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Identification and characterization of amino-piperidinequinolones and quinazolinones as MCHr1 antagonists

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Abstract—Several potent, functionally active MCHr1 antagonists derived from quinolin-2(1*H*)-ones and quinazoline-2(1*H*)-ones have been synthesized and evaluated. Pyridylmethyl substitution at the quinolone 1-position results in derivatives with low-nM binding potency and good selectivity with respect to hERG binding.
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Melanin concentrating hormone (MCH) is a cyclic 19-membered orexigenic neuropeptide found in the lateral hypothalamus and zone incerta that regulates feeding behavior and energy expenditure in mammals.[1,2] ICV injection of MCH stimulates food intake in rodents[3] causing an increase in body weight,[4] whereas mice lacking the gene encoding MCH are hypophagic, lean and have increased metabolic rates.[5] Furthermore, MCH receptor knockout mice have been shown to be hyperphagic on regular chow yet less susceptible to diet induced obesity and to have reduced fat mass.[6,7] Infusion (ICV) of MCH did not induce food intake or obesity in the receptor knockout mice.[7] These pharmacological data suggest that oral administration of brain-permeable MCHr1 antagonists may provide a novel therapy for obesity. Indeed, recently reported MCHr1 antagonists from several structural types have been reported to show in vivo efficacy in animal models.[8–14] As discussed in earlier communications, aminopiperidine benzamides[11–13] and coumarins[14] have been optimized from screening hits based on an ^{125}I-MCH binding assay. For example, coumarin-based 4-aminopiperidine **1** was identified as a potent MCHr1 antagonist ($IC_{50} = 2$ nM in the binding assay and 28 nM in a functional assay measuring inhibition of MCH-mediated Ca^{2+} release) and proved to be efficacious in rodent weight loss models.[14] However, even the most attractive compounds from these series in terms of both potency and pharmacokinetics required surprisingly high brain and plasma concentrations in order to provide prolonged receptor antagonism in vivo. Thus, for compound **1**, plasma concentrations of the order of 2 μg/mL (5 μM) were necessary in order to provide comparable brain levels leading to sustained weight loss. These high circulating plasma concentrations present a safety challenge in terms of off-target effects. In consequence, improved MCHr1 potency, selectivity, and brain penetration characteristics will be required in order to develop a safe, orally bioavailable anti-obesity therapeutic. In particular, several compounds based on analogs **1** and **2** showed severe cardiotoxicity in anesthetized dogs.[12–14] While the mechanism of these effects has yet to be established, we reasoned that more potent MCHr1 antagonists would provide a better opportunity to achieve an acceptable therapeutic index for efficacy relative to cardiovascular effects. In addition, it was also of interest to screen the compounds in an assay measuring binding to the hERG potassium channel as one predictor of cardiovascular liabilities.[15]

Keywords: Obesity; MCH antagonist; Quinolone; hERG.
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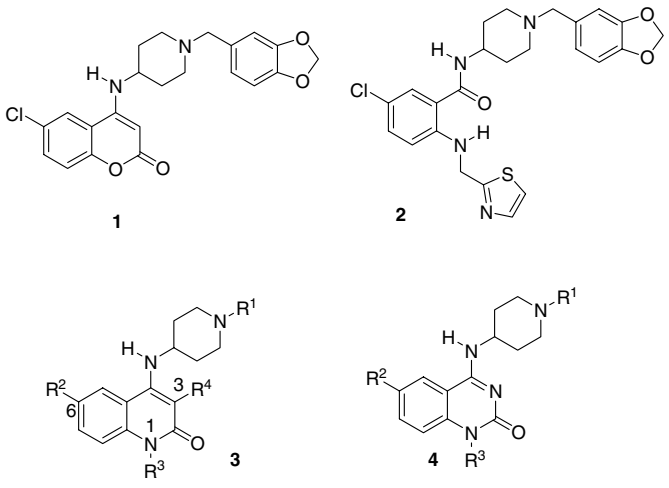
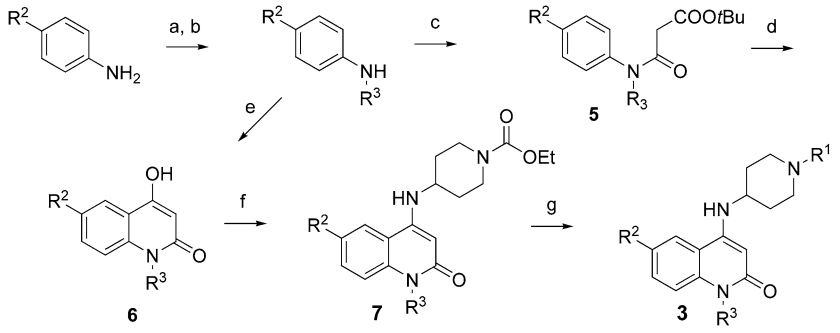


