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Synthesis and structure–activity relationships of retro bis-aminopyrrolidine urea (rAPU) derived small-molecule antagonists of the melanin-concentrating hormone receptor-1 (MCH-R1). Part 2

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Abstract—The design, synthesis, and SAR of a series of retro bis-aminopyrrolidine ureas are described. Compounds from this series exhibited considerable binding affinity (K_i = 1 nM) and functional activity at MCH-R1, acceptable *CYP*2D6 inhibition, and good rat brain exposure.

Mammalian melanin-concentrating hormone (MCH) is a 19 amino acid cyclic peptide[1] which selectively binds and activates two 7-transmembrane G protein-coupled receptors, namely MCH-R1 and R2.[2] Recent research suggests that MCH-R1 is involved in the modulation of energy homeostasis, food intake, and body weight.[3] Animal models and other data suggest that, in man, blockade of MCH-R1 may lead to a clinical treatment for chronic obesity. A number of groups have recently reported on their efforts toward the development of selective small-molecule MCH-R1 antagonists.[3,4]

In the previous paper we discussed our efforts to optimize the retro bis-aminopyrrolidine urea scaffold (rAPU), a series of potent and functional MCH-R1 antagonists.[5] This series was derived from the bis-aminopyrrolidine ureas (APU)[4i] and had significantly less activity in the hERG patch-clamp assay. The series also exhibited greater metabolic stability in human liver microsomes which translated to greater oral bioavailability in rat. Experiments to investigate the SAR of the terminal aryl ring of the biaryl moiety revealed that *para* lipophilic substitution was favored, resulting in a dramatic increase in MCH-R1 potency. However, two problems were identified: significant *CYP*2D6 inhibition and low brain penetration. With these issues in mind, the left- and right-hand sides of the rAPU were modified to identify compounds equipotent to APU **1** (MCH-R1 K_i = 2 nM) with the same low liability for inhibition of *CYP2D6*, while retaining the superior properties demonstrated by previous rAPUs (Fig. 1).

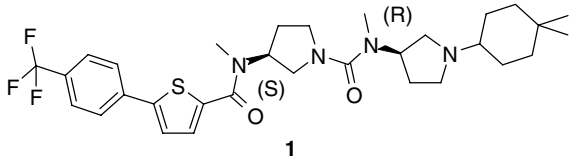

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Figure 1. Aminopyrrolidine urea **1**.

Keywords: Retro bis-aminopyrrolidine urea; Melanin-concentrating hormone receptor-1; Antagonists; CYP2D6.
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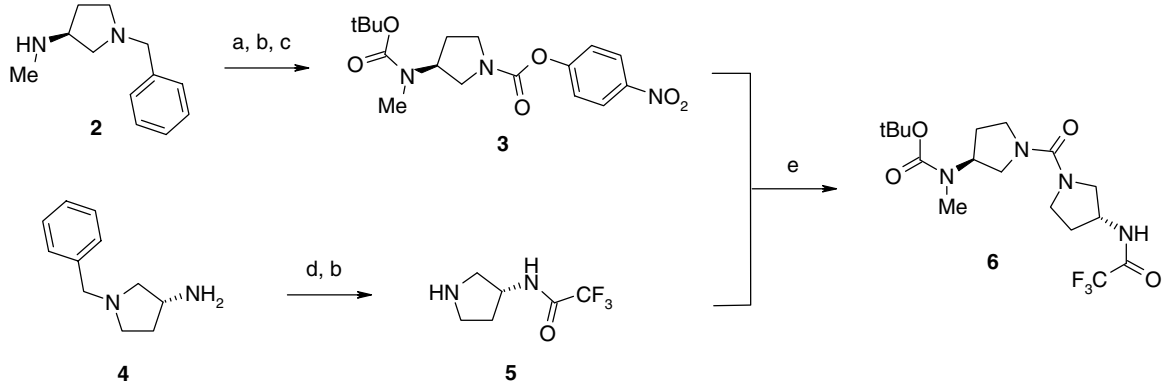
To increase the efficiency of functionalizing both ends of the rAPU core, the previously reported synthesis[5] was modified to generate the key intermediate **6** (Scheme 1). (3*S*)-(−)-1-Benzyl-3-(methylamino)pyrrolidine (**2**)[6] was first protected as the *tert*-butylcarbamate using standard conditions. Subsequent N-debenzylation followed by reaction with 4-nitrophenyl chloroformate afforded carbamate **3** in good yield. Pyrrolidine **5** was prepared in two steps from commercially available (3*R*)-(−)-1-benzyl-3-aminopyrrolidine (**4**).[7] Trifluoroacetamide protection followed by N-debenzylation afforded **5** in quantitative yield. Coupling of carbamate **3** and pyrrolidine **5** yielded the orthogonally protected urea core **6**. Theoretically the two ends of the rAPU core could be functionalized in either order; however, intermediates first elaborated on the left-hand side were much easier to purify. Scheme 2 depicts the chosen synthetic route to compounds in Tables 1 and 2. Deprotection of the amine with trifluoroacetic acid, coupling to the appropriate carboxylic acid, followed by base promoted deprotection of the trifluoroacetamide afforded primary amines **7**. N-alkylation via reductive amination afforded rAPUs **8**. Alternatively, urea **6** deprotection followed by coupling to 5-bromo-2-thiophene carboxylic acid using EDCI/HOBt afforded **9** in good yield. Trifluoroacetamide deprotection followed by reductive amination with 4,4-dimethylcyclohexanone yielded the secondary amine **10**. Suzuki coupling of bromothiophene **10** with a variety of arylboronic acids afforded rAPUs **11**. The furan **12** was synthesized in an analogous manner starting with 5-bromo-2-furan-carboxylic acid.

