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Synthesis of functionalized 1,8-naphthyridinones and their evaluation as novel, orally active CB1 receptor inverse agonists

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Abstract—Synthesis, SAR, and binding affinities are described for a new class of 1,8-naphthyridinone CB1 receptor specific inverse agonists. Food intake, knockout mouse, and pharmacokinetic evaluation of **14** indicate that this compound is an effective orally active modulator of CB1.
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Modulation of the cannabinoid receptor 1 (CB1; predominantly found in the central nervous system) has been demonstrated to have powerful effects on feeding behavior in both humans and other animals.[1] CB1 agonists such as Δ^9-THC stimulate food intake, while CB1 inverse agonists such as SR141716A[2] suppress food intake demonstrating the utility of CB1 inhibition for the treatment of obesity.[3]

Our interest in the discovery and development of novel therapeutics for obesity prompted a high-throughput screen of the Merck chemical collection. This effort led to the discovery of compound **1** which exhibited relatively high-affinity binding to cloned human CB1 with an IC_{50} value of 526 nM (Fig. 1).

We recently described medicinal chemistry optimization of screening lead **1** to afford the diaryl pyridine derivative **2** which achieved a greater than 400-fold improvement in CB1 binding affinity.[4] Unfortunately, the high in vitro potency of **2** did not equate with high efficacy in vivo, which was a consequence of its poor CNS

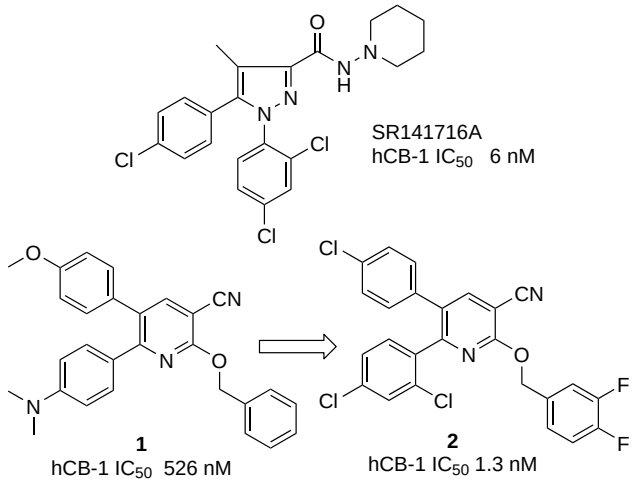

Figure 1. SR141716A and early Merck leads.

Keywords: Cannabinoid; CB1; Inverse agonist; Anorexigenic; Obesity; CB2.
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exposure (brain to plasma ratio = 0.03–0.26 at 0.25–4 h post-dosing).

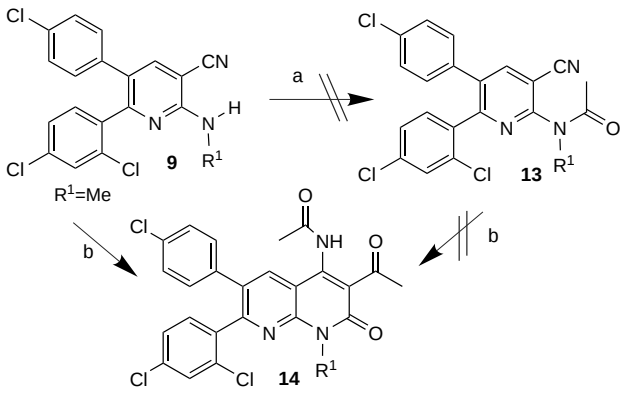
We theorized that improved pharmacokinetic properties might be achieved by alteration of certain physical properties for compounds in this series (e.g., the high log *D* value).[5] One goal therefore became to reduce the lipophilicity of the series. From earlier work it appeared that the biaryl pyridine core was fairly well optimized for potency, but that there was considerable flexibility to alter the substitution pattern on the *b*-face of the pyridine ring to thereby affect the pharmaceutical properties of the series.

The presence of the 3-cyano group on the pyridine would provide the framework to generate an additional fused ring to the pyridine. It was hoped that heterobicyclic systems would maintain good potency but would provide more drug-like physical properties. We first examined the 1,8-naphthyridinone core system.[6] Additional heterobicyclic systems will be the subject matter of future publications.

