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Privileged structure based ligands for melanocortin receptors—Substituted benzylic piperazine derivatives

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Abstract—Replacement of the aryl piperazine moiety in compound **1** with a variety of substituted benzylic piperazines (**6**) yields compounds that afford melanocortin receptor 4 (MCR4) activity. Analogs with ortho substitution on the aromatic ring afforded the highest affinity. Resolution of the stereocenter of the benzylic piperazine based privileged structure revealed that the *R*-enantiomer was more active.

The melanocortin receptor (MCR) family is composed of 5 closely related receptors; all of which belong to the Type I segment of the G-Protein Coupled Receptor (GPCR) family.[1] We were interested in identifying and optimizing selective tools to facilitate pharmacological characterization of MCRs with a primary focus on finding ligands selective for MCR4.[2,3] We recently reported on our initial efforts, which used a privileged structure based approach, for the identification of compounds with the desired activity profile.[4] We had found that a privileged structure, containing both hydrophobic aromatic and polar functionalities (**2**), coupled with a dipeptide address element (**3**), provided activity and selectivity for the receptor of interest (Fig. 1). In order to increase the dynamic range of our current series, we sought to better understand what structural elements were required and/or tolerated in privileged structures that afford MCR activity.

We have previously shown that both substituted monocyclic and substituted bicyclic aryl piperazines are privileged structures that provide MCR activity when coupled with the dipeptide address element **3**.[5] In both cases described above, the piperazine moiety was directly attached to the phenyl ring, thus limiting overall flexibility in this region. We were curious to see if MCR activity would be retained when the piperazine moiety was attached to a carbon chain that emanates from the phenyl ring (Fig. 2). The resulting benzylic piperazines would be interesting in that they offer an increased level of flexibility both in regard to privileged structure conformation and synthesis. Herein, we report the results of our continued progress toward the development of privileged structures that can be used in the construction of ligands with MCR activity.

Synthesis of the desired benzylic piperazines begins as outlined in Scheme 1. Condensation of a variety of benzaldehydes (**7**) with cyanide ion and *N*-Boc piperazine, using a modified Strecker protocol, affords the desired amino nitriles **8** in good yield.[6] These intermediates could be carefully reduced with LAH at −50 °C to afford the 1° amines **9**.[7] Functionalization of amines **9** was easily accomplished by alkylation (**12**), acylation (**11**, **13**), or sulfonylation (**10**) using standard reaction conditions as shown in Scheme 2. In some cases, reduction of the initial product of acylation (**13**) with BH_3 afforded a 2° amine (**14**) that could be further derivatized providing fully substituted amines **15–17**.

Keywords: Melanocortin receptors; Priveleged structures.
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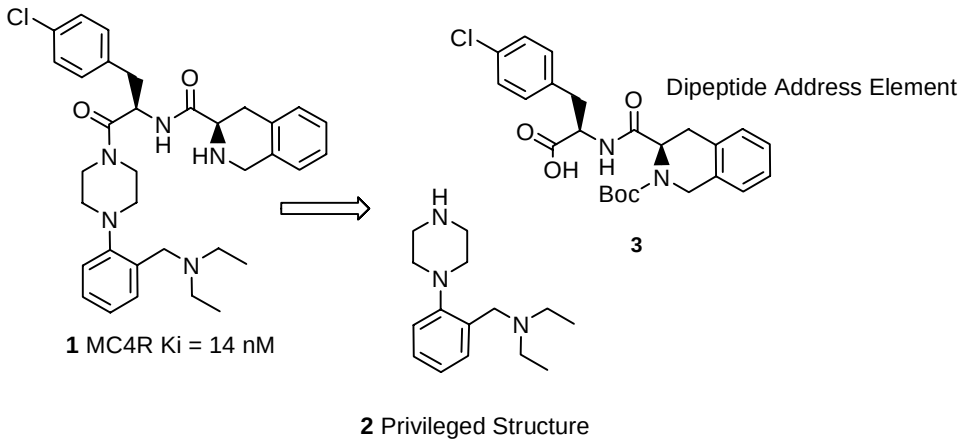


Figure 1. Disconnective analysis of lead series.

