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# Aryl Piperazine Melanocortin MC4 Receptor Agonists

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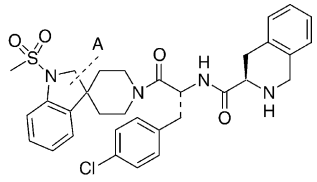
**Abstract**—Incorporation of substituted phenyl piperazine privileged structures into a known MC4 specific dipeptoid consensus sequence resulted in a series of potent ($EC_{50}=24$ nM) and selective MC4-R agonists. We report the SAR of this series of compounds using in vitro cAMP functional assays in cells transfected with the MC4 or other melancortin receptors.


Melanocortin receptors belong to the seven transmembrane spanning G-protein coupled receptor superfamily and are activated by peptide ligands derived from proteolysis of proopiomelanocortin polypeptide. Melanocortin receptor activating peptide ligands include the α, β, and γ melanocyte stimulating hormones and ACTH.[1] Recently melanocortin MC3-R and MC4-R receptors have been the subject of intense interest for the possible development of therapeutic agents to treat obesity. The melanocortin MC4-R receptor is found in the hypothalamus, a center of the brain long known to control weight and food intake. Experiments with MC4-receptor knock-out mice and with potent non-selective peptide MC4-R agonists and antagonists suggest that this receptor plays an important role in energy homeostasis, metabolism and food intake.[2a–d] MC4-R antagonists are of interest in the treatment of cachexia or wasting diseases associated with cancer, AIDS, and other conditions.[3] MC4-R agonists are of interest as potential therapeutics for the treatment of obesity and diabetes. MC4-R specific agonists are also reported to stimulate penile erections in rodents.[7] Non-specific melanocortin peptide agonists have been shown to stimulate erections in primates and humans,[5,6] therefore MC4-R agonists are of interest as potential treatments of human sexual dysfunction. Patents claiming MC4-R agonist activity have appeared using 'privileged structures' formerly employed in growth hormone secretagogue compounds attached to a MC4-R consensus dipeptide: D-Tic-D-(*p*-Cl)-Phe-X.[4a,b] For example, the spiroindoline privileged structure found in MC4-R agonist **1** is a key structural component of the growth hormone secretagogue MK-677. In our assays, **1** is a full agonist on the MC4-R receptor with an $EC_{50}$ of 190 nM and much weaker agonist activity on MC1-R, MC3-R and MC5-R. We have studied the incorporation of several known GPCR privileged structures, as well as new moieties with MC4-R activity into the D-Tic-D-(*p*-Cl)-Phe-X sequence in order to gain insight into the design of MC4-R novel ligands. Since the completion of the initial work reported here, a preliminary report and patent applications for related compounds have appeared.[7–9]




**1**

Our initial agonist screen measured cAMP accumulation in HEK cells. These cells were stably transfected with a plasmid construct expressing human MC4-R from a cytomegalovirus promoter ($B_{max}$ [$^{125}$I-NDP-MSH] = 40 fmol/mg protein; $K_D$ [$^{125}$I-NDP-MSH] = 0.41 nM). Initial screening was completed by stimulation with a test compound at a concentration of 30 uM. Activity was expressed as fold-stimulation over baseline, with α-MSH ($EC_{50}=6$ nM) yielding 80-fold stimulation over baseline. The more active compounds were compared directly against α-MSH stimulation, and $EC_{50}$ values were generated for compounds showing a full dose

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response curve in the concentration range tested. All active compounds were tested for cAMP accumulation on HEK cells which were not transfected with the MC4 receptor to evaluate for non-receptor mediated cAMP production. Key compounds were then tested for selectivity against HEK293 cell lines expressing human MC1-R, human MC3-R, mouse MC4-R, human MC5-R, and the cell line Y1(ATCC #CCL-79) endogenously expressing mouse MC2-R (Table 1).