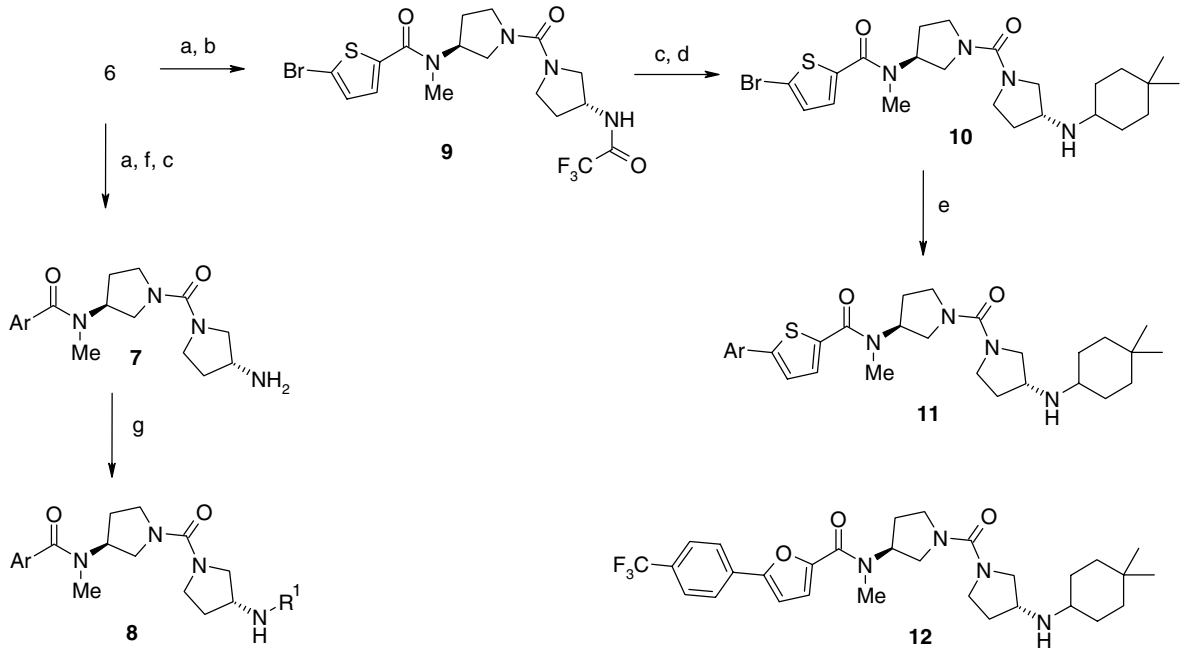
For tertiary methyl amines, as exemplified by **14a**–**c**, the methylation step was conveniently performed at an earlier point in the synthesis (Scheme 3). Trifluoroacetamide **6** was methylated with methyl iodide in quantitative yield. Carbamate deprotection, amide coupling, a second deprotection, and a final reductive amination afforded rAPUs **14**. The trifluoroethyl derivative **17** was prepared in four steps from **6**. Borane reduction of the trifluoroacetamide afforded amine **15**. N-methylation using formaldehyde via reductive amination yielded tertiary amine **16**. Deprotection followed by amide coupling afforded the desired trifluoroethyl derivative **17**. Scheme 4 was employed when reductive amination was not an option to prepare the tertiary amine of the right-hand side (**23a**–**f**). Alcohol **21** was prepared in four steps. Coupling of the arylcarboxylic acid to (3*S*)-(−)-1-benzyl-3-(methylamino)pyrrolidine (**2**) afforded **18**. N-debenzylation of **18** yielded amine **19**. Derivatization with 4-nitrophenyl chloroformate afforded carbamate **20**. The urea core was synthesized via 4-nitrophenol displacement with (*S*)-(−)-3-hydroxypyrrolidine and afforded alcohol **21** in 32% (four steps). Reaction of **21** with methanesulfonyl chloride at low temperature afforded mesylate **22**. Displacement of mesylate **22** with a variety of amines afforded rAPUs **23**.[8]