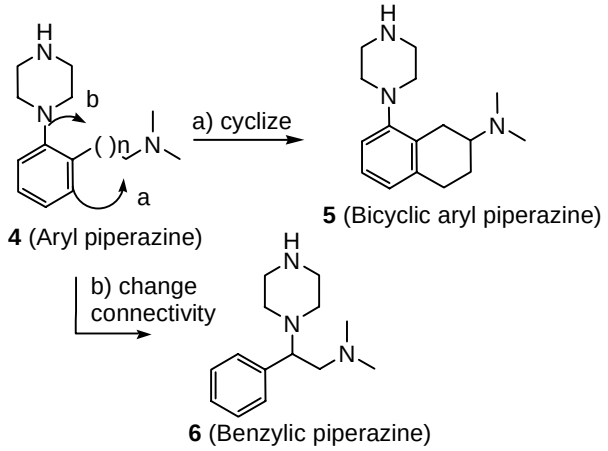
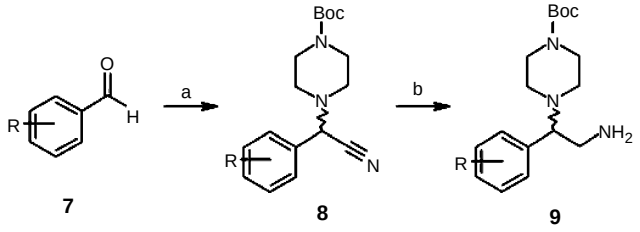


Figure 2. Evolution of priveleged structure connectivity.

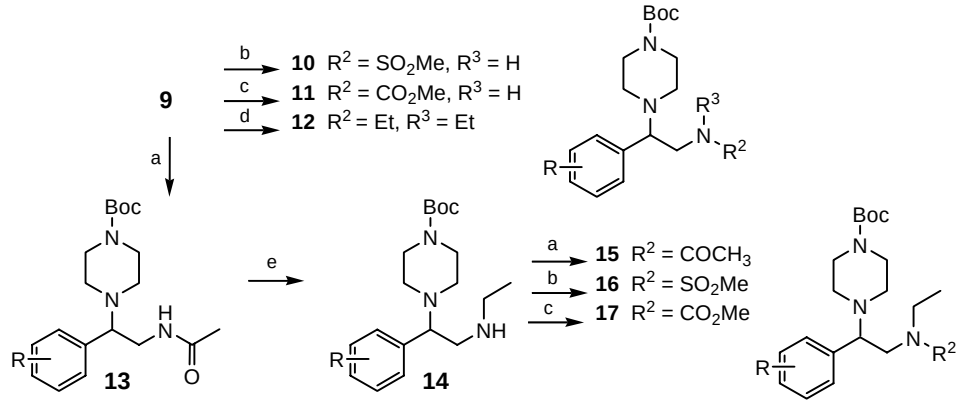


Scheme 1. Reagents and conditions: (a) Boc-piperazine, TMSCN, and ZnI_2: (b) LAH, −50 °C.

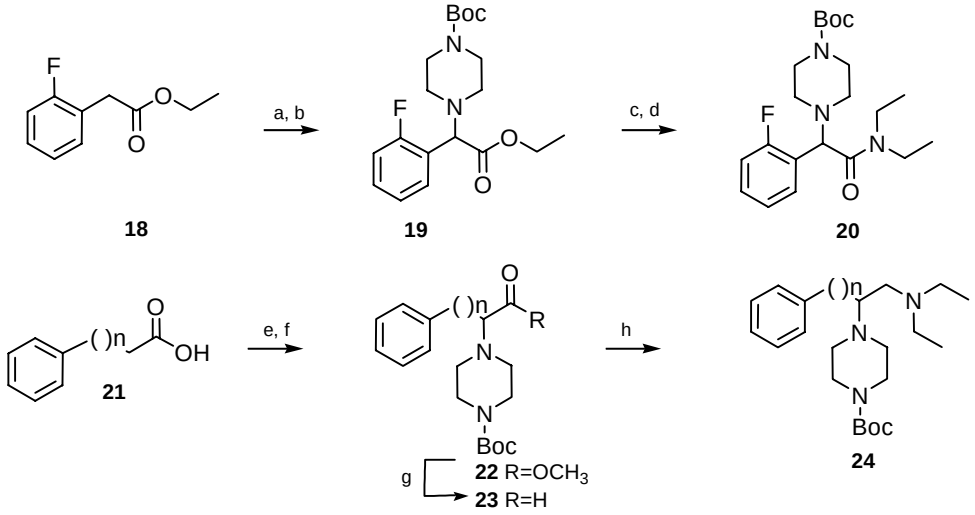
An analog containing an α-amino amide moiety was prepared as described in Scheme 3. The commercially available ethyl ester of *o*-fluoro phenylacetic acid (**18**) was brominated with *N*-bromosuccinimide forming the α-bromo derivative, which was then allowed to react with *N*-Boc-piperazine yielding **19**. This material was then hydrolyzed with sodium hydroxide and the resulting acid was transformed into the diethyl amide **20** using standard conditions. Compounds derived from other homologs of phenyl acetic acid were prepared using similar chemistry. In these cases, the commercially available phenyl carboxylic acids (**21**) were first transformed with thionyl chloride into the acid chlorides, which were then brominated with NBS and catalytic HBr, to provide the desired α-halo acid chlorides in good yield. Allowing this material to first react with methanol and then *N*-Boc piperazine gave good yields of the desired homologs **22**. The ester moiety in these compounds was then partially reduced with DIBAH to give aldehydes **23**, which were then subjected to reductive amination with diethylamine giving amines **24**.