The first compounds described herein are based on the generic structure **3**[16] which has clear similarities to our earlier series in that the quinolone (systematically, 1-alkylquinolin-2(1*H*)-one) core serves as a replacement for the coumarin ring system and also provides a configurationally restrained version of the *ortho*-amino benzamide[13] series. We have developed synthetic routes placing emphasis on derivatives substituted at the 1- and 3-positions thereby exploring space not readily accessed in series **1** and **2**. In addition, we have also prepared related compounds based on the quinazoline-2(1*H*)-one ring system (**4**).

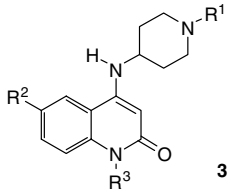
We began by developing synthetic routes to quinolin-2(1*H*)-one derivatives **3** that permit ready variation at the 1-, 3-, and 6-positions. In one such route (Scheme 1), several N-substituted anilines were prepared, coupled to mono-*t*-butylmalonate in the presence of EDCI, and the products, **5**, cyclized to **6** by treatment with P_2O_5.[17] In some instances (R^3 = Me) condensation of the aniline with malonic acid afforded **6** directly. 4-Hydroxyquinolin-2(1*H*)-ones (**6**) were activated toward nucleophilic substitution by conversion to the corresponding triflate and then allowed to react with a protected 4-aminopiperidine to give **7**. Deprotection followed by reaction with the appropriate benzyl or cinnamyl halide afforded **3a**–**3j** (Table 1). 3-Substituted derivatives **3k**–**3o** (Table 2) were synthesized according to Scheme 2. Thus, 6-chloroisatoic anhydride was treated with malonic ester in the presence of base to give 4-hydroxyquinolin-2(1*H*)-one **8a**. Conversion to the corresponding tosylate followed by reaction with 1-piperonyl-4-aminopiperidine and subsequent N-methylation gave **3n**. A similar malonate condensation was conducted with an N-methylated isatoic anhydride and the product treated with pyrrolidine to give **8b**, which was then converted to **3o**. The 3-cyano analogues were obtained via coupling methyl 2-amino-5-chlorobenzoates with cyanoacetic acid followed by cyclization to give **9**. Conversion of **9b** to the corresponding chloride followed by reaction with the appropriate 1-benzyl-4-aminopiperidine afforded **3l** and **3m**. Treatment of **9a** with $POCl_3$ afforded 2,4-dichloroquinoline derivative **10**[18] which underwent nucleophilic displacement with 1-piperonyl-4-aminopiperidine; subsequent acid hydrolysis afforded quinolone **3k**.

Formal replacement of the 3-methine of the 1-alkylquinolin-2(1*H*)-one series with the N-atom affords the quinazoline-2(1*H*)-one series of compounds **4**. The synthesis of these analogs began with the *ortho*-lithiation of *N*-Boc-4-chloroaniline[19] followed by amidation with *t*-butylisocyanate. Upon heating, intramolecular displacement of the adjacent *t*-BuO group took place

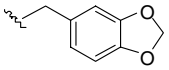
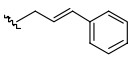
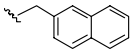
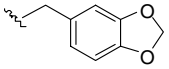
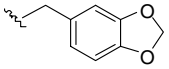
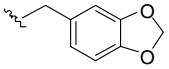
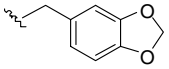
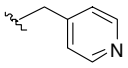
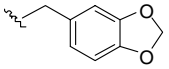
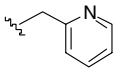
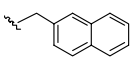
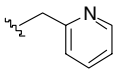
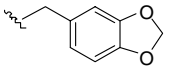