The rAPUs described herein (Tables 1–3), obtained as single diastereomers, were tested in the MCH competitive binding assay.[9] The functional antagonism was measured of all compounds with K_is less than 10 nM,[5] in general a 3-fold reduction in potency was observed. Replacement of the biphenyl for phenyl thiophene in the rAPU series did not result in the same dramatic increase in potency as was achieved for the APU series (a 17-fold increase in binding affinity was observed for **1** compared to its biphenyl analog).[4i] Most of the rAPU phenyl thiophene analogs were up to 3-fold less active (Table 1). In addition, as demonstrated by **8a** and **8b**, compounds with *para*-ethyl or *para*-methoxy substitution were about 3-fold more potent than the trifluoromethyl analog **8c**, suggesting that electron-donating substituents are favored.

Even though the introduction of the thiophene moiety did not generally result in a large increase in potency, it did result in highly potent compounds when combined with the gem dimethylcyclohexyl right-hand side described previously[4i] (as summarized in Table 2). Compounds **11a** and **11b**, with left-hand side *para*-ethyl and *para*-methoxy, respectively, highlight the importance of the *para* substitution. Both compounds have an affinity equal to 1 nM, which is significantly better relative to the unsubstituted analog, **11h**, with an affinity of 25 nM. Increasing the size of the *para* substituent also led to a drop in affinity with the ethoxy analog **11g** being 8-fold less potent than **11b**. This supports the hypothesis that the receptor binding pocket around the biaryl motif is sensitive to sterics. Two other promising analogs are

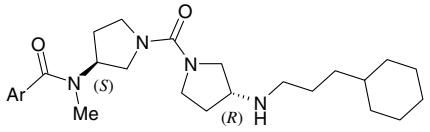


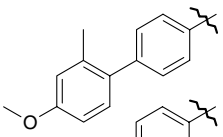
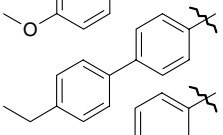
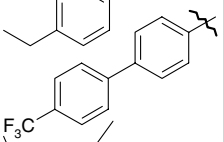
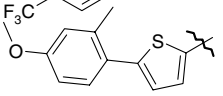
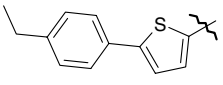
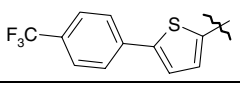
Scheme 1. Reagents and conditions: (a) di-*tert*-butyl dicarbonate, TEA, DCM, rt, 2 h, quantitative; (b) 10% Pd/C, ammonium formate, EtOH, Δ_R, 2 h, 76% (**3**) and quantitative (**5**); (c) 4-nitrophenyl chloroformate, TEA, THF, 0 °C to rt, 2 h, 68%; (d) ethyl trifluoroacetate, TEA, MeOH, rt, 12 h, quantitative; (e) TEA, DMF, 90 °C, 2.5 h, 66%.



Scheme 2. Reagents and conditions: (a) TFA, DCM, rt, 1 h, quantitative; (b) 5-bromo-2-thiophene carboxylic acid, EDCI, HOBt, TEA, DCM, rt, 18 h, 85%; (c) potassium carbonate, 10% aqueous EtOH, 80 °C, 16 h, 71–95%; (d) 4,4-dimethylcyclohexanone, $Na(OAc)_3BH$, MeOH, rt, 18 h, 69%; (e) arylboronic acid, $Pd(PPh_3)_4$, toluene, EtOH, 2 M sodium carbonate, 80 °C, 18 h, 38–50%; (f) biarylcarboxylic acid, EDCI, HOBt, TEA, DCM, rt, 16 h, 63–72%; (g) aldehyde or ketone, $Na(OAc)_3BH$ or BH_3–pyridine, MeOH, rt, 12 h, 20–60%.