Resolution of the chiral benzylic piperazines described in this paper was accomplished in two ways. Benzylic piperazines devoid of substitution on the aromatic ring were prepared via synthesis starting from optically active phenylglycinols as shown in Scheme 4. Synthesis of the *R*-enantiomer began with the alkylation of the amino moiety contained in *S*-phenylglycinol **25** with excess ethyl iodide affording intermediate **26** in good yield. This material was then subjected to the action of methanesulfonyl chloride in ether and the resulting mesylate was allowed to react with *N*-Boc-piperazine at room temperature for 18 h. This afforded the desired intermediate benzylic piperazine **27** in good yield after workup and chromatography. Analysis of the material produced in this fashion by chiral chromatography (Chiralpak AD, hexane–TFA 0.05%) indicated that product was obtained in >98% ee. This transformation presumably occurs via an intermediate aziridium ion, which is then displaced at the benzylic center resulting in an overall inversion of stereochemistry. Synthesis of the *S*-enantiomer was accomplished in the same way starting with *R*-phenylglycinol. Alternatively, the desired privileged structures could be resolved on a gram scale by chiral chromatography (Chiralcel OD (4.6 × 250 mm) column, eluting with 5% ethanol in heptane at 1 mL/min) of the corresponding phthalimide derivatives (**29**). This sequence is illustrated in Scheme 4 using the *o*-fluoro derivative **28**. The resolved phthalimides (**29**) were then deprotected with excess hydrazine and the liberated amine (**30**) was functionalized in an analogous fashion to the racemic analogs.

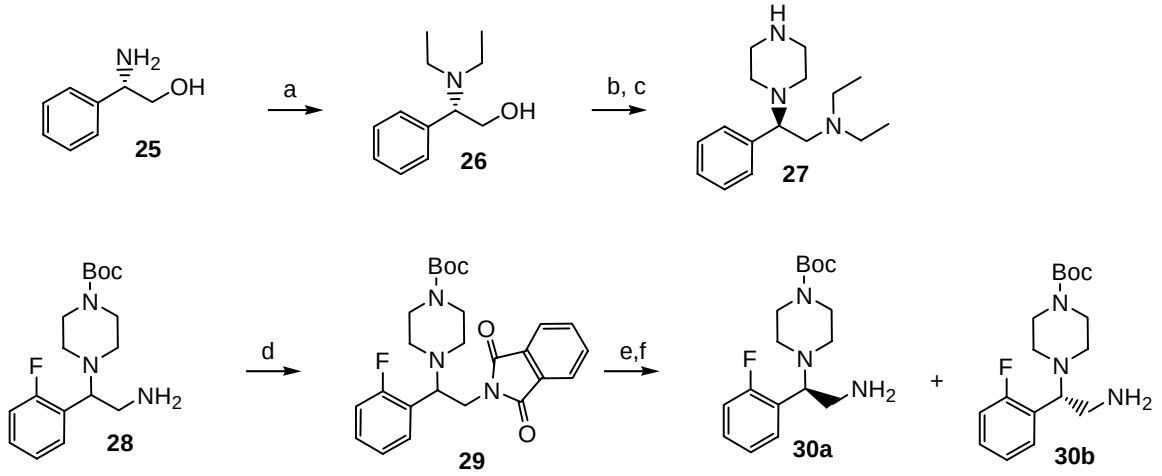
Final compounds described in this paper were constructed from the aforementioned intermediates as outlined in Scheme 5. The piperazine-containing



Scheme 2. Reagents: (a) acetyl chloride; (b) methanesulfonyl chloride; (c) methylchlorofomate; (d) EtBr, K_2CO_3; and (e) BH_3.

