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Privileged structure based ligands for melanocortin-4 receptors—Aliphatic piperazine derivatives

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Abstract—Aliphatic carbocyclic replacement of the benzyl group of compound **1** yielded compounds with high affinity for the melanocortin-4 receptor (MC4R). Compounds with a cyclohexyl group showed a consistent high affinity, while different polar groups with less basicity were good replacements for the original diethyl amines. Substitution of the polar group found in these privileged structures with an aliphatic moiety produced compounds with high affinity for MC4R.

The five melanocortin receptors form a family of type I G-protein-coupled receptors (GPCRs). Each member of the melanocortin receptor family has been cloned and characterized.[1] The MC4R is expressed in the hypothalamus and is thought to play a critical role in regulating metabolism, feeding, and reproductive behavior.[2] We have been interested in developing tools for the pharmacological characterization of the melanocortin receptors (MCRs) with a primary focus on finding ligands selective for MC4R.[3] Our approach has been to employ a privileged structure-based paradigm for the generation of the initial active leads.[4,5] In these studies, we have found that compounds presenting an aromatic hydrophobic and a polar group can be coupled with a dipeptide address element to afford products with activity and selectivity for the MC4R (Fig. 1).[3] We have shown that there is a fair amount of flexibility in the privileged structure with monocyclic and bicyclic aryl piperazines, and substituted benzylic piperazines affording interesting activity.[6]

This structural flexibility initially encountered in the privileged structure motif suggested that additional opportunity might exist for finding active constructs with varied physicochemical properties. In all of our previous investigations we operated under the assumption that an aromatic group and a polar group were required elements for privileged structure design and function. In this paper, we examined if aliphatic privileged structures (with different steric and hydrophobic requirements) would influence the melanocortin activity. Simultaneously we explored whether a polar group was required in the privileged structure. Herein we present our findings in the further optimization of this family of melanocortin ligands.

The preparation of privileged structures with aliphatic hydrophobic groups is outlined in Scheme 1. Treatment of the carboxylic acids (**4**, X = X′ = H) with thionyl chloride formed the acid chloride followed by bromination with NBS and HBr to provide the α-bromo acid chloride, which was then reacted with MeOH to afford the α-bromo methyl esters (**4**, X = Br, X′ = Me).[7] For the compound derived from ethyl propionate, bromination was accomplished by forming the enolate with LHMDS followed by reaction with NBS. The final tertiary amines **5** were then obtained by nucleophilic displacement of the bromine with *N*-Boc-piperazine, transformation to the aldehyde ($LiAlH_4$ reduction then Swern oxidation), and subsequent reductive amination with the desired amine using $NaBH(OAc)_3$.[8]

The α,α-disubstituted cyclopentyl derivative was synthesized from cyclopentanone using a Strecker synthesis[9] as

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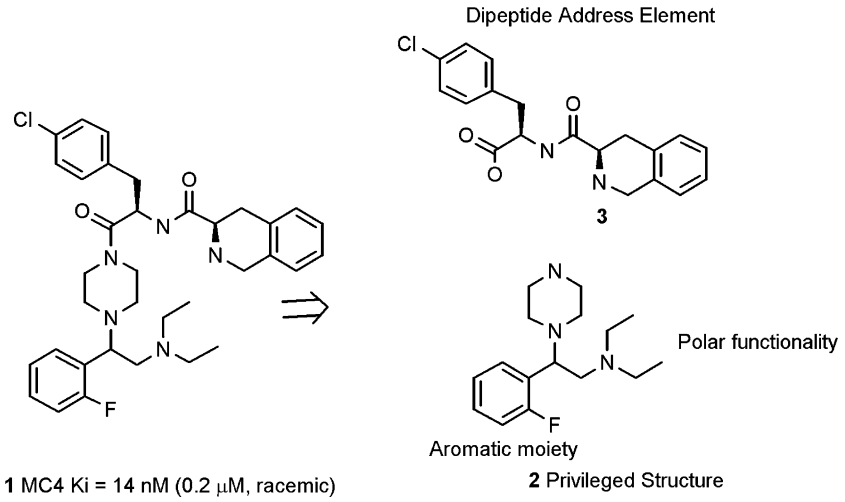
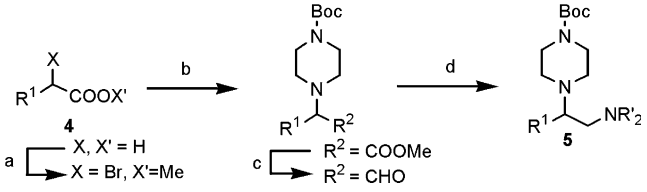


Figure 1. Disconnective analysis of lead series.