Table 1. Binding affinities of rAPUs **8a**–**f** toward MCH-R1



Compound	Ar	K_i[a] (nM) (pK_i ± SEM)
8a		5.7 (8.3 ± 0.1)
8b		4.9 (8.3 ± 0.1)
8c		22 (7.7 ± 0.2)
8d		2.2 (8.7 ± 0.1)
8e		21 (7.7 ± 0.1)
8f		60 (7.2 ± 0.2)

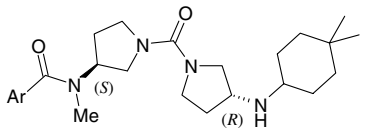
[a] K_i values (n = 2–6).[9]

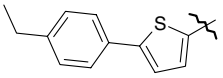
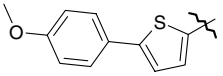
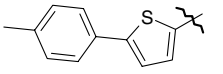
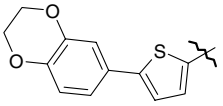
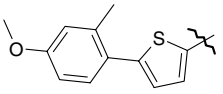
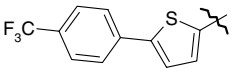
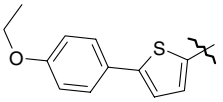
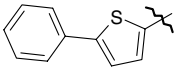
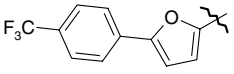
the 2-methyl-4-methoxy (**11e**) and the benzodioxane (**11d**) compounds both of which had K_i = 2 nM. Replacement of the thiophene with furan (**11f** and **12**) led to a 1000-fold loss of potency. This loss in potency could be attributed to one of two features of the furan ring: the increase in hydrophilicity or the decrease in ring size leading to a disfavored orientation of the pendant aryl ring.

Unfortunately, whilst the reversal of the aminopyrrolidine had enabled us to identify compounds with similar potency to the original APU series, it had a detrimental effect on *CYP*2D6 inhibition. rAPU **11f** had a *CYP*2D6 IC_{50} of 900 nM compared to 14 μM for compound **1**. Indeed, with the exception of **11g**, most of the compounds in this series showed low micromolar inhibition of *CYP*2D6 which remained a concern. It was anticipated that brain/plasma ratios would likely be below 1 for our initial compounds and thus higher doses would be required to achieve adequate brain levels for efficacy. The resulting peripheral concentration was expected to reach levels which would significantly inhibit *CYP*2D6.

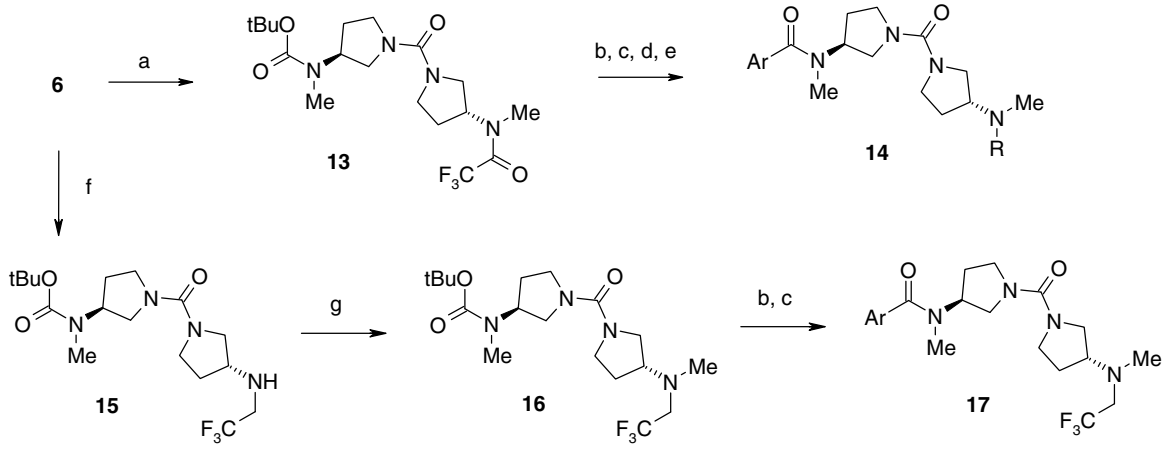
Utilizing the most potent left-hand side, the *p*-ethyl biaryls, a final round of optimization was designed. The issue of *CYP*2D6 inhibition was addressed in an attempt to identify the most suitable compounds for further development (Table 3). Lipophilicity is well documented[10] as being an important contributory factor in cytochrome P450 inhibition. Compounds **11a**–**h** have *c*log *P*s in the range of 5.0–6.8. Our first strategy was to investigate this property by reducing the size of the right-hand side substituent in order to reduce the *c*log *P*. An additional strategy was to investigate tertiary amines. One obvious difference between the APU series, which is devoid of *CYP*2D6 inhibition issues, and the rAPU series is the nature of the terminal amine. In the