Scheme 1. Reagents and conditions: (a) For R^3 = –CH_2Ar: ArCHO, CH_2Cl_2, AcOH, $NaBH(OAc)_3$, 25 °C, 3 h, 60–94%; (b) For R^3 = Me: i—EtOCOCl, DIEA, DMAP, THF, 25 °C, 70–85%; ii—$LiAlH_4$, THF, 60 °C, 60–70%; (c) $HOOCCH_2COOtBu$, EDCI, CH_2Cl_2 25 °C, 3 h, 60–85%; (d) P_2O_5, $MeSO_3H$, 100 °C, 15 min, 50–71%; (e) $CH_2(COOH)_2$, $POCl_3$, 25 °C, 16 h, 20–25%; (f) i—NaH, Tf_2NPh, MeCN, 50 °C, 1 h; ii—1-carbethoxy-4-aminopiperidine, MeCN, 100 °C, 7 h, sealed tube, 50–84%; (g) i—HBr, HOAc, sealed tube, 110 °C, 1 h; ii—$ArCH_2Br$, $NaCO_3$, DMF, 25 °C, 16 h, 40–80%.

Table 1. MCHr1 binding affinity and functional activity of quinolin-2(1*H*)-one compounds



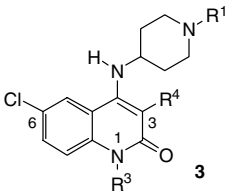
3

Compound	R^2	R^3	R^1	MCH binding IC_{50} (nM)	MCH FLPR IC_{50} (nM)	hERG binding K_i (nM)
3a	Cl	H		4 ± 1	26 ± 2	112 ± 6
3b	Cl	Me		10 ± 2	88 ± 4	30 ± 2
3c	Cl	Me		5 ± 1	100 ± 7	215 ± 8
3d	MeO	Me		3 ± 1	20 ± 3	778 ± 15
3e	CHF_2O	Me		5 ± 2	34 ± 4	>2000
3f	CF_3	Me		34 ± 2	104 ± 21	1800 ± 100
3g	Cl	Bn		0.8 ± 0.1	42 ± 5	280 ± 12
3h	Cl			1.1 ± 0.1	29 ± 5	355 ± 30
3i	Cl			0.9 ± 0.1	55 ± 10	88 ± 7
3j	Cl			1.2 ± 0.2	20 ± 7	550 ± 40

affording **11**. Alkylation of the *N*-1 lactam, followed by acid induced removal of the *t*-butyl group, gave the quinazoline-2,4(1*H*,3*H*)-diones **12**.[20] Treatment of **12** with $POCl_3$ gave the corresponding 4-chloro-compounds which were converted to the final products, **4**, under comparable conditions to those used in the quinolone series. In order to probe conformational effects in the diamine moiety, a bicyclic alternative to the 4-aminopiperidine linker was also investigated (Scheme 4). Hydrogenation of *N*-carbethoxy-4-tropinone (**13**) afforded the *endo*-alcohol, which was converted to the mesylate **14**, then subjected to S_N2 displacement with sodium azide followed by hydrogenation (H_2, Pd/C) to provide the *exo*-amine **15**. The *endo*-stereoisomer **16** was accessed via hydrogenation of oxime **17**.[21] Representative quinazoline-2-(1*H*)-ones **4g-i** (Table 3) were then synthesized from the tropane-based amines **15** and **16** in a manner analogous to Scheme 3.