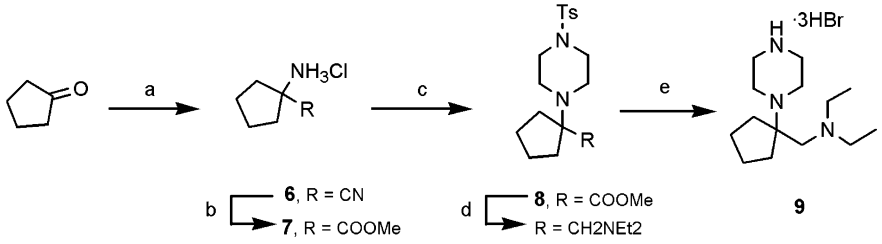


Scheme 1. Reagents: (a) 1—$SOCl_2$ CH_2Cl_2; 2—NBS, HBr, CH_2Cl_2; 3—MeOH, 45–90%; (b) Boc-piperazine, K_2CO_3, *n*-Bu_4NI, MeCN, 35–45%; (c) 1—$LiAlH_4$, THF, 90–95%; 2—$(COCl)_2$ DMSO, NEt_3, CH_2Cl_2, 80%; (d) R′$_2$N, $NaBH(OAc)_3$; 1,2-DCE, 50–85%.

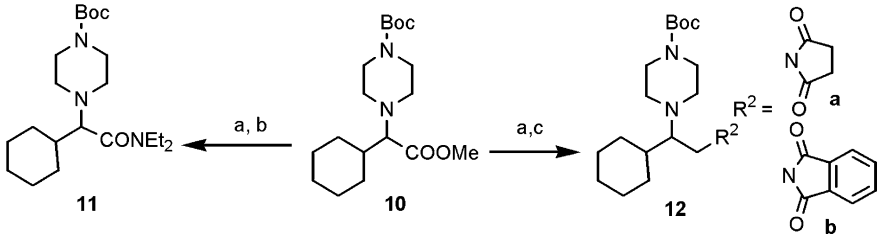
shown in Scheme 2. Hydrolysis of the intermediate amino nitrile **6** provided the ethyl ester **7** which was allowed to condense with *N*,*N*-bis-(2-chloroethyl)-*p*-toluenesulfonamide giving rise to the piperazine intermediate **8**. Further transformation to the diethylamine and deprotection of the tosyl-protecting group afforded **9**.

Different polar groups were prepared as replacement for the tertiary amine. Using the cyclohexyl ester intermediate **10** as a precursor (Scheme 3), the amide **11** was prepared by transformation of the ester moiety to the acid followed by amide formation. The succinimide **12a** and phthalimide **12b** were obtained by reduction of **10** to the alcohol and subsequent Mitsunobu reaction with succinimide and phthalimide, respectively.

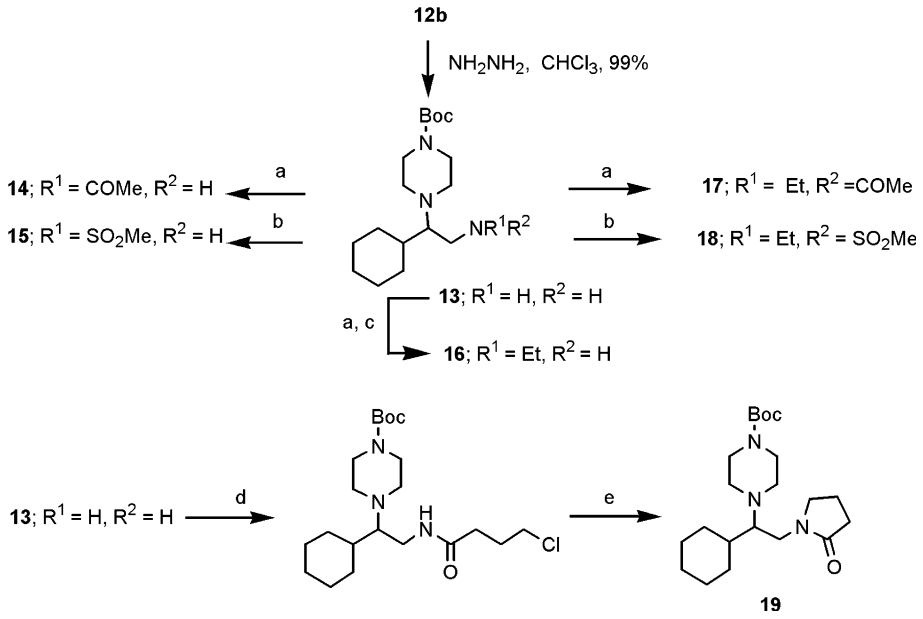
The *N*-Boc-piperazine-phthalimide derivative **12b** was a key intermediate for the preparation of most of the remaining compounds as shown in Scheme 4. Treatment of this derivative with hydrazine afforded the primary amine **13** ($R^1 = R^2 = H$), which was used to prepare different sulfonamides, amides, and carbamates. Reaction of **13** with acetyl chloride or methanesulfonyl chloride yielded the secondary amide **14** (R^1 = COMe, R^2 = H) and the secondary sulfonamide **15** ($R^1 = SO_2Me$,



