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Design, synthesis, and SAR studies on a series of 2-pyridinylpiperazines as potent antagonists of the melanocortin-4 receptor

Joe A. Tran,[a] Joseph Pontillo,[a] Beth A. Fleck,[b] Dragan Marinkovic,[a] Melissa Arellano,[a] Fabio C. Tucci,[a] Marion Lanier,[a] John Saunders,[a] Wanlong Jiang,[a] Caroline W. Chen,[a] Alan C. Foster[c] and Chen Chen[a,*]

[a]*Department of Medicinal Chemistry, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*Department of Pharmacology, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[c]*Department of Neuroscience, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*

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Abstract—A series of 2-pyridinylpiperazines derived from β-Ala-(2,4-Cl)Phe dipeptide was synthesized for the study of their SARs and possible interactions with the MC4 receptor. Compounds such as **11k** (K_i = 6.5 nM) possessed high potency.

We have previously demonstrated that the basic nitrogen of the benzylamines **1** (Fig. 1), which possibly interacts with Asp122 of the human melanocortin-4 receptor (*h*MC4R), is very important for high affinity binding.[1] Thus, the benzylamines bearing an alkyl side chain such as 2-thienylethyl (**1a**, K_i = 1.8 nM) or 1-methoxy-2-propyl group (**1b**, K_i = 8.8 nM) possess potent affinity in a competition binding assay.[2] Since the phenyl ring of the benzylamines **1** could reside in an area close to the transmembrane domain seven (TM-7) of *h*MC4R, and interacts with several lipophilic residues such as Phe284, Leu288, and Ile289 based on a computational receptor model,[1] we designed and synthesized a series of cyclohexyl derivatives exemplified by **2a** and **2b** as potent *h*MC4R antagonists.[3] Interestingly, while compound **2a** (K_i = 11 nM) bearing a basic side chain exhibits good binding affinity as expected, compound **2b** (K_i = 4.2 nM), with an amide group, also possesses high potency. One possible explanation for these results is that, instead of the charge-charge attraction between the basic amine of **2a** and an acidic residue of the receptor, the amide of **2b** may pick up the interaction with Asp122 through hydrogen-bonding. However, the piperazine of **2** is weakly basic (estimated pK_a value is 6.8 on the basis of calculation), it might participate in the interaction of **2b** with the receptor. Therefore, we decided to explore the role of the weakly basic piperazine by installing the more basic 2-piperazinepyridine moiety (calculated pK_a is 9.2). In addition, the more hydrophilic pyridine will reduce the lipophilicity of its phenyl analogs such as **3** (c log D = 3.1). Here we report the synthesis and structure–activity relationship studies of a series of 2-pyridinylpiperazines, derived from β-Ala-D-(2,4-Cl)-Phe dipeptide, bearing an amine or amide side chain, as potent *h*MC4R antagonists.

The synthesis of the targeted compounds started from the 2-chloro-3-pyridinylcarboxaldehyde **4**, which was condensed with *N*-Boc-piperazine at an elevated temperature to give the 2-aminopyridine **5**. Deprotection of **5** with trifluoroacetic acid in dichloromethane, followed by a standard peptide coupling protocol with D-*N*-[*N*-(*tert*-butoxycarbonyl)-β-alanine]-2,4-dichlorophenylalanine, or D-*N*-acetyl-2,4-dichlorophenylalanine, afforded the key intermediate **6a**, or **6b**. Reductive amination of the aldehyde **6a** with ammonia provided the primary benzylamine **7**, which was subjected to coupling reactions with various carboxylic acids to give the amides **8a**–**l** after TFA-deprotection. Coupling reactions of **7** with different chloroformates in the presence of triethylamine, followed by TFA treatment, afforded

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* Corresponding author. Tel.: +1 858 617 7600; fax: +1 858 617 7967; e-mail: cchen@neurocrine.com