Compounds were screened in a binding assay for MCHr1 using receptor obtained from human neuronal IMR 32 cells[22] and for functional antagonism of MCH-mediated Ca^{2+} release.[23] Binding potencies of compounds at the hERG potassium channel[24] were also determined[25] and data are compiled in Tables 1–3. In the 1-alkylquinolin-2(1*H*)-one series (**3**) substitutions at the piperidine 1-position and the 6-position of the quinolinone ring led to similar trends as in the coumarin[14] and benzamide series.[12,13] Thus, with chlorine at the 6-position potent MCHr1 binding was observed when the piperidine N-1 substituents were piperonyl (**3a**), cinnamyl (**3b**), and naphthyl (**3c**) with potencies improved slightly compared to their coumarin counterparts[14] (Table 1). In addition to chloro, other small hydrophobic groups at the 6-position such as alkyl (not shown), alkoxy (**3d**), and the fluorinated derivatives **3e** and **3f** conferred high potency. The relative potencies in the functional assay were in good agreement with the binding data. In general, high potency (30–800 nM) in the hERG binding assay was also observed but this was significantly reduced for the latter two compounds.

Table 2. MCHr1 binding affinity and functional activity of 3-substituted quinolin-2-(1*H*)-ones and quinazoline-2-(1*H*)-ones



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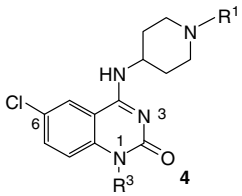

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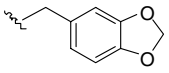
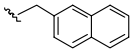
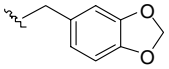
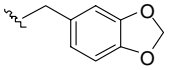
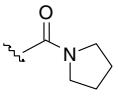
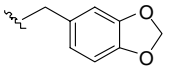
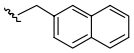
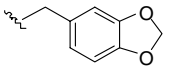
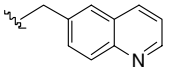
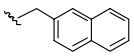
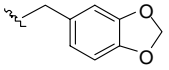
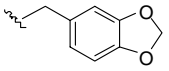
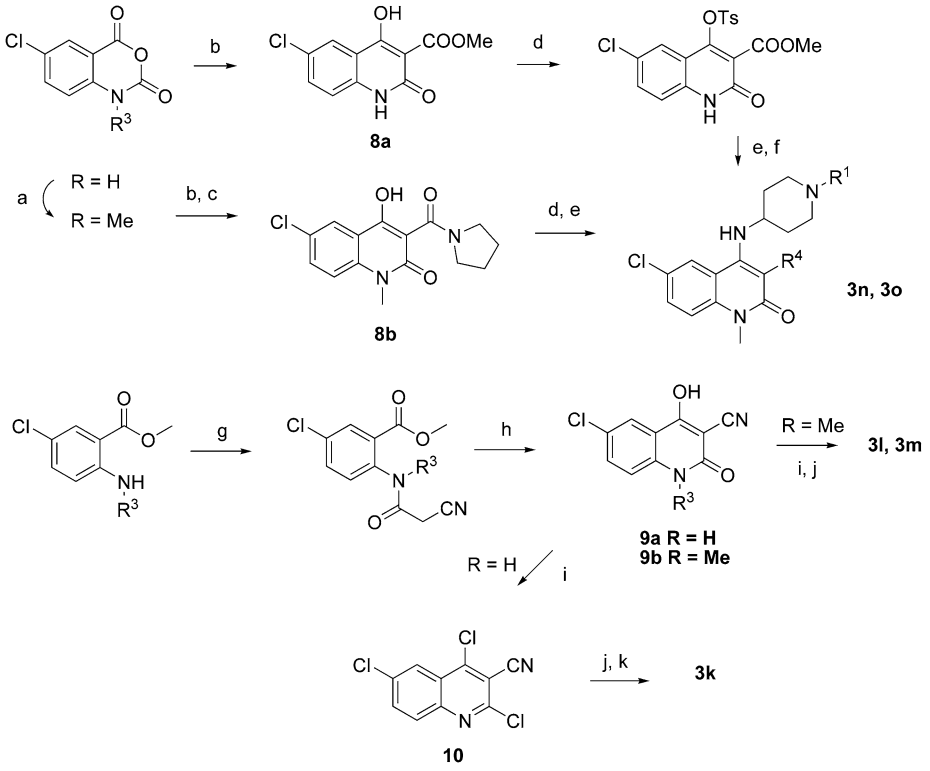
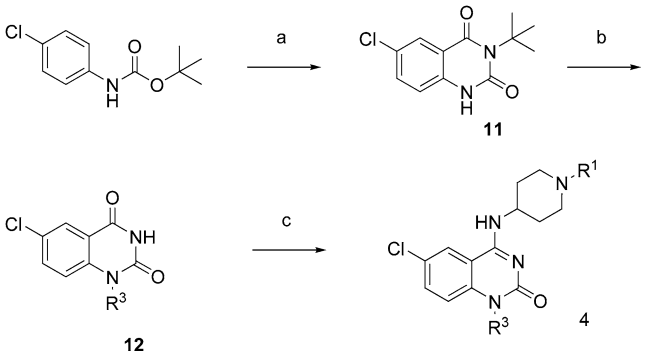
Compound	R^3	R^4	R^1	MCH binding IC_{50} (nM)	MCH FLPR IC_{50} (nM)	hERG binding K_i (nM)
3k	H	CN		15 ± 3	20 ± 1	420 ± 30
3l	Me	CN		12 ± 2	194 ± 116	>2,000
3m	Me	CN		24 ± 4	69 ± 9	>2000
3n	Me	COOMe		97 ± 33	452 ± 39	1440 ± 150
3o	Me			16 ± 7	53 ± 3	>2000
4a	Me			7 ± 2	72 ± 11	160 ± 15
4b	Me			9 ± 1	47 ± 5	360 ± 15
4c	Me			12 ± 2	66 ± 3	1750 ± 100
4d	CH_2CF_3			5 ± 2	62 ± 9	150 ± 5
4e	CH_2CF_3			2 ± 1	29 ± 4	445 ± 30
4f	*n*-Pentyl			12 ± 3	50 ± 12	135 ± 15