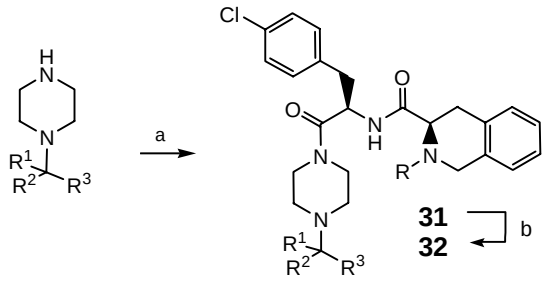


Scheme 3. Reagents: (a) NBS; (b) Boc-piperazine; (c) NaOH; (d) Et_2NH, EDCI; (e) $SOCl_2$; (f) NBS, HBr; (g) DIBAH; and (h) Et_2NH, $NaBH_3CN$.



Scheme 4. Reagents: (a) EtI, K_2CO_3, (b) MsCl, (c) Boc-piperazine, (d) phthalic anhydride, (e) chiral chromatography, and (f) hydrizine.

privileged structures were allowed to react with the previously described dipeptide **3** in the presence of HATU[8] to afford the penultimate derivatives (**31**). Treatment with TFA provided the desired deprotected compounds **32** which could be used as the corresponding TFA salt or subjected to ion exchange to obtain the HCl salt. In the cases where the piperazines were not prepared in chiral form, coupling with dipeptide **3** afforded diastereomeric pairs that were not separated for biological evaluation.



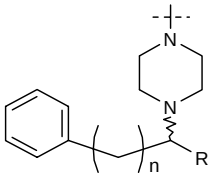
Scheme 5. Reagents: (a) **3**, HATU; (b) TFA, DCM.

Compounds described for this report were evaluated for binding affinity at human melanocortin 4 receptors (hMCR4) by determining competitive inhibition of ^{125}I-NDP MSH binding.[9,10] Selected compounds were evaluated for selectivity across human melanocortin receptors 1, 3, and 5 in a similar fashion.[11] We first examined the effects that benzylic attachment would have on this series of compounds. Compound **33** (Table 1) which lacks a polar group (thought to be key for activity) afforded modest affinity for hMCR4 with a K_i of 2 μM. Substitution on the benzylic carbon with a simple methyl group yielded compound **34**, which showed a 3-fold increase in activity (K_i 0.7 μM) relative to the unsubstituted analog **33**. Conversion of this methyl group into a diethylaminomethyl moiety, a functional group that has been very productive in our previous series, yielded compound **35**, whose activity increased 8-fold (K_i of 0.09 μM) relative to the methyl analog **34**. Stepwise elongation of the linker between the phenyl group and the tertiary carbon provided analogs **36**, **37**, and **38** which had comparable affinities of 0.04 μM, 0.13, and 0.11 μM, respectively.

As the binding affinities of the above-mentioned homologs were similar, we chose to focus on optimizing the more synthetically accessible benzylic analog **35** (Table 2). We next examined simple aromatic substitution and a trifluoromethyl group was the first substituent evaluated. The data reveal that *ortho* substitution (**39**) was more active than *meta* (**40**) or *para* (**41**) substitution by 4.7- and 3.6-fold, respectively. Ortho substitution provided a 2-fold benefit relative to the unsubstituted analog **35**. *Ortho* substitution of the phenyl ring was also beneficial for other substituents such as chloro, fluoro, and methyl. Of the single substitutions examined in the ortho position, the fluoro analog (**44**) appeared to be optimal with a K_i of 0.02 μM. The only disubstituted aromatic analogs examined (**46** and **47**) both utilized fluorine as replacements of hydrogen and neither showed an activity advantage relative to their monosubstituted counterpart **44**.

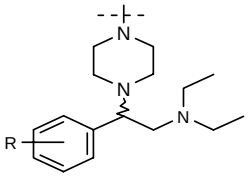
We next turned our attention to the polar functionality found in this series of privileged structures through examination of substitution on the amine moiety (Table 3). Relative to the dialkylamine moiety found in compound **44** (K_i 0.02 μM), simple acylation (**50**) resulted in a 5-fold erosion of activity (K_i 0.46 μM). Activity was also diminished (K_i 0.47 μM) by sulfonylation (**49**). Conversion of the dimethylamino moiety to a carbamate (**48**) also decreased activity similar to that observed from acylation. Interestingly, N-alkylation of the acylated and sulfonylated compounds (**49** and **50**, respectively) with an ethyl moiety provided tertiary amides **52** and **53**, that were more potent than their nonalkylated counterparts and only 2-fold less active

Table 1. Phenyl alkyl piperazines



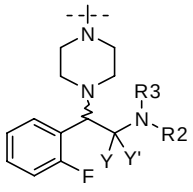
Compound	*n*	R	MC4R K_i (nM)[12]
33	0	H	2000
34	0	CH_3	700
35	0	$CH_2N(CH_2CH_3)_2$	90
36	1	$CH_2N(CH_2CH_3)_2$	40
37	2	$CH_2N(CH_2CH_3)_2$	130
38	3	$CH_2N(CH_2CH_3)_2$	110