Table 2. Binding affinities of rAPUs **11a–h**, **12** toward MCH-R1



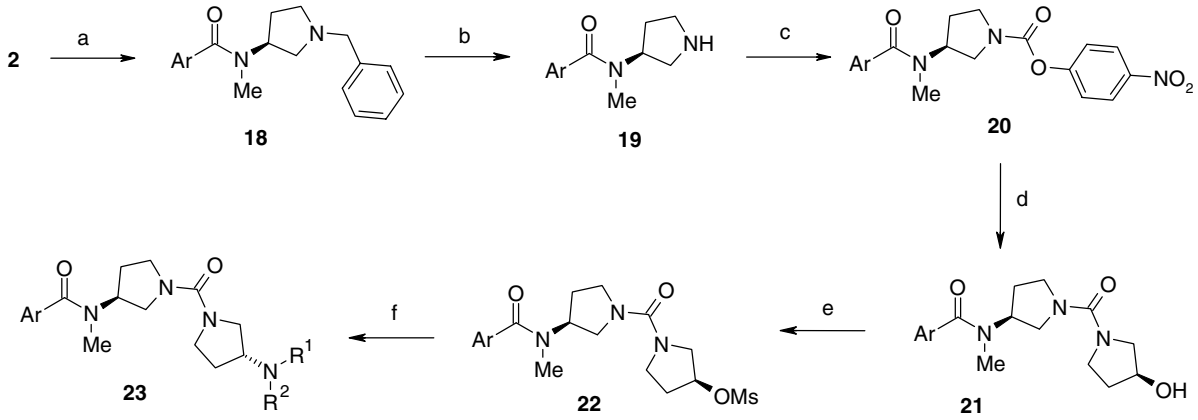
Compound	Ar	K_i[a] (nM) (pK_i ± SEM)	*CYP*2D6 IC_{50}[b] (μM) (pIC_{50} ± SEM)
11a		0.7 (9.2 ± 0.01)	3.2 (5.5 ± 0.1)
11b		0.9 (9.0 ± 0.1)	5.6 (5.3 ± 0.1)
11c		1.6 (8.8 ± 0.04)	3.5 (5.5 ± 0.1)
11d		1.9 (8.7 ± 0.01)	4.5 (5.4 ± 0.1)
11e		2.4 (8.6 ± 0.2)	4.5 (5.4 ± 0.1)
11f		3.7 (8.4 ± 0.00)	0.9 (6.1 ± 0.1)
11g		16 (7.8 ± 0.01)	12.5 (4.9 ± 0.02)
11h		25 (7.6 ± 0.2)	2.3 (5.7 ± 0.01)
12		3600 (5.4 ± 0.1)	ND

[a] K_i values (n = 2–6).[9]
[b] IC_{50} values (n = 2 or 3).[14]



Scheme 3. Reagents and conditions: (a) methyl iodide, potassium carbonate, DMF, 80 °C, 14 h, 98%; (b) TFA, DCM, rt, 1 h, quantitative; (c) biarylcarboxylic acid, EDCI, HOBt, TEA, DCM, rt, 16 h, 50–72%; (d) potassium carbonate, 10% aqueous MeOH, 70 °C, 16 h, quantitative; (e) aldehyde or ketone, $Na(OAc)_3BH$ or BH_3–pyridine, MeOH, rt, 12 h, 20–60%; (f) BH_3, THF, Δ_R, 6 h, 94%, (g) $HCHO_{(aq)}$, BH_3–pyridine, MeOH, rt, 16 h, 65%.

APU series this amine is exclusively tertiary by incorporation into the pyrrolidine ring, whereas in the rAPU series previous analogs had predominantly been secondary amines. It has been reported[11] that the presence of a hydrogen bond donor on the ligand is crucial for binding and inhibition potency of the *CYP*2D6 enzyme.