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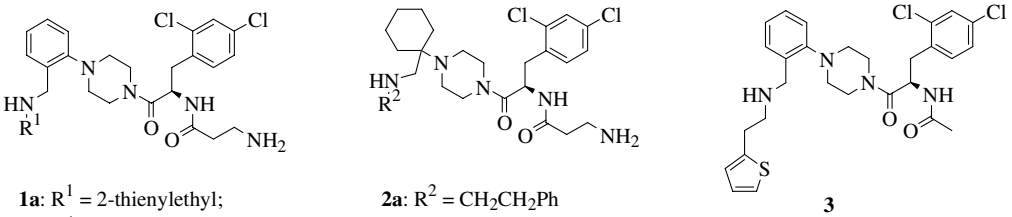


1a: R^1 = 2-thienylethyl;
1b: R^1 = 1-methoxy-2-propyl;
1c: R^1 = 2'-methoxyphenethyl;

2a: $R^2 = CH_2CH_2Ph$
2b: $R^2 = COCH_2C_6H_4OMe\text{-}4$
2c: $R^2 = COC_6H_4OMe\text{-}4$
2d: $R^2 = CONHC_6H_4OMe\text{-}4$

3

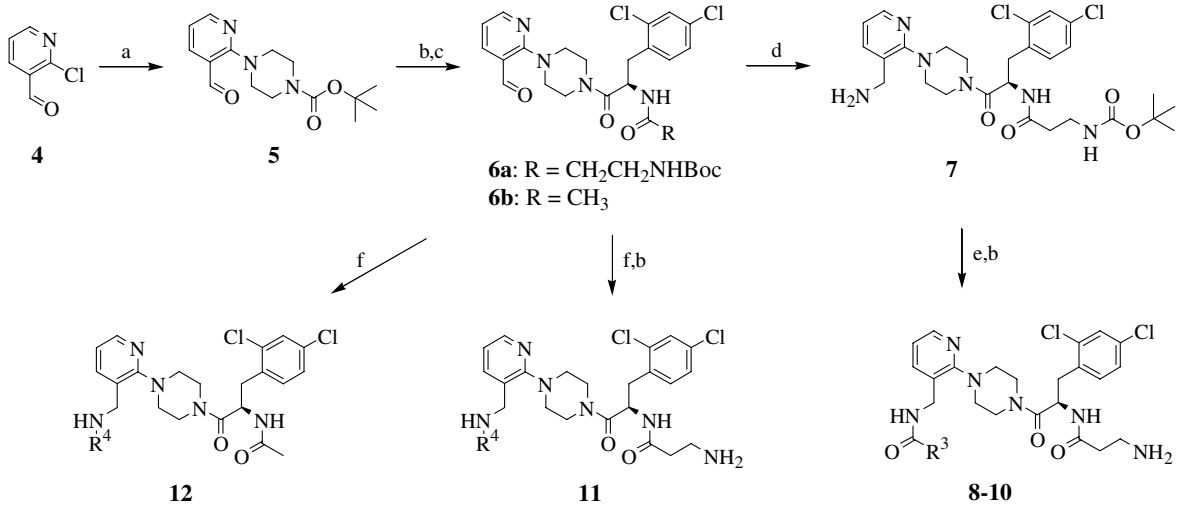
Figure 1. Small molecule antagonists of the melanocortin-4 receptor.

the carbamates **9a**–**c**. Condensations of **7** with several alkyl or arylisocyanates provided, after deprotection, the ureas **10a**–**h** in excellent yields (Scheme 1).

Alternatively, reductive aminations of **6a** with various primary amines afforded the secondary amines **11a**–**l** after TFA treatment. Similarly, compounds **12a**–**c** were obtained from **6b**.