Table 2. Substituted benzyl piperazines



Compound	R	MC4R K_i (nM)[12]
39	*o*-CF_3	40
40	*m*-CF_3	150
41	*p*-CF_3	200
42	*o*-Cl	70
43	*o*-CH_3	70
44	*o*-F	20
45	*o*-OCH_3	190
46	2,6-Difluoro	150
47	2,4-Difluoro	100

Table 3. Amine substitution



Compound	Y,Y′	R^2	R^3	MC4R K_i (nM)[12]
48	H,H	$COOCH_3$	H	483
49	H,H	SO_2CH_3	H	472
50	H,H	$COCH_3$	H	467
51	H,H	$COOCH_3$	Et	261
52	H,H	SO_2CH_3	Et	157
53	H,H	$COCH_3$	Et	106
54	Oxo	CH_2CH_3	Et	125

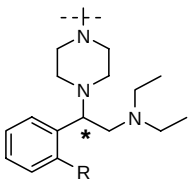
than their tertiary amine counterpart **44**. Changing the point of acylation from an external position to an internal arrangement provided glycinamide derivative **54**, which was roughly 4-fold less potent (K_i 0.125 μM) than the corresponding tertiary amine analog **44**.

Finally, we examined the effect that the absolute configuration of the privileged structure had on the activity of these compounds (Table 4). The data revealed a 4-fold difference in the phenyl antipodes as seen by comparing **55** and **56**. As this pair was from intermediates with known absolute stereochemistry, we are able to assign the stereochemistry of the benzylic carbon of most potent isomer **56** as *R* while the same center of the less potent isomer **55** was assigned the *S* configuration. Similar differences in activity were observed for the fluoro analogs, which were resolved chromatographically (see text). In this case, the faster eluting isomer **57** afforded more activity than the slower eluting isomer **58**. Based on the activity difference observed for both sets of compounds, we have assigned the faster eluting isomer of the fluoro analog **57** as having the *R* configuration at the benzylic center and the slower isomer having the *S* configuration.

The above data demonstrate that appropriately substituted benzylic piperazines act as privileged structures when coupled to address element **3**. Of interest is the fact that the unsubstituted benzyl piperazine **33** affords measurable activity, and functionalization of the benzylic position with even a simple methyl group (**34**) affords a compound with submicromolar activity. Consistent with our earlier results, we found that the incorporation of a polar group proximal to the aromatic group enhances activity for this series. It appears that distance between the phenyl moiety and the benzylic center to which the polar group is attached is not very restrictive. This may indicate that the putative binding site for this pharmacophore is size tolerant. Substitution of the phenyl moiety with halogens, especially fluorine, had a measurable benefit, which suggests that this moiety likely occupies a hydrophobic area in the receptor.

The activity afforded by both the phenyl and benzyl piperazine series demonstrates the variety allowed in privileged structures that yield MCR activity. These observations underscore the practicality of employing privileged structures as key components of GPCR ligands. While the use of privileged structures has proven useful for the generation of active compounds, we have found that efficient optimization requires an accurate understanding of the pharmacophores and topography displayed by the lead privileged structures. This information can then be used in the design and construction of a portfolio of physicochemically diverse, yet functionally equivalent, subunits. Identification of these privileged structure families would in principle allow the ready optimization of the address elements of interest. Further development of this hypothesis and its application to the further refinement of ligands for the MCRs will be the subject of future reports.

Table 4. Resolved examples



Compound	R	*	MC4R K_i (nM)[12]
55	H	*S*-isomer	190
56	H	*R*-isomer	50
57	F	Isomer 1	14
58	F	Isomer 2	111

Acknowledgment

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9. The details of the binding assay have been recently described. See Ref. 3.
10. Functional activity was determined by measuring cAMP release with a standard luciferase assay employing HEK 293 cells stably transfected with hMC4R. Compounds (both single antipodes and diastereomeric pairs) described in this paper were agonists with relative efficacies 60–100% of the maximum response obtained with NDP-α-MSH. Of the diastereomeric pairs that were separated, we were unable to detect any antagonist activity in the less active diastereomer.
11. Compounds of this report were found to be generally selective for MC4R relative to the related receptors MC1 and MC3. Moderate to good selectivity was observed for MC4R relative to MC5R.
12. Each data point represents the average of at least two determinations with an average error of the binding assay being 15%.