Scheme 2. Reagents: (a) KCN, NH_4Cl H_2O (b) HCl, MeOH; (c) $TsN(CH_2CH_2Cl)_2$, *i*-Pr_2NEt, DMF, 10% over three steps; (d) 1—$LiAlH_4$, THF, 99%; 2—$(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, 99%; 3—Et_2NH, $NaBH(OAc)_3$, 1,2-DCE; (e) HBr, HOAc, 38% over 2 steps.



Scheme 3. Reagents: (a) 1—$LiAlH_4$, THF, 96%; 2—CrO_3, H_2SO_4, acetone, 70%; (b) Et_2NH, HOBt, EDCl, CH_2Cl_2, DMF, 58%; (c) succinimide or phthlalimide, DEAD, PPh_3, THF, 75–80%.



Scheme 4. Reagents: (a) MeCOCl, NEt_3 CH_2Cl_2, 63%; (b) $MeSO_2Cl$, NEt_3, CH_2Cl_2; 70%; (c) BH_3·THF, THF, 86%; (d) $Cl(CH_2)_3COOH$ HOAt, HATU, *i*-Pr_2NEt, DMF, 69%; (e)^{t}BuOK, THF, 54%.

R^2 = H), respectively. Furthermore, the primary amine **13** ($R^1 = R^2$ = H) was transformed to the ethylamine **16** (R^1 = Et, R^2 = H) (acetylation with acetyl chloride and reduction with BH_3·THF) and converted to the tertiary amide **17** (R^1 = Et, R^2 = COMe) and tertiary sulfonamide **18**(R^1 = Et, $R^2 = SO_2Me$), respectively. Scheme 4 also shows the preparation of the cyclic amide **19** from the same intermediate **13** ($R^1 = R^2$ = H) by amidation with 3-chlorobutaric acid and intramolecular cyclization promoted by base.

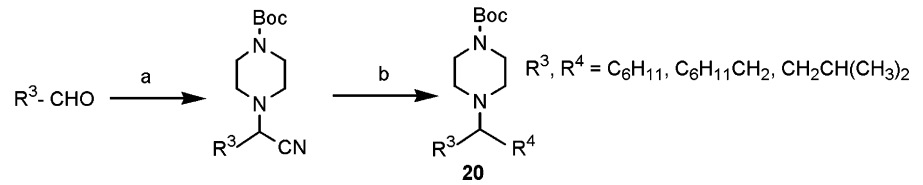
The enantiomers of phthalimide **12b** were separable on a gram scale with chromatography on a chiral AD column, allowing for the preparation of specific antipodes of different compounds.[10] The absolute stereochemistry of the enantiomers was not determined; rather it was tracked by the order of elution and designated as isomer **a** (first eluting) and isomer **b** (second eluting).

Finally, a set of compounds was prepared where the polar group was replaced by an all-carbon moiety as shown in Scheme 5. The route began with a Strecker synthesis using *N*-Boc-piperazine as the amine counterpart, followed by displacement of the cyano group with various Grignard reagents,[11] providing the all-aliphatic A-domains **20**.