As one of several approaches we explored formal opening of the indoline ring of **1** at position A and replacement of the tetravalent carbon with nitrogen. The resulting 2-substituted phenyl piperazine[4b] is also a known GPCR privilidged structure. The modified dipeptoid, compound **2** was a full agonist on the MC4-R with $EC_{50}$ of 520 nM. Deletion of the N–Me resulted in compound **3** with similar activity ($EC_{50}$ = 380 nM) (see Scheme 1). Conservative modifications (**4**–**8**) of the sulfonamide resulted in similar or less potent compounds. The receptor tolerated extension of the sulfonamide from the aryl ring by one carbon. Extended analogues with branching one carbon removed from the nitrogen were tolerated **9** (380 nM) but requirements for steric interactions were severe with the addition of an another methyl **10** greatly decreasing agonist activity. Acyl derivatives (**11**–**20**) of the aniline nitrogen reported here were also weak partial agonists (Scheme 1).

Heterocycles were explored which incorporated the ability to form multidentate hydrogen bonds as in histidine found in MC4-R peptide ligands or the sulfonamide **3** (Scheme 2). The formal replacement of the sulfonamide with a triazole attached via a methylene linkage resulted in a compound **21** which was a full agonist on the MC4 receptor with an $EC_{50}$ of 80 nM. Preliminary attempts to increase potency of this molecule by the simple attachment of electron withdrawing (**22**, **23**) or donating (**29**) substituents or by addition of hydrophobic bulk (**25**) were not productive. However, modification of this compound by attachment to the triazole at the 4-position gave a full agonist **26** with $EC_{50}$ of 24 nM. This compound shows greater than 100-fold specificity for activation at the MC4-R receptor compared to human MC1-R, MC2-R, MC3-R and MC5-R as shown in Table 2.

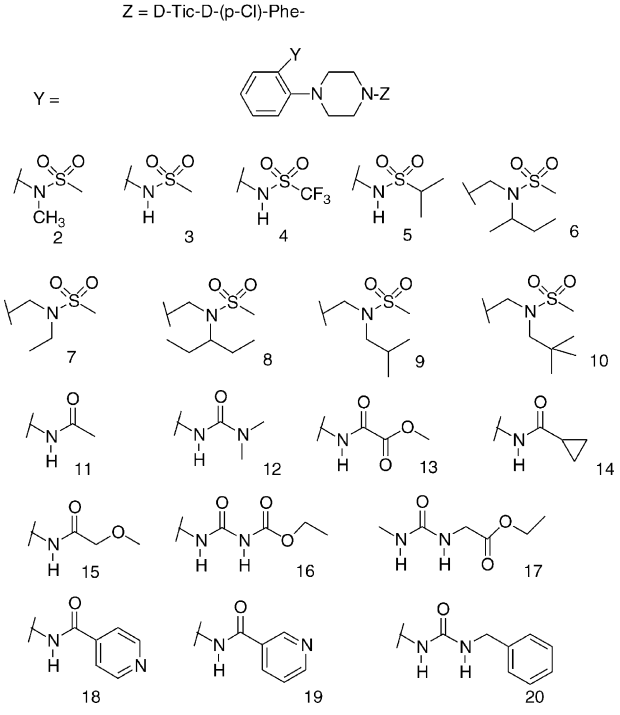
Recently, a patent application[7] and preliminary reports[8] have appeared for a related agonist **27** which in our assay has an $EC_{50}$ of 4 nM and is therefore about 6-fold more potent than **26** and is nearly equipotent in in vitro cAMP assays to the endogenous ligand, α-MSH. However agonist **27** had only a modest short-lived effect in suppressing food intake in fasted mice compared to the activity of MT-II or α-MSH when given by icv administration, and little effect on insulin levels or metabolism levels measured by oxygen consumption.[10] Patent applications claiming aryl piperazine MC4 ligands with the D-Tic-D-(*p*-Cl)-Phe consensus sequence have recently appeared without reported biological activity.[9]

Aryl piperazines were synthesized as described in Schemes 3 and 4. Deprotection and standard peptide couplings produced the target compounds. Displacement of the mesylate **34** with the cyanoethyl modified triazole[11] followed by base induced elimination provided **37** which was incorporated into target structure **26**.

