, EtOAc, rt.

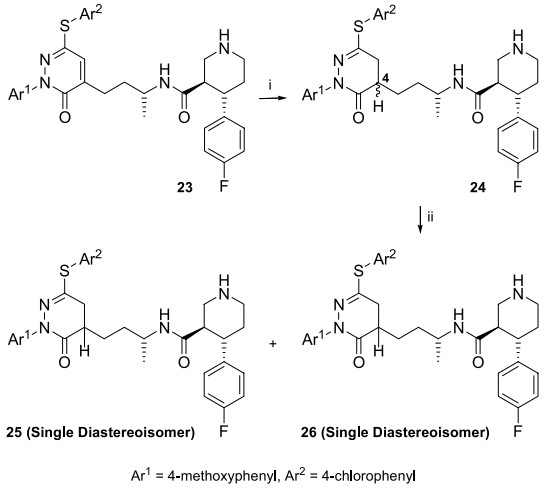
conditions, and facilitate late stage ease of removal. Accordingly, palladium-catalyzed hydrogenolysis of **4** followed by BOC protection afforded piperidone **5**. Formation of the enol triflate **6** and subsequent Suzuki-Miyaura[5] cross-coupling with an arylboronic acid afforded 4-aryl tetrahydropiperidine **7**. Reduction of the tetrasubstituted alkene functionality under atmospheric pressure of hydrogen furnished aryl piperidine **8** as a single *cis* diastereoisomer. Ultimately, a *trans* disposition of the carboxamide and aryl groups was required, and although a direct conversion of **7** to *trans*-**8** was not achievable in our hands, we discovered that **7** could be converted to an enriched mixture of *trans*-**8**/*cis*-**8** ($\geqslant$3:1, respectively) in the presence of magnesium metal in methanol.[6] Exposure of *trans*-**8**/*cis*-**8** to sodium in methanol afforded exclusively *trans*-**8**, which upon base catalyzed hydrolysis of the ester functionality furnished the racemic *trans* carboxylic acid **9**.

3-Aryl piperidine **11** was prepared in an analogous fashion to **9**, starting with commercially available piperidone **10** (Scheme 1).

4-Aryl pyrrolidine **14** was prepared according to a concerted cycloaddition strategy (Scheme 1). Thus, azomethine-ylide [3+2] cycloaddition of benzyl-(methoxymethyl)[(trimethylsilyl)methyl]amine and a *trans* arylacrylate in the presence of a catalytic amount of trifluoroacetic acid afforded *trans* pyrrolidine **12**.[7] Replacement of the *N*-benzyl protecting group with a Boc derivative for the aforementioned reasons, followed by base catalyzed hydrolysis of the ester functionality provided the racemic *trans* carboxylic acid **14**.

Investigation of an aryl piperazine variant of **1** was also of interest, and the requisite coupling fragment was prepared according to the general method delineated below (Scheme 2). Arylation of known piperazine **15** according to the Evans–Chan[8] protocol followed by saponification of the ester functionality afforded the racemic carboxylic acid **17**.

Acylation of **18**[4] with racemic acid **9** afforded an equimolar mixture of the target pyridazinone **19** and the related *trans* diastereoisomer **20** (Scheme 3). These compounds were conveniently separated by normal phase flash chromatography on silica gel stationary phase. Acid catalyzed removal of the Boc group afforded pyrid-



Scheme 4. Reagents and conditions: (i) Zn, AcOH, 120 °C, 56–71%; (ii) preparative normal phase chiral HPLC, Chiralcel® OD stationary phase, 50% EtOH/hexanes mobile phase, 36% (**25**, faster eluting diastereoisomer), 34% (**26**, slower eluting diastereoisomer).

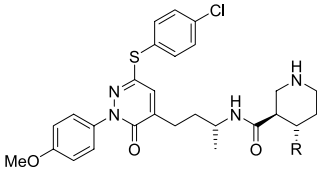
azinones **21** and **22**, respectively. Pyridazinones **27–55** were prepared uneventfully according to this general method.

The synthetic strategy to dihydropyridazinones **25** and **26** is presented in Scheme 4. Reduction of **23** in the presence of zinc metal and acetic acid provided **24** as a statistically equivalent mixture of C-4 epimers,[9] which was resolved by preparative normal phase chiral high performance liquid chromatography (HPLC). Dihydropyridazinones **60–69** were prepared in an analogous fashion.