The synthesized compounds were tested for their ability to compete with [^{125}I]-NDP-MSH at *h*MC4R expressed in HEK 293 cells in a binding assay as previously described.[4] The amides **8a**–**l** displayed K_i values of 310–2800 nM regardless of the R^3-group, implying this group is not in the contact with the receptor. For example, the 4-methoxybenzoyl **8e** (K_i = 1500 nM) and the 4-methoxyphenylacetyl **8k** (K_i = 550) displayed similar binding affinity. In contrast, cyclohexylpiperazine analogs **2c** (K_i = 250 nM) and **2b** (K_i = 4.2 nM) exhibited about 40-fold difference in potency, suggesting the aromatic ring of **2b**, but not **2c**, offers extra binding energy. To further explore this point, several carbamates **9a**–**c** and a series of ureas **10a**–**h** were synthesized and examined. The carbamates **9a**–**c** exhibited K_i values of 440–1,800 nM, and the ureas **10a**–**h** displayed K_i values of 560–1300 nM (Table 1). These results were no better than that of the carboxamides **8a**–**l**, including acetamide **8a** (K_i = 2800 nM). In comparison, the urea **2d** (K_i = 36 nM) of cyclohexylpiperazine had about 9-fold reduction in potency, suggesting the conformation of this side chain is an important factor for **2**. These results indicate that the aromatic ring in the side chain of **8**–**10** did not participate in the interaction of these compounds with the receptor. They are clearly different from the cyclohexyl compounds such as **2a**–**d** in which the phenylacetamides (i.e., **2b**) are much better than the benzoylamides (i.e., **2c**) and arylureas (i.e., **2d**), but the phenethylamine **2a** had comparable binding value to **2b**.[3]

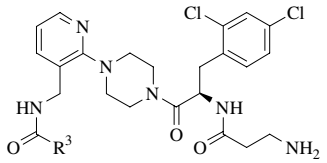
A series of amines **11a**–**l** then was studied. We have previously demonstrated that a proper *N*-alkyl group can improve binding affinity of a primary benzylamine over 60-fold.[1] While an aromatic ring connected by an ethylene such as 2-thienylethylamine is an optimal side chain for both benzylamine **1a** and cyclohexylmethylamine **2a**, a smaller 1-methoxy-2-propyl group is found to be a suitable replacement for **1a**. Thus, the racemic compound **1b** (K_i = 8.8 nM) is a potent *h*MC4R antagonist. However, while this group was incorporated into the pyridine series, the corresponding analog **11c** (K_i = 87 nM) exhibited 10-fold reduction in binding from **1b**, although it was still better than the lipophilic trifluoroethyl **11a** (K_i = 170 nM) and the hydrophilic hydroxyethyl **11b** (K_i = 520 nM). Its O-demethylated analog **11d** (K_i = 420 nM) was only moderately active, and an additional methyl group on **11d** did not alter its binding affinity (**11e**,



4 **5** **6a**: R = CH_2CH_2NHBoc **6b**: R = CH_3 **7** **12** **11** **8-10**

Scheme 1. Reagents and condition: (a) *N*-Boc-piperazine/DMSO/Δ; (b) TFA/CH_2Cl_2; (c) *N*-(RCO)-D-(2,4-Cl)Phe-OH/EDC/HOBt; (d) NH_3/$NaBH(OAc)_3$; (e) R^3COOH/EDC; or R^3COCl/Et_3N or isocyanate; (f) $R^4NH_2/NaBH(OAc)_3$.

Table 1. SAR of amides, carbamates and ureas at *h*MC4R



8-10

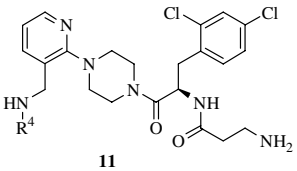
Compound	R^3	K_i (nM)
8a	Me–	2800
8b	$MeOCH_2$–	2800
8c	Ph–	1800
8d	4-FC_6H_4–	2200
8e	4-$MeOC_6H_4$–	1500
8f	2-Furanyl–	2600
8g	2-Thienyl–	1800
8h	2-$FC_6H_4CH_2$–	450
8i	2-$MeOC_6H_4CH_2$–	360
8j	4-$FC_6H_4CH_2$–	1000
8k	4-$MeOC_6H_4CH_2$–	550
8l	2-$ThienylCH_2$–	310
9a	PhO–	1200
9b	2-$MeOC_6H_4O$–	1800
9c	BnO–	440
10a	*i*-PrNH–	1300
10b	PhNH–	710
10c	2-FC_6H_4NH–	1100
10d	2-$MeOC_6H_4NH$–	1300
10e	BnNH–	1000
10f	2-$FC_6H_4CH_2NH$–	970
10g	2-$MeOC_6H_4CH_2NH$–	560
10h	2-$ThienylCH_2NH$–	750