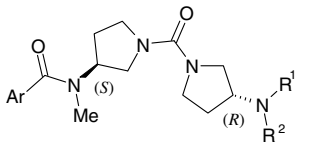
Scheme 4. Reagents and conditions: (a) carboxylic acid, HBTU, HOBt, DIEA, DMF, rt, 18 h; (b) 10% Pd/C, ammonium formate, EtOH, Δ_R, 2 h; (c) 4-nitrophenyl chloroformate, TEA, THF, 0 °C to rt, 2 h; (d) (*S*)-3-hydroxypyrrolidine hydrochloride, TEA, DMF, 80 °C, 4 h, 32% (four steps); (e) methanesulfonyl chloride, TEA, DCM, −40 °C, 1 h, 52–89%; (f) amine, DMA, 60–90 °C, 24 h, 30–50%.

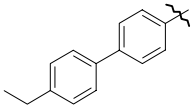
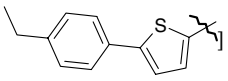
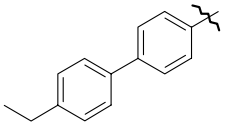
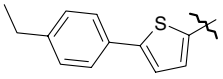
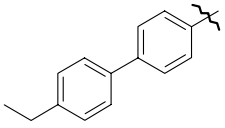
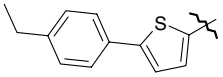
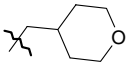
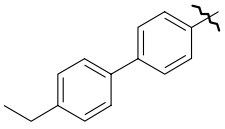
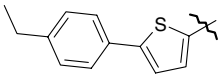
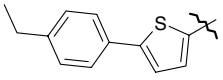
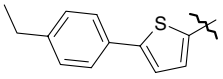
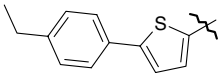
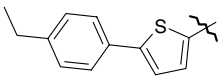
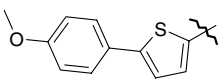
Conversion to a tertiary amine, even though it would still be protonated at physiological pH, may alter the steric environment of the amine enough to prevent this key interaction.

The primary amine **7a** had moderate affinity for the MCH-R1 receptor with a K_i of 18 nM. In addition, it did not inhibit *CYP*2D6. A significant increase in MCH-R1 affinity was observed with a more lipophilic secondary amine. Amines substituted with a saturated ring such as cyclopentyl (**8g**) or pyran (**23a**, **8h**) gave rise to potent analogs, $\leqslant$5 nM. Branched acyclic side chains also had good binding affinity, for example **23e**. Removing the cyclic moiety and replacing it with a less lipophilic substituent led to a drop in potency. For example, the methylamine analog **14c** and the isopropyl analog **8i** were 3- and 5-fold less potent than **8g**, respectively. Extending the chain length by introducing a methylene linker led to a 4-fold drop in potency (compound **8k**). As seen previously, some of the secondary amines exhibited significant *CYP*2D6 activity: the cyclopentyl compound **8g** had a *CYP*2D6 IC_{50} of 2.6 μM. An improvement to 5.1 and 5.8 μM was observed when a pyran, with or without branching, was introduced (**23a** and **8k**, respectively), presumably due to the presence of the more hydrophilic oxygen atom. Small lipophilic substituents also decreased the *CYP*2D6 activity; the isopropyl (**8i**) and methyl (**14c**) secondary amines had IC_{50}s of 9.2 and 18.7 μM, respectively. Modification of the amines to give tertiary methylamines resulted in compounds with greatly improved *CYP*2D6 activity. For example, **23a** and **14a** both had K_is of around 2–3 nM and had *CYP*2D6 IC_{50}s equal to 5.1 and >30 μM, respectively. This is probably due to the removal of a favorable H-bonding interaction. Unfortunately, the tertiary amines were generally less metabolically stable than their parent secondary amines. We rationalized that reducing the basicity of the amine, either by the introduction of a β-oxygen atom or trifluoromethyl substituent, may lead to a drop in *CYP*2D6 activity. These effects have been observed previously;[12] for *CYP*2D6 substrates decreasing the pK_a of an amine from 9.5 to 4.5 by switching a β-methyl to β-trifluoromethyl resulted in a 20-fold drop in the *CYP*2D6 K_m. In our case we were pleased to observe the desired effect of decreasing the *CYP*2D6 activity, but the decreases in basicity also resulted in moderate to large drops in MCH-R1 binding affinity. The morpholine derivative **23d** was a relatively poor MCH-R1 binder compared to its piperidine analog. The branched acyclic analog **23f** retained some potency (14 nM) compared to **23e**. However, incorporation of the hydroxyethyl moiety (compound **8l**) led to a further drop in potency to 32 nM. This could be attributed not only to the lower basicity of the amine, but also to increased hydrophilicity of the side chain. The most dramatic loss in potency was observed with the trifluoroethyl substituted analog **17** which had a binding affinity of 500 nM. This is probably due to the decreased basicity of the amine. The microsomal stability of compounds with ethyl and methoxy left-hand side substitution was lower than for *para*-trifluoromethyl. It was also apparent that there was a decrease in stability for phenyl thiophene containing compounds; identical substitutions had scaled intrinsic clearances of 83 mL/min/kg for phenyl thiophene **8i** versus 22 mL/min/kg for its biphenyl analog **8j**. The *p*-methoxy derivative **23b** also had higher scaled intrinsic clearances (91 mL/min/kg) than **23a** (55 mL/min/kg).