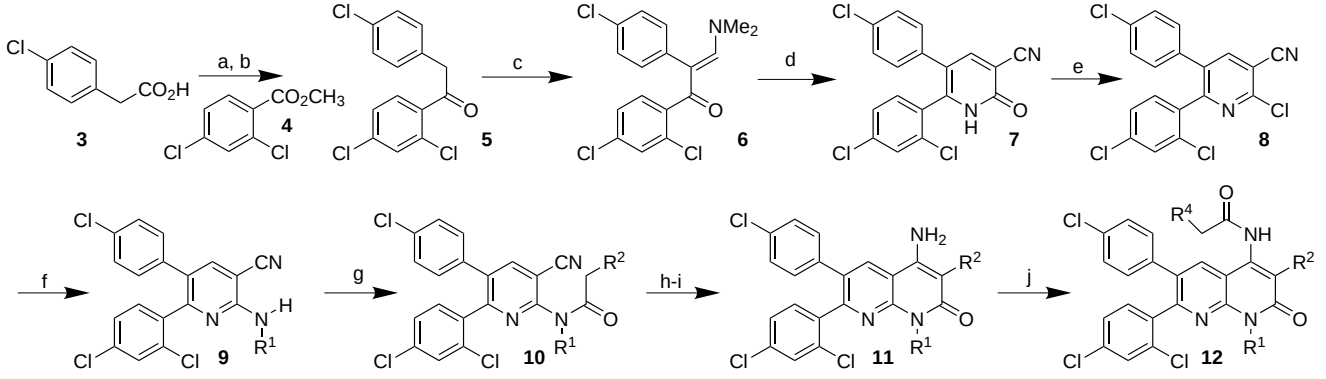
Scheme 1 illustrates the general synthesis of these compounds.[7] The dianion generated from acid **3** added to ester **4** with loss of CO_2 afforded ketone **5**. Condensation of **5** with dimethylformamide dimethylacetal afforded vinylogous amide **6**, which in turn reacted with cyanoacetamide under basic conditions to generate the 2-pyridone **7**. The 2-chloropyridine **8** was prepared by chlorination of **7** with $POCl_3$ and then treated with various amines to generate the 2-aminopyridines **9**. Substituted 2-aminopyridines can often be quite difficult to acylate and this was indeed the case with compounds of general structure **9**. When **9** was exposed to acid chlorides at room temperature, the reactions failed to provide the desired 2-acyl pyridines in acceptable yields. However, deprotonation of the 2-amino group with MeMgBr first, followed by exposure to various acid chlorides, allowed rapid (<1 h) formation of the 2-acyl derivatives at room temperature. When the R^2 group on the newly installed acyl substituent was a leaving group such as a chlorine, a nucleophilic displacement could be carried out providing additional diversity. Exposure of the 2-acyl pyridine to a strong base such as NaH or LiHMDS allowed annulation of the new ring to the pyridine core providing the 4-amino-1,8-naphthyridinone **11**. The amino group could be further acylated with acetic anhydride at elevated temperature or by treatment with various acid chlorides to give **12**.

That the 2-aminopyridine was unreactive to acetylation is clearly shown in Scheme 2. When **9** (R^1 = methyl) was treated to a large excess of Ac_2O (co-solvent) in pyridine at 80 °C for 18 h, no reaction was observed. When 1.2 equiv of DMAP were added and the reaction was run at 85 °C, the 2-acetyl pyridine **13** was still not formed in significant quantities. Instead, an unexpected derivative was formed in an unoptimized 50% isolated yield. It was determined that the reaction had provided an unprecedented ring annulation process allowing novel construction of 1,8-naphthyridinone **14** directly from the aminopyridine.[8]

The reaction appears to be general for substrates of general structure **9** allowing the preparation of various *N*-substituted analogs quite readily. It is noteworthy that when the 2-acetyl pyridine **13** (R^1 = Me) is treated to the above reaction conditions, no significant formation of **14** is observed.



Scheme 2. Reagents and conditions: (a) Ac_2O, Pyr, 80 °C; (b) Ac_2O, Pyr, DMAP, 85 °C, 50%.