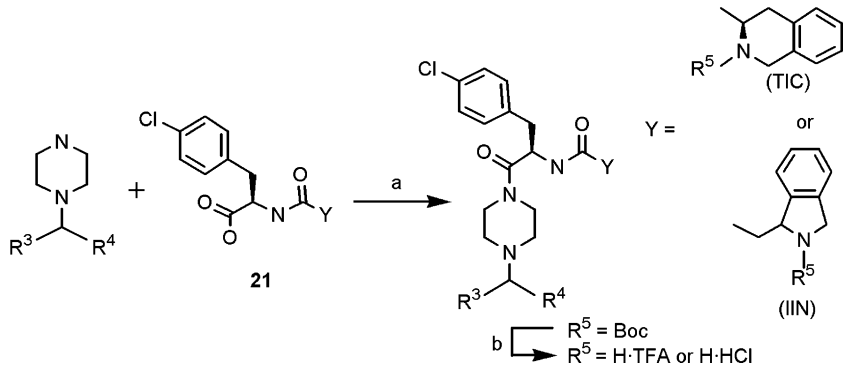
The final compounds for this study were constructed from the privileged structures (previous deprotection of the Boc group with TFA) and the dipeptide **21** in the presence of HATU as previously described.[3] The dipeptide **21** incorporated 2 different moieties (Y in Scheme 6): tetrahydroisoquinoline (TIC) and isoindol (IIN).[12] Treatment of the penultimate intermediates with TFA provided the desired compounds as the corresponding salt. These materials were either used as isolated or subjected to salts exchange with HCl (Scheme 6). In the cases where the piperazines were prepared as racemates, the resultant diasteroisomeric pairs were not separated.

The primary assays were conducted as follows: affinities were determined by competitive inhibition of ^{125}I-NDP-α-MSH binding in HEK293 cells stably transfected with human MC4 receptors.[13,14]

We initially focused on analogs in which the *o*-fluoro benzylic group of **1** was replaced with an aliphatic substituent. We observed a significant loss of affinity when the aryl group was replaced by a methyl group, as in **22** (Table 1).[15] However, the introduction of carbocyclic moieties, as in **23**–**27**, afforded compounds with equal or higher affinity than that of the parent benzylic derivative **1** (K_i 0.2 μM). Both the cyclopentyl **23** and cyclohexyl **24** compounds showed low nanomolar affinities: 4 nM. Other groups like cycloheptyl (**25**) and cyclohexylmethyl (**26**) had comparable affinities (13

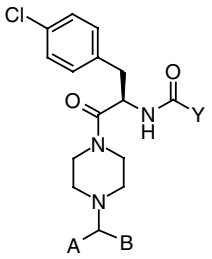


Scheme 5. Reagents: (a) TMSCN, Boc-piperazine, MeOH, 70–80%; (b) R^4MgBr, THF, 50–60%.



Scheme 6. Reagents: (a)HOAt, HATU, *i*-Pr_2NEt, CH_2Cl_2, DMF, 55–85%; (b) TFA, DCM or HCl, EtOAC, 99%.

Table 1.



Compound	A	B	Y	K_i (nM)[15]
1	*o*-FPh	CH_2NEt_2	TIC	20
22	Me	CH_2NEt_2	TIC	3427
23	C_5H_9	CH_2NEt_2	TIC	4
24	C_6H_{11}	CH_2NEt_2	TIC	4
25	C_7H_{13}	CH_2NEt_2	TIC	13
26	$C_6H_{11}CH_2$	CH_2NEt_2	TIC	13
27			TIC	70
28	C_6H_{11}	CH_2NEt_2	IIN	3
28a[a]	C_6H_{11}	CH_2NEt_2	IIN	3
28b[a]	C_6H_{11}	CH_2NEt_2	IIN	5
29	$C_6H_{11}CH_2$	CH_2NEt_2	IIN	5
30	$C_6H_{11}CH_2$	$CH_2NC_4H_8$	IIN	12
31	$C_6H_{11}CH_2$	CH_2morpholine	IIN	10
32	C_6H_{11}	$CONEt_2$	IIN	148
33	C_6H_{11}	CH_2NHSO_2Me	IIN	26
34	C_6H_{11}	CH_2NEtSO_2Me	IIN	3
34a[a]	C_6H_{11}	CH_2NEtSO_2Me	IIN	6
34b[a]	C_6H_{11}	CH_2HEtSO_2Me	IIN	7
35	C_6H_{11}	$CH_2NEtCOMe$	IIN	8
35a[a]	C_6H_{11}	$CH_2NEtCOMe$	IIN	2
35b[a]	C_6H_{11}	$CH_2NEtCOMe$	IIN	3
36	C_6H_{11}	CH_2succinimide	IIN	10
36a[a]	C_6H_{11}	CH_2succinimide	IIN	10
36b[a]	C_6H_{11}	CH_2succinimide	IIN	18
37	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	IIN	18
38	C_6H_{11}	C_6H_{11}	IIN	35
39	C_6H_{11}	$CH_2CH(CH_3)_2$	IIN	54
40	$C_6H_{11}CH_2$	$C_6H_{11}CH_2$	IIN	326