Pyridazinones **1** (Table 1), **2** (Table 2), and **3** (Table 3) were disclosed in an earlier report,[4] and are displayed here for comparative purposes. Investigation of the aryl substituent attached to piperidine ring revealed a marked preference for 4-substitution compared to 3-substitution, as exemplified by compounds **23** and **27** versus **30** and **28**, respectively (Table 1). In the case of fluorine substitution, the 2-fluoro analog **31** was also examined and showed binding affinity comparable to that of **23**, but intermediary functional activity between **23** and **30**. Notably, while compounds such as **29**, **32**, and **34** all demonstrated comparable binding affinity to hMC4R relative to **23**, they were significantly less efficacious hMC4R agonists. Since the binding and functional assays are inherently different, it was not unexpected to observe compounds which showed differences in binding affinity to, and activation of the hMC4R. However, the particularly large disconnect in IC_{50} versus EC_{50} for compounds such as **32**, **34**, and especially **29** was interesting considering the subtle structural differences between the compounds. The binding affinity (IC_{50} = 3.2 nM) and lack of functional activity (2% at 10 μM) observed with **29** suggested that this compound might be a functional antagonist of hMC4R. Pyridazinone **29** was indeed found to be a functional antagonist demonstrating $K_B = 3.1 \pm 0.7$ nM.[12] In the 3-aryl piperidine and 4-aryl pyrrolidine series, a much closer correlation between hMC4R binding affinity and functional activity was observed (Tables 2 and 3). The 4-fluoro and 4-chloro modifications were found to display the optimum blend of hMC4R binding affinity, functional activity, and receptor agonism, and overall, the 4-fluorophenyl substitution pattern was preferred in each of the three series. A more detailed hMCR activity profile of pyridazinones **23**, **40**, and **47** is shown in Table 4. Pyridazinone **23** showed the best combination of hMC4R binding affinity, functional activity, and functional selectivity (vs hMC3R and hMC5R) relative to **40** and **47**, although these differences while statistically significant were subtle.

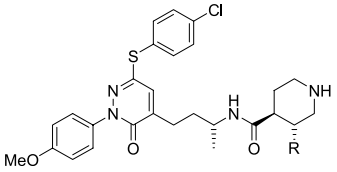
Replacement of the piperidine ring in **23** with a piperazine variant resulted in a significant attenuation of hMC4R functional activity, irrespective of the chirality of the carboxamide group (**52** and **53**, Table 5). While

Table 1. Binding affinity and functional activity of compounds at the human MC4R[9,10]



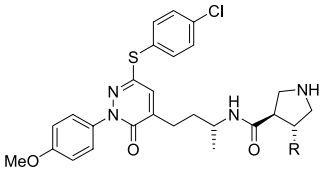
Compound	R	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
1	Ph	33	180 (77%)
27	Ph-4-Cl	2.4	210 (39%)
28	Ph-3-Cl	43	680 (37%)
29	Ph-2,4-diCl	3.2	2% at 10 μM
23	Ph-4-F	11	61 (71%)
30	Ph-3-F	490	29% at 10 μM
31	Ph-2-F	13	550 (58%)
32	Ph-4-CF_3	3.8	>5000 (24%)
33	Ph-3-CF_3	100	40% at 10 μM
34	Ph-4-Me	4.7	1100 (47%)
35	2-Naphthyl	17	12% at 10 μM
36	1-Naphthyl	89	22% at 10 μM
37	3-Thienyl	130	490 (82%)

Table 2. Binding affinity and functional activity of compounds at the human MC4R[10,11]



Compound	R	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
2	Ph	130	240 (85%)
38	Ph-4-Cl	58	150 (101%)
39	Ph-2,4-diCl	36	2400 (30%)
40	Ph-4-F	66	110 (101%)
41	Ph-2-F	100	550 (75%)
42	Ph-4-CF_3	200	710 (75%)
43	Ph-4-CN	45	120 (81%)
44	CH_2Ph	750	36% at 10 μM
45	CH_2Ph-4-F	390	630 (62%)

Table 3. Binding affinity and functional activity of compounds at the human MC4R[10,11]