Compounds N-methylated at the 1-position (R^3) were found to have comparable potency to the unsubstituted compounds (see for example **3a** and **3c**, Table 1 and **3k** and **3l** of Table 2). Other alkyl groups (not shown) as well as benzylic groups (compound **3g**, for example) were well tolerated. Particularly noteworthy were the pyridyl–methyl moieties which imparted a boost in potency suggesting that these compounds can access an additional receptor binding pocket not available to their coumarin counterparts. These analogs (**3h**–**3j**) all exhibited approximately 1 nM binding affinities but, in general, their functional activity was not improved to the same degree possibly due to impaired cell permeability. In addition, hERG ion channel binding remained unacceptably high (Table 1). Substitution of the 3-position (R^4) with electron-withdrawing groups resulted in approximately 2- to 5-fold losses in MCH1r binding potency and significant lowering of the hERG binding potency provided the quinolone nitrogen was alkylated (Table 2 compounds **3l**–**3n**).

Replacement of the quinolin-2(1*H*)-one ring system with quinazoline-2(1*H*)-one did not adversely affect MCH binding potency (compare, for example, **4a** with **3c**) but the hERG binding profile was not improved. However, substitution of the piperidine N-1 position by a quinolyl group (compound **4c**) resulted in a considerably improved (140-fold) MCH1r/hERG selectivity. At the 1-position, alkyl and fluoroalkyl substituents were well tolerated (for example, compounds **4d** and **4e**). Even compounds bearing aliphatic groups with multiple degrees of freedom such as *n*-pentyl (**4f**) retained low nanomolar MCH binding potency and further illustrate the steric tolerance at this site. In addition, locking the



Scheme 2. Reagents and conditions: (a) MeI, Na_2CO_3, DMF, 25 °C, 16 h, 78%; (b) Dimethylmalonate, NaH, DMF, 120 °C, 3 h, 47–55%; (c) Pyrrolidine, DMF, 130 °C, 1 h, 90%; (d) TsCl, DIEA, MeCN, 25 °C, 75–85%; (e) 1-piperonyl-4-aminopiperidine, DIEA, DMF, 50 °C, 8 h, 60–65%; (f) NaH, MeI, DMF, 25 °C, 8 h, 40%; (g) $HOOCCH_2CN$, EDC, THF, 16 h, 60%; (h) NaOMe, MeOH, 60 °C, 2 h, 70%; (i) $POCl_3$, 90 °C, 1 h, 30–70%; (j) 1-benzyl-4-aminopiperidine, DIEA, DMF, 75 °C, 2 h, 55–60%; (k) 1 N HCl, THF, 70 °C, 24 h, 50%.