K_i = 480 nM). The 1-methoxy-2-butyl analog **11f** (K_i = 200 nM) exhibited slightly reduced potency from **11c**, and its O-demethylated derivative **11g** (K_i = 710 nM) again displayed over 3-fold reduction in binding. As expected, the more hydrophilic **11h** had further reduction in binding affinity, and the 2-fluorobenzyl **11i**, however, showed similar potency to that of **11c**. Further extension of the 2-fluorobenzyl group of **11i** resulted in compound **11j** with 10-fold improvement (K_i = 12 nM). The 2-methoxyphenethyl **11k** (K_i = 6.7 nM), exhibiting the best affinity of this series, was slightly better than that of the 2-thienylethyl **11l** (K_i = 15 nM) which had similar binding affinity to **11j**. However, the binding affinity of these two compounds was 4- and 10-fold, respectively, lower than that of benzylamines **1a** (K_i = 1.8 nM) and **1c** (K_i = 1.6 nM) (Table 2).

Similarly, the acetamides **12b** (K_i = 10 nM) and **12c** (K_i = 21 nM) exhibited K_i values close to that of **11k** and **11l**, while **12a** (K_i = 180 nM) was slightly less potent than the β-alanine derivative **11c**. The pyridine compound **12c** was again about 9-fold less active than the benzene analog **3** (K_i = 2.4 nM).

Apparently, the 2-piperazinepyridine bearing an amide side chain at the 3-position such as **8k** (K_i = 550 nM) could not mimic the piperazinecyclohexane attached by an amide at the 1-position such as **2b** (K_i = 4.2 nM). In contrast, the pyridine analogs **11** of benzylamines

Table 2. SAR of amine at *h*MC4R



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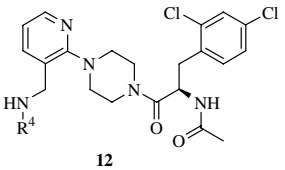

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Compound	R^4	K_i (nM)
11a	CF_3CH_2–	170
11b	$HOCH_2CH_2$–	520
11c	$MeOCH_2CH(Me)$–	87
11d	$HOCH_2CH(Me)$–	420
11e	$HOCH_2C(Me)_2$–	480
11f	$MeOCH_2CH(Et)$–	200
11g	$HOCH_2CH(Et)$–	710
11h	$(HOCH_2)_2CH$–	1500
11i	2-$FC_6H_4CH_2$–	130
11j	2-$FC_6H_4CH_2CH_2$–	12
11k	2-$MeOC_6H_4CH_2CH_2$–	6.7
11l	2-$ThienylCH_2CH_2$–	15
12a	$MeOCH_2CH(Me)$–	180
12b	2-$MeOC_6H_4CH_2CH_2$–	10
12c	2-$ThienylCH_2CH_2$–	21

1a–**c** were only moderately less potent. For example, the K_i values of **11k** (K_i = 6.7 nM) and **11l** (K_i = 15 nM) were 4- and 8-fold higher than that of **1c** (K_i = 1.6 nM) and **1a** (K_i = 1.8 nM), respectively. Computational analysis of the conformations of piperazinebenzylamine and the corresponding pyridine analog indicates that the almost planar acylpiperazine ring is orthogonal to the aromatic ring, while the dihedral angle of the pyridine analog could be slightly smaller (Fig. 2). This difference in conformations could partly explain the different binding affinities of these two series of compounds. Alternatively, the pyridine ring is less lipophilic and π-electron-deficient in comparison with benzene, therefore, its interaction with an aromatic residue through π-stacking, or aliphatic side chains of the receptor via van der Walls interaction, would be less favored.[5] One aromatic acidic acid Phe284 of *h*MC4R is located at the top TM-7, which is involved in the interaction with a small molecule agonist based on mutagenesis studies.[4a]