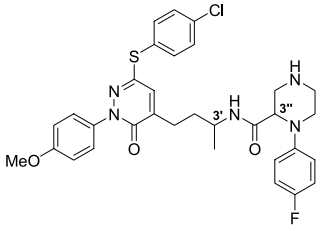


Compound	R	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
3	Ph	98	400 (103%)
46	Ph-2-F	160	710 (82%)
47	Ph-4-F	70	110 (84%)
48	Ph-2,4-diF	60	200 (81%)
49	Ph-4-Cl	45	230 (70%)
50	Ph-4-Me	28	750 (67%)
51	Ph-4-OMe	19	430 (67%)

Table 4. Human MCR activity profile of **23**, **40**, and **47**[10,11]

Compound	Receptor	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
23	MC3	940 ± 92	6 ± 2% at 10 μM
	MC4	**11** ± 1.2	**61** ± 19 (**71** ± 3%)
	MC5	840 ± 92	7 ± 1% at 10 μM
40	MC3	40% at 4 μM	45 ± 5% at 10 μM
	MC4	**66** ± 20	**110** ± 17(**101** ± 5%)
	MC5	1200 ± 210	1 ± 1% at 10 μM
47	MC3	>3000	2400 ± 790 (24 ± 2%)
	MC4	**70** ± 13	**110** ± 35 (**84** ± 3%)
	MC5	1400 ± 150	5 ± 1% at 10 μM

Table 5. Binding affinity and functional activity of **52**–**55** at the human MC4R[10,11]

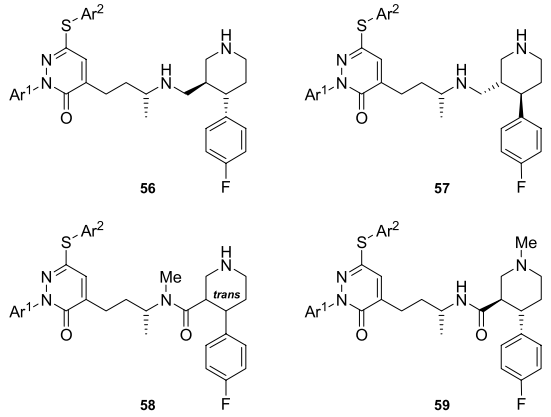


Compound	C-3′ stereo	C-3″ stereo	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
52	*R*	*R* or *S*	950	6% at 10 μM
53	*R*	*S* or *R*	28	1100 (75%)
54	*S*	*R* or *S*	1100	9% at 10 μM
55	*S*	*S* or *R*	1000	38% at 10 μM

53 demonstrated comparable hMC4R binding affinity relative to **23**, ~20-fold attenuation in functional activity was observed, again highlighting the difference between binding to, and activation of the hMC4R. Reversal of the stereochemical orientation of the methyl group in the tether amplified this drop in potency (**54** and **55**). Elimination of the carbonyl functionality (amide) resulted in attenuated hMC4R functional activity (**56**), which was further dramatized in the related *trans* diastereoisomer **57** (Table 6). Pyridazinones **56** and **57** were prepared according to the Fukuyama method.[13] Thus, the *trans* racemic primary alcohol derived from borane reduction of carboxylic acid **9** (Ar = 4-fluorophenyl) was coupled with the 2,4-dinitrobenzenesulfonamide derivative of amine **18**. Subsequent diastereoisomer separation and desulfonylation afforded diamines **56** and **57**. Capping the carboxamide functionality or the piperidine moiety with a methyl group did not lead to compounds with substantially improved hMC4R binding affinity or functional activity (**58** and **59**, respectively). *N*-Methyl amide **58** was prepared by acylation of the amino methyl derivative of **18** with carboxylic acid **9** (Ar = 4-fluorophenyl), while **59** was prepared using a reductive amination reaction between **23** and paraformaldehyde.

To optimize the functional selectivity of the pyridazinone series of compounds, we conceptualized the idea of introducing additional chirality to this lead design,

Table 6. Binding affinity and functional activity of **56**–**59** at the human MC4R[10,11]



Ar^1 = 4-methoxyphenyl, Ar^2 = 4-chlorophenyl

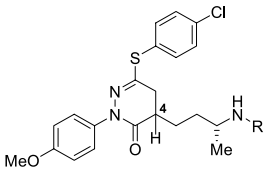
Compound	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
56	28	470 (60%)
57	290	9% at 10 μM
58[a]	93	410 (94%)
59	4.7	320 (59%)