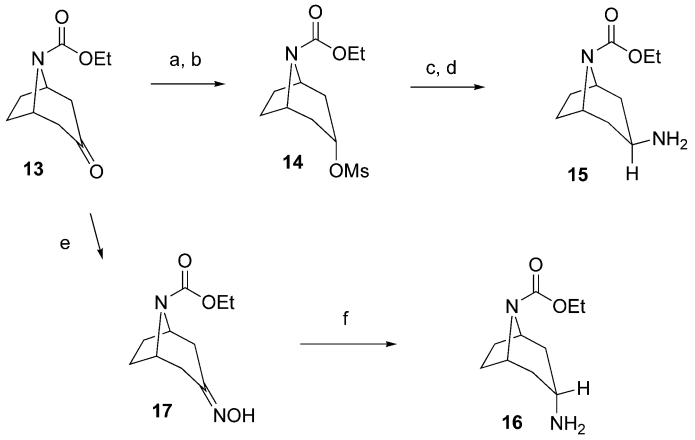


Scheme 3. Reagents and conditions: (a) *t*-BuLi, THF, −78 to −20 °C, 2 h; then *t*BuNCO, −20 to 70 °C, 12 h, 98%; (b) i—RI, NaH, DMF, 25 °C, 3 h, 80–90% or CF_3CH_2OTf, $CsCO_3$, MeCN, 50 °C, 48 h, 50–65%; ii—HBr, HOAc, 25 °C, 3 h, 95%; (c) i—$POCl_3$, DIEA, 100 °C, 4 h, 45–75%; ii—1-carbethoxy-4-aminopiperidine, Et_3N, DMF, 50 °C, 12 h, 25–55%; iii—HBr, HOAc, 100 °C, sealed tube, 2 h; iv—$ArCH_2Cl$, Et_3N, DMF, 50 °C, 24 h, 55–80%.

conformation of the diamine linker in the case of bridged diamines **4g**–**4i** afforded potent compounds in the binding and functional assays but again no significant improvements in hERG selectivity could be achieved. The *exo*-stereochemical configuration resulted in the more potent compounds in the MCHr1 binding assays (compare **4g** and **4i**, Table 3).

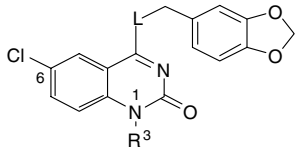
Pharmacokinetic analyses of representative members of the 1-alkylquinolin-2(1*H*)-one and quinazoline-2(1*H*)-one series were conducted (Table 4). While oral administration of compounds **3h** and **3m** from the former series and **4b** from the latter provided comparable plasma levels to those achieved by related benzamide[12,13] and coumarin[14] compounds, brain permeation was considerably lower. Compound **3h** exhibits the highest brain levels but the C_{max} value is 2-fold lower than that of efficacious coumarin **1** and the AUC is some 6-fold lower. The low brain/plasma AUC ratios (0.29 or less) imply that any efficacious dose will result in a considerable concentration of compound remaining in the systemic circulation with consequent cardiovascular risk. Hence, despite their superior potencies in vitro, it is unlikely that this compound class will provide anti-obesity agents with an improved efficacy and/or safety window compared to the benzamide and coumarin derivatives that we have described previously.[26]

In conclusion, quinolone and quinazolinone MCHr1 antagonists have been designed, synthesized, and their potency, selectivity, and PK properties determined. Several compounds from these series are considerably more potent than their coumarin[14] and benzamide[12,13] counterparts and in some cases exhibit sub-nanomolar MCHr1 binding potency and >500-fold selectivity with respect to hERG binding. However, these properties are compromised by relatively low MCH1r functional activity and poor brain penetration in the DIO mouse, following oral dosing.