[a] Resolved diasteroisomers.

nM) compared to those of **23** and **24**. In contrast, the achiral α,α-disubstituted cyclopentyl **27** presented a roughly 10-fold decrease in affinity (70 nM).

Previously we had shown that address elements incorporating an isoindole (IIN) moiety in place of the tetrahydroisoquinoline (TIC) provide active molecules with melanocortin activity.[12] We therefore opted to try this address element with these new privileged structures. It was interesting to find that the substitution afforded comparable (although slightly higher) activity (**28** and **29**) in comparison with the analogous TIC derivatives (**24** and **26**). At this point, we chose to continue our investigation with the more potent isoindole containing address element.

We next turned our attention to the nature of the polar group. It was observed that other amine polar groups such as pyrrolidine and morpholine (compounds **30**–**31**) did not produce a significant variation in the affinity. The introduction of a diethylcarboxamide group (**32**) was accompanied by a decrease in affinity (148 nM). Also, *N*-methylsulfonamide **33** showed a decrease in affinity with a K_i of 26 nM compared with **28**. The N-ethylation of **33** afforded the tertiary sulfonamide **34** which provided an 8-fold improvement (3 nM) relative to **33**. This trend was continued with the tertiary amide **35**, showing a similar affinity. Finally, the succinimide, **36**, afforded a K_i of 10 nM. As with our related series,[6] we wanted to learn the effect that the absolute configuration of the privileged structure had on melanocortin affinity. We prepared the two diasteroisomers of the final compounds (only the diasteroisiomeric pairs for four compounds **28**, **34**, **35**, and **36** are shown).[16] Both isomers had comparable MC4R affinity.[17] Unlike their aromatic counterparts, these aliphatic privileged structures did not show a strong enantiopreference.

Finally we turned our attention to the compounds in which an aliphatic group replaced the polar group. Unlike our previous benzylic piperazine series where these modifications decreased affinity,[6] we found that the all-aliphatic compounds (**37**, **38**, and **39**) only suffered a 5- to 10-fold decrease in activity when compared with the related congeners, that is, **28** and **29**. Notably, these hydrophobic privileged structures still provided a good potency range (18–54 nM). The compound **40** with larger aliphatic groups showed a more significant decrease in affinity (326 nM).

Data herein illustrate that the use of an aliphatic carbocyclic piperazine-based privileged structure can provide molecules with potent MC4R affinity.[6] As we have seen with previous privileged structures, the polar groups are an important component for activity, but in contrast to the aromatic series, less basic or nonbasic polar groups showed excellent affinity. That privileged structures devoid of polar functionality provide active constructs suggests that our initial hypothesis regarding requirements for privileged structure design may have been oversimplified. In fact, these results suggest that considerable dynamic range with regard to required privileged structure functionality exists and that careful systematic evaluation of all new privileged structures is needed. These results demonstrate the usefulness of the privileged structure approach to find ligands with melanocortin activity. Further refinements of these structures will be presented in due course.

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13. Functional activity was determined by measuring cAMP release with a standard luciferase assay employing HEK 293 cells stably transfected with hMC4 (data not shown). Compounds of this paper were agonists with relative efficacies 60–100% of the maximum response obtained with NDP-α-MSH.
14. Compounds of this report were found to be generally selective for MC4 relative to the related receptors MC1 and MC3. Moderate to good selectivity was observed for MC4 relative to MC5 (data not shown).
15. Each data point represents the average of at least two determinations with an average error of the binding assay being 15%.
16. The numeration of the 2 epimers comes from the order of elution in the chiral separation of intermediate **9b**.
17. This same trend was also observed in other less-active derivatives.