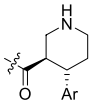
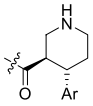
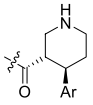
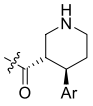
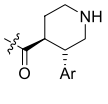
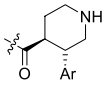
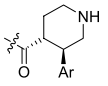
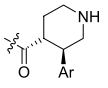
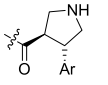
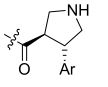
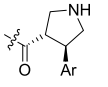
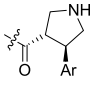
[a] 1:1 mixture of *trans* diastereoisomers.

with a prerequisite that overall molecular weight be conserved. With this condition in mind, installation of a stereogenic center at the C-4 position of the pyridazinone core emerged as a seductive option, since the vectoral disposition of the SAR-sensitive C-4 grouping would be different from a center of tetrahedral (sp^3) geometry versus trigonal (sp^2) geometry. This led to the C-4,5-dihydropyridazinone modification, which for completeness, was investigated in two stereochemical series. The first, involved **23**, **40**, and **47**, which possessed the preferred stereochemical disposition between the carboxamide and aryl groups on the piperidine/pyrrolidine nucleus, and the second, involved the related *trans* diastereoisomers, which featured an antipodal arrangement of these groups (Table 7). Since the reductions of **23**, **40**, and **47** were stereo-random, a set of two diastereoisomers was generated for each compound, and these were epimeric at the C-4 position. Dihydropyridazinones **26**, **63**, and **67**, which originated from **23**, **40**, and **47**, respectively, showed improved hMC4R binding affinity compared to their respective pyridazinone counterparts. In the case of **26**, ~7-fold improvement in hMC4R binding affinity was observed relative to **23**, whereas for **63** versus **40** and **67** versus **47**, the changes in hMC4R binding affinity were less pronounced. HMC4R functional activity and receptor agonism were essentially unchanged by partial saturation of the pyridazinone core. The full hMCR activity profile of **26**, **63**, and **67** is illustrated in Table 8, which demonstrates the inherent potential of the dihydropyridazinone modification to modulate hMC4R binding affinity, functional activity, receptor agonism, and subtype selectivity (Table 8).

In conclusion, we have described the design and asymmetric synthesis of a new class of non-peptidyl, MC4R

Table 7. Binding affinity and functional activity of compounds at the human MC4R[10,11]



Compound	C-4 stereo	R[a,b]	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
25	*R* or *S*		26	390 (72%)
26	*S* or *R*		1.5	40 (67%)
60	*R* or *S*		560	20% at 10 μM
61	*S* or *R*		690	4000 (32%)
62	*R* or *S*		540	480 (100%)
63	*S* or *R*		22	30 (98%)
64	*R* or *S*		1300	2000 (16%)
65	*S* or *R*		280	1400 (33%)
66	*R* or *S*		47	1200 (90%)
67	*S* or *R*		18	66 (98%)
68	*R* or *S*		780	4000 (26%)
69	*S* or *R*		430	740 (48%)

[a] Absolute stereochemistry.
[b] Ar signifies 4-fluorophenyl.

agonists derived from a dihydropyridazinone architecture. This SAR study has transformed the MC4R agonists **1**, **2**, and **3** to the more potent and subtype selective agonists **26**, **63**, and **67**, respectively.

Table 8. Human MCR activity profile of **26**, **63**, and **67**[10,11]

Compound	Receptor	Binding IC_{50} (nM)	cAMP EC_{50} (nM) (%max)
26	MC1B[14]	230 ± 0.0	12 ± 7% at 10 μM
	MC3	420 ± 47	5 ± 2% at 10 μM
	MC4	**1.5** ± 0.2	**40** ± 7.0 (**67** ± 2%)
	MC5	1100 ± 140	2400 ± 390 (27 ± 2%)
63	MC1B[14]	1100 ± 64	220 ± 41 (33 ± 8%)
	MC3	4700 ± 92	1100 ± 280 (21 ± 3%)
	MC4	**22** ± 2.8	**30** ± 5.2 (**98** ± 3%)
	MC5	3100 ± 290	7 ± 2% at 10 μM
67	MC1B[14]	290 ± 93	240 ± 31 (18 ± 2%)
	MC3	1600 ± 1000	10 ± 2% at 10 μM
	MC4	**18** ± 3.6	**66** ± 19 (**90** ± 3%)
	MC5	690 ± 132	5 ± 1% at 10 μM

References and notes

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