Scheme 4. Reagents and conditions: (a) H_2, RaNi, EtOH, 70 °C, 12 h, 95%; (b) MsCl, Et_3N, CH_2Cl_2, 25 °C, 2 h, 88%; (c) NaN_3, DMF, 100 °C, 5 h, 100%; (d) H_2, Pd/C, MeOH, 1 atm, 12 h, 25 °C, 73%; (e) $NH_2OH{\cdot}HCl$, Na_2CO_3, EtOH, H_2O, 80 °C, 1 h; 80%; (f) H_2, PtO_2, MeOH, HOAc, 25 °C, 50 psi, 48 h, 75%.

Table 3. MCHr1 binding affinity and functional activity of quinazoline-2(1*H*)-ones with bicyclic amine linkers



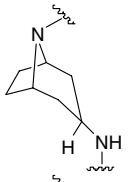
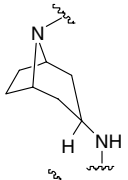
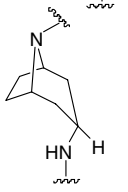
Compound	R^3	Diamine	MCH binding IC_{50} (nM)	MCH FLPR IC_{50} (nM)	hERG binding K_i (nM)
4g	Me		9 ± 4	53 ± 6	540 ± 50
4h	CH_2CF_3		4 ± 1	31 ± 7	120 ± 20
4i	Me		68 ± 24	136 ± 6	310 ± 20

Table 4. Pharmacokinetic parameters of some quinolin-2(1*H*)-one and quinazoline-2(1*H*)-ones dosed at 10 mg/kg in DIO mice

	3h	**3m**	**4b**
Plasma AUC (μg h/ml)	2.1	5.9	5.1
Brain AUC (μg h/ml)	0.73	0.62	0.65
Plasma $T_{1/2}$ (h)	2.3	1.9	1.6
Brain $T_{1/2}$ (h)	2.5	2.0	1.9
Plasma C_{max} (μg/ml)	0.68	1.6	2.2
Brain C_{max} (μg/ml)	0.2	0.16	0.23
Brain/plasma AUC	0.29	0.11	0.13

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22. Displacement of [^{125}I]-MCH from MCHr1 expressed in IMR-32 (I3.4.2) cells (MCH binding $K_d = 0.66 \pm 0.25$ nM, $B_{max} = 0.40 \pm 0.08$ picomol/mg).
23. Inhibition of MCH-mediated Ca^{2+} release in whole IMR-32 cells (MCH $EC_{50} = 62.0 \pm 3.6$ nM). All values are mean values ± SEM and are derived from at least three independent experiments (all duplicate).
24. HEK-293 cells that were stably transfected with hERG cDNA were obtained according to Zhou, Z.; Gong, Q.; Ye, B.; Fan, Z.; Makielski, J. C.; Robertson, G. A. *Biophys. J.* **1998**, *74*, 230. Membrane homogenates were prepared from cell pellets, suspended in 50 mM Tris–HCl buffer (pH 7.4) containing 10 mM KCl and 1 mM $MgCl_2$, and centrifuged at 4 °C.
25. The hERG binding assay was conducted as described in: (a) Finlayson, K.; Sharkey, J. In *Optimization in Drug Discovery*; Yan, Z., Caldwell, G. W., Eds.; Humana Press: Totowa, NJ, 2004; pp 353–368; (b) Diaz, G. J.; Daniell, K.; Leitza, S. T.; Martin, R. L.; Su, Z.; McDermott, J. S.; Cox, B. F.; Gintant, G. A. *J. Pharmacol. Toxicol. Methods* **2004**, *50*, 187.
26. It is unclear why these compounds exhibit less desirable brain permeation properties than earlier series. For the compounds listed in Table 4, the respective calculated *S*log*P* and polar surface areas are **3h** (5.1, 67.8), **3m** (3.8, 77.8), and **4b** (3.9, 66.4). These properties are not atypical for compounds that pass the blood–brain barrier. Moreover, compounds **3h**, **3m**, and **4b** were found to have moderate to high permeabilities in PAMPA and Caco assays (with no evidence that the compounds are efflux substrates). It did not prove possible to conduct receptor occupancy experiments in vivo. Thus, it is unclear why such high total brain concentrations of MCH1r antagonists, relative to in vitro potency, are required in order to observe efficacy[12–14] though high binding to brain lipids is one possibility.