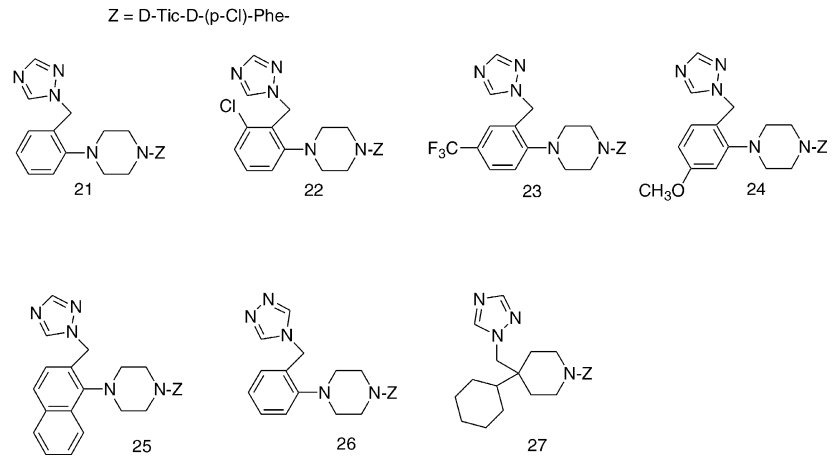
**Table 1.** Agonist activity in cAMP accumulation assay

| Compd | $EC_{50}$ (nM)[a] | % Stimulation[b] | Fold stimulation[c] |
|---|---|---|---|
| **1** | 190 | 100 | |
| **2** | 520 | 100 | |
| **3** | 380 | 100 | |
| **4** | 4400 | 34 | |
| **5** | 440 | 85 | |
| **6** | 1000 | 100 | |
| **7** | 690 | 100 | |
| **8** | 2200 | 100 | |
| **9** | 380 | 100 | |
| **10** | 2400 | 100 | |
| **11** | 1900 | 64 | |
| **12** | | | 7 |
| **13** | 1600 | 55 | |
| **14** | | | 21 |
| **15** | 600 | 34 | |
| **16** | 650 | 24 | |
| **17** | 2100 | 68 | |
| **18** | | | 30 |
| **19** | 1100 | 83 | |
| **20** | 1000 | 62 | |
| **21** | 80 | 100 | |
| **22** | 470 | 70 | |
| **23** | 680 | 100 | |
| **24** | 110 | 100 | |
| **25** | 170 | 100 | |
| **26** | 24 | 100 | |
| **27** | 4 | 100 | |

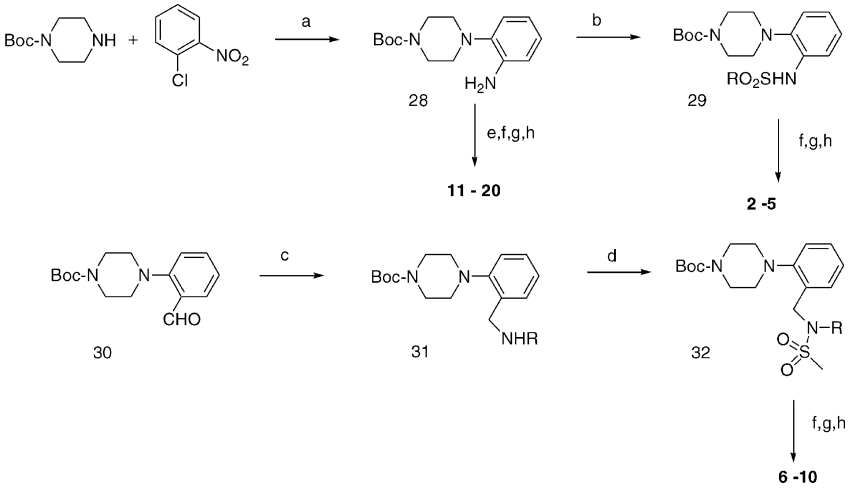
[a]Values are means of three experiments.
[b]Relative to stimulation observed for α-MSH.
[c]Fold stimulation, @30 μM reported for weak screening hits.