With the availability of a number of compounds having good potency, we addressed the brain penetration issue. One likely explanation for the low brain penetration in this series relative to the APU series is the presence of a secondary amine. Choosing compounds was complicated by the lower microsomal stability of the tertiary amines and thus brain/plasma levels were measured by iv cassette dosing.[13] Results for representative compounds tested are shown in Table 4. Secondary amines typically had low penetration as exemplified by **23a** and **8h**, and the addition of even a methyl group was enough to substantially improve the brain/plasma ratio, as shown with **14a**. In the **14a** cassette, we also measured the appearance of metabolite **23a** in plasma and brain. The amount of **23a** in plasma was significant, 2.4 ng/g compared to 6.7 ng/g for the parent compound, confirm-

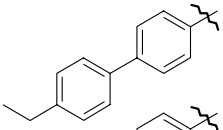
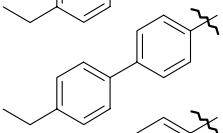
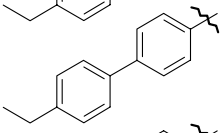
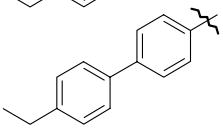
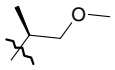
Table 3. Binding affinities toward MCH-R1, CYP2D6 inhibition and predicted oral bioavailabilities of rAPUs



Compound	Ar	R^1	R^2	K_i[a] (nM) ($pK_i \pm$ SEM)	*CYP*2D6 IC_{50}[b] (μM) ($pIC_{50} \pm$ SEM)	Scaled intrinsic clearance (mL/min/kg)
7a		H	H	18 (7.7 ± 0.03)	>30	ND
8g			H	2 (8.8 ± 0.01)	2.6 (5.6 ± 0.1)	60
8h			H	5 (8.3 ± 0.1)	>10	18
8i			H	11 (8.0 ± 0.1)	9.2 (5.0 ± 0.03)	83
8j			H	11 (8.0 ± 0.00)	15.6 (4.8 ± 0.03)	22
8k			H	8 (8.1 ± 0.1)	5.8 (5.2 ± 0.03)	43
8l			H	32 (7.5 ± 0.1)	>30	ND
14a			Me	3 (8.6 ± 0.1)	>30	210
14b		Me	Me	5 (8.3 ± 0.1)	>30	240
14c		Me	H	6 (8.2 ± 0.1)	18.7 (4.7 ± 0.04)	55
17			Me	500 (6.3 ± 0.2)	>30	ND
23a			H	2 (8.6 ± 0.1)	5.1 (5.3 ± 0.02)	55
23b			H	10 (8.0 ± 0.1)	>30	91

(*continued on next page*)

Table 3 (*continued*)

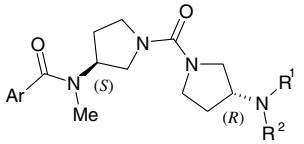
Compound	Ar	R^1	R^2	K_i[a] (nM) ($pK_i \pm$ SEM)	*CYP*2D6 IC_{50}[b] (μM) ($pIC_{50} \pm$ SEM)	Scaled intrinsic clearance (mL/min/kg)
23c			—	4 (8.4 ± 0.04)	10.4 (5.0 ± 0.00)	88
23d			—	69 (7.2 ± 0.1)	>30	ND
23e			H	4 (8.4 ± 0.1)	4.3 (5.4 ± 0.01)	150
23f			H	14 (7.9 ± 0.01)	24.5 (4.6 ± 0.01)	ND