While the orthogonal conformation of the phenyl- and pyridinyl-piperazines could account for the difference in binding affinity between these two series, the lower affinity of amides such as **8i** than the amine such as **11k** needs further understanding. The lack of SAR of the amides could not be simply explained by the loss of a charge–charge interaction of the amides **8**–**10** since it has been demonstrated the aromatic ring on the N-side chain plays a role in receptor binding. A reasonable explanation is the lack of cation–π interaction.[6] Thus, in the case of a 2-pyridinylpiperazine with an amine side chain, such as **11k**, the protonated amine under physiological conditions has a strong interaction with the π-system of the aromatic pyridine ring. This interaction might position the phenethyl side chain of **11k** to a conformation required for the pharmacophore. It will cost energy for **8i** to possess a similar conforma-

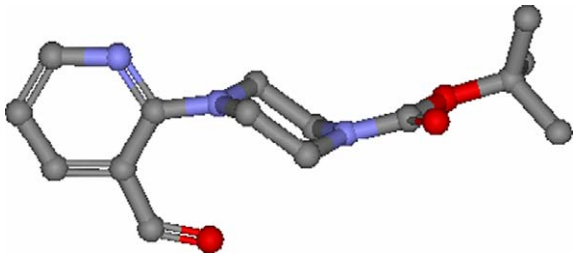

Figure 2. Orthogonal relationship between pyridine and piperazine represented by **5** (generated by DS ViewerPro 5.0, Accelrys).

tion due to a much weaker amide-π interaction.[7] In the cyclohexyl series, the amine nitrogen of **2a** presumably resides at the top of the cyclohexyl ring as an active conformation, and the amide functionality of **2b** could be slightly favored to do so, because of the weak interaction of the partially positively charged protons of the cyclohexyl ring with the electron-negative amide, which, in combination with an amide-acid hydrogen bond, could compensate for the loss of a strong charge–charge attraction. Thus, the amide **2b** possessed slightly better binding affinity than the amine **2a**. In comparison to **2b** (K_i = 4.2 nM), in which the 2-methoxyphenyl group is connected through a methylene to the amide, the directly connected **2c** (K_i = 250 nM) and the NH-linked **2d** (K_i = 36 nM) push the aromatic ring to a less favored position for receptor interaction. One of the amino acid residues of the receptor interacting with this aromatic moiety could be Phe184 at the top of TM-4 based on a receptor model. This residue has been demonstrated to interact with α-MSH based on mutagenesis studies.[8]

Compounds such as **11k** from this series were also highly selective. Thus, **11k** had K_i values of 5000, 6.7, and 2100 nM at *h*MC3R, *h*MC4R, and *h*MC5R, respectively. All compounds had no significant stimulation of cAMP production at the human MC4 receptor expressed in HEK 293 cells at 10 μM concentration.

However, despite the reduction in lipophilicity, compounds such as **12b** possessed low metabolic stability possibly due to their structural features such as high flexibility. For example, **12b** had an intrinsic clearance CL_{int} of greater than 3500 ml/min kg in an in vitro rat liver microsomal assay, predicting a zero percentage of bioavailability in this species. In comparison, the dibasic molecule **11k** with a calculated log *D* of 1.1 had a CL_{int} of 390 ml/min kg, which would give an oral bioavailability of 15% assuming complete absorption. The poor metabolic stability prevents the further development of these compounds.

In conclusion, a series of 2-pyridinylpiperazines was synthesized to study the detailed structure–activity relationships and their interactions with the MC4 receptor. While these compounds possessing a basic moiety displayed high binding affinity, similar to the benzylamine analogs, analogs bearing an amide side chain were much less potent. These results are different from the cyclohexylpiperazines, in which an amide side chain resulted in a compound with similar to or better than that with an amine group in binding affinity. Compounds from this series possessed high binding affinity. For example, **12b** had a K_i value of 10 nM at *h*MC4R. While it was slightly less potent than its phenyl analog 3 (K_i = 2.4 nM),[9] the reduced lipophilicity caused by the hydrophilic pyridine provided **12b** with a desirable calculated log *D* value of 2.7.

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