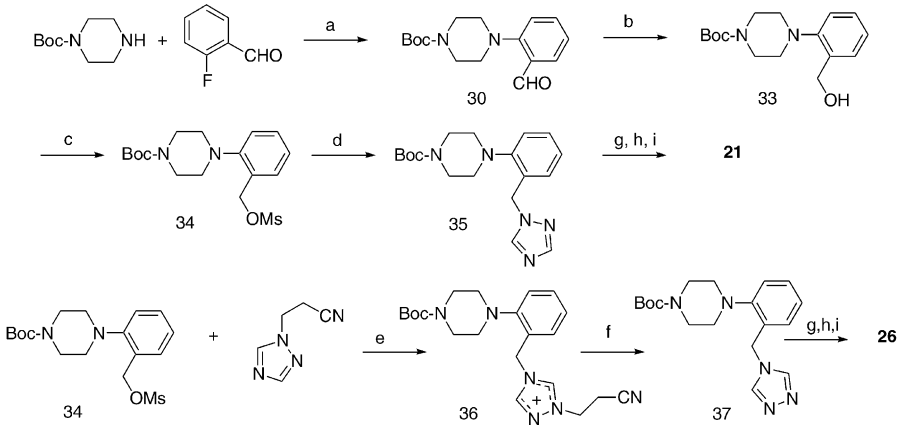

**Scheme 1.**




**Scheme 2.**




**Scheme 3.** (a) $K_2CO_3$, DMSO 130° (89%), $H_2$ Raney Ni, EtOH (91%); (b) $RSO_2Cl$, $Et_3N$, $CH_2Cl_2$ (63%); (c) $RNH_2$, $Na(OAc)_3BH$ (75%); (d) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$ (63%); (e) RCOCl, DIEA, DIEA, $CH_2Cl_2$; (f) TFA, $CH_2Cl_2$; (g) dipeptide, HOBt, EDC, $CH_2Cl_2$; (h) TFA, $CH_2Cl_2$, prep-HPLC.




**Scheme 4.** (a) $K_2CO_3$, DMSO 130 °C (93%); (b) $NaBH_4$, MeOH, 0 °C (74%); (c) MsCl, $Et_3N$, 0 °C (96%); (d) triazole sodium salt, $CH_2Cl_2$, 25 °C (55%); (e) $CH_3CN$, 25° C; (f) aq NaOH (70% over two steps); (g) TFA, $CH_2Cl_2$; (h) dipeptide, HOBt, EDC, $CH_2Cl_2$; (i) TFA, $CH_2Cl_2$, prep-HPLC.

**Table 2.** Selectivity testing for stimulation of melanocortin receptors, in vitro cAMP accumulation in transfected HEK or Y1 cells

| Compd | hMC1 $EC_{50}$ (nM) | mMC2(Y1) $EC_{50}$ (nM) | hMC3 $EC_{50}$ (nM) | hMC4 $EC_{50}$ (nM) | mMC4 $EC_{50}$ (nM) | hMC5 $EC_{50}$ (nM) |
|---|---|---|---|---|---|---|
| α-MSH | 1.6 | > 10,000 | 1.4 | 6.1 | 8.8 | 29 |
| **21** | > 3000 | > 10,000 | > 3000 | 80 | 59 | > 3000 |
| **26** | > 3000 | > 10,000 | > 3000 | 24 | 38 | > 3000 |
| **27** | 1400 | > 10,000 | 1400 | 4.1 | 7.6 | 2700 |

The strategy of incorporating GCPR privileged structures into dipeptide consensus sequences with affinity for specific peptide ligand receptors is a viable approach for producing new series of agonists. Compound **26** and the related series of phenyl piperazine MC4-R agonists have provided the starting point and SAR for the development of two novel series[12] of antagonists and agonists that will be reported in due course.

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