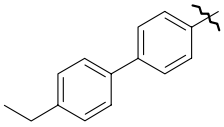
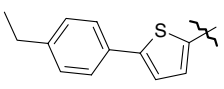
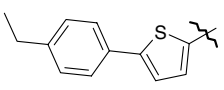
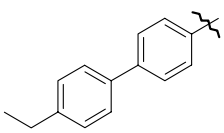
[a] K_i values (n = 2–6).[9]
[b] IC_{50} values (n = 2 or 3).[14]

ing that **14a** was indeed metabolically unstable in rats. Brain levels were also measured and **23a** was found to be 60× less abundant (at 0.35 ng/g) than **14a**. The best compound, **23c**, had comparable brain/plasma ratio and potency to **1** but the microsomal stability was marginal and the plasma and brain levels in rats were both about 4× lower than **1**. We therefore placed this series on hold in favor of generating efficacy and toxicology data from the APU series.

In summary, we have thoroughly explored the scope of the rAPU series as potent and functional MCH-R1 antagonists. Extensive SAR was performed around the biarylcarboxamide, *p*-ethyl biphenyl and *p*-ethyl phenyl thiophene emerged as promising replacements for *p*-trifluoromethylbiphenyl. SAR carried out around the basic nitrogen moiety highlighted some interesting compounds. In particular, tertiary amines from this series exhibited desirable *CYP*2D6 activity and rat brain expo-

Table 4. B/P ratios of rAPUs iv cassette PK



Compound	Ar	R^1	R^2	Average plasma concn[a] (ng/g)	Average brain concn[a] (ng/g)	B/P ratio[a]
8h			H	14.5	5.1	0.4
14a			Me	6.7	21.8	3.4
23a			H	30.5	17.3	0.6
23c			—	6.2	38.3	6.5

[a] Average plasma and brain concentrations and brain/plasma ratios estimated by the cassette dosing procedure.[13]

sure and the best compound was the piperidine derivative **23c**.

Acknowledgments

We are indebted to Mr. John Harman, Mr. Chris DeVore, and Mr. Shawn Ayube for LC–MS support. We also wish to thank Ms. Monica Mistry, Mr. Jason Haelewyn, and Mr. Rajesh Huntley for pharmacology support and Dr. John Saunders for valuable discussions. This work was partly supported by NIH Grant 2-R44-DK059107-02.

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6. (3*S*)-(−)-1-Benzyl-3-(methylamino)pyrrolidine is commercially available from Tokyo Chemical Industry (TCI) America, Portland, Oregon. The e.e. was determined to be >97%.
7. (3*R*)-(−)-1-Benzyl-3-aminopyrrolidine is commercially available from Tokyo Chemical Industry (TCI) America, Portland, Oregon (e.e. 98%).
8. Typical experimental procedure for preparation of rAPUs via mesylate displacement: synthesis of **23c**. To a solution of the mesylate **22** (30 mg, 0.06 mmol) in DMA (0.5 mL) was added piperidine (100 μL, 1 mmol). The reaction mixture was heated at 60 °C for 20 h then cooled to room temperature. The mixture was diluted to 1 mL with methanol and purified by preparative HPLC. Concentration of the pure fractions in vacuo afforded the TFA salt of **23c** (32 mg, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$ + one drop CD_3OD) δ 7.59 (d, 2H, J = 8.1 Hz),

7.48 (d, 2H, J = 8.4 Hz), 7.41 (d, 2H, J = 8.1 Hz), 7.25 (d, 2H, J = 8.4 Hz), 3.20–3.87 (m, 10H), 3.05 (m, 2H), 2.95 (m, 2H), 2.66 (q, 2H, J = 8.1 Hz), 1.63–2.42 (m, 10H), 1.23 (t, 3H, J = 8.1 Hz); MS (m/z) 489.3 $(M+H)^+$.

9. On each assay plate, a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall K_i values were highly reproducible, the standard error of the mean (SEM) being reported. All compounds described were assayed in 2–6 independent experiments.
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13. In the cassette dosing experiments a group of three male rats were dosed with four NCEs and a positive control. Plasma and brain samples were collected at appropriate intervals, processed, and analyzed by HPLC/MS. Calibration curves were generated and were used to quantify concentrations of the NCEs in plasma and brain. B/P ratios were determined and values >1 were presumed sufficient for the NCE to readily cross the BBB.
14. The *CYP*2D6 inhibition assay was carried out in the presence of the fluorescent substrate, AMMC. Quinidine was used as positive control. All compounds described with an $IC_{50} < 30$ μM were assayed in 2 or 3 experiments.