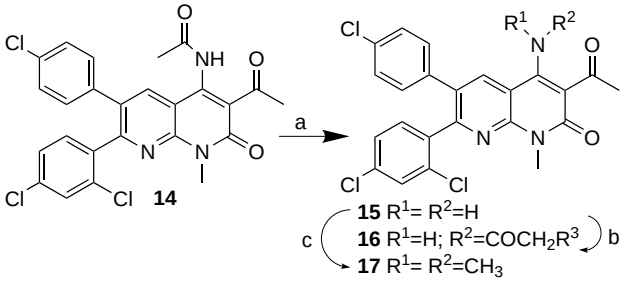
Scheme 1. Reagents and conditions: (a) $NaN(TMS)_2$, THF, −20 °C; (b) ester **4**, 79%; (c) $(CH_3)_2N(OCH_3)_2$, DMF, 90 °C, 98%; (d) 2-cyanoacetamide, DMF, $NaOCH_3$, 95 °C, 90%; (e) $POCl_3$, reflux, 81%; (f) $R^1CH_2NH_2$ (R^1 = alkyl or aryl), THF, 25–65 °C, 90–100%; (g) MeMgBr, THF, then $ClC(O)CH_2X$, 67–93%; (h) optional nucleophilic displacement, DMF, KI, 64–100%; (i) NaH or $LiN(TMS)_2$, THF and/or DMF, 0–25 °C, 73–85%; (j) Ac_2O, 85 °C, or $ClC(O)CH_2R^4$, 64–79%.

The most expeditious way to explore the effect of varied substitution at the 4-position on CB1 binding from analog **14** was by cleavage of the *N*-acetamide group. This could be readily accomplished via treatment with TFA in aqueous ethanol at elevated temperature. The 4-aminonaphthyridinone was readily functionalized by acylation or alkylation (Scheme 3).

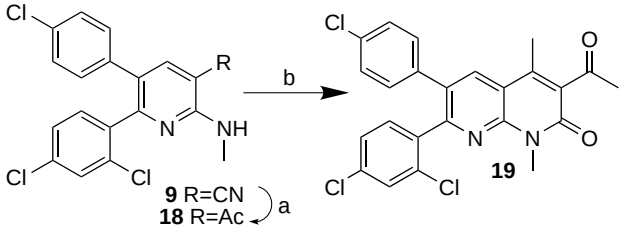
Alternatively, the nitrogen at this position could be replaced with carbon by changing the nature of the ring annulation step (Scheme 4). Instead of ring annulation by attack on a cyano group which forms an amino-substituted ring, aldol condensation on a ketone would leave a methyl group. Conversion of the 3-cyano to an acetyl group was carried out via treatment of two equivalents of MeMgBr on intermediate **9** followed by hydrolysis in aqueous HCl. Subsequent exposure of ketone **18** to similar reaction conditions to produce **14** afforded 4-methylnaphthyridinone **19** in 59% yield.

In the hope of simplifying the general structure it was of interest to prepare naphthyridinones that were unsubstituted on the nitrogen atom. Unfortunately, direct cyclization of 2-*N*-acyl pyridines (**10** $R^1 = H$) was not possible as deacylation usually occurred after exposure of these derivatives to strong bases such as NaH. Scheme 5 shows that by utilizing a 2,4-dimethoxybenzyl-protecting group the 1,8-naphthyridinone system could be generated as above and deprotected in neat trifluoroacetic acid.

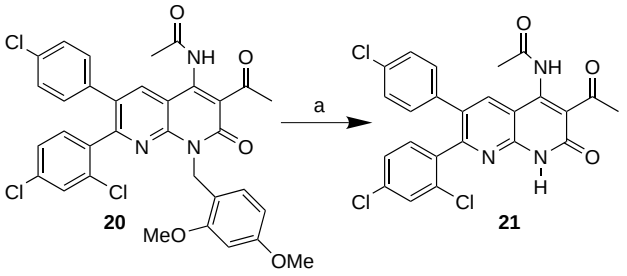
Binding affinities were determined using a standard protocol.[9] Table 1 summarizes the SAR for the 1,8-naphthyridinone compounds. Most potent compounds showed high selectivity for CB1 over CB2. All naphthyridinone compounds tested were functional inverse



Scheme 3. Reagents and conditions: (a) TFA, EtOH, H_2O, 130 °C, 77%; (b) $ClC(O)CH_2R^3$, 11–55%; (c) NaH, THF, MeI, 0–25 °C, 16%.



Scheme 4. Reagents and conditions: (a) 2 equiv MeMgBr, THF, 50 °C, 78%; (b) Ac_2O, Pyr, DMAP, 90 °C, 59%.



Scheme 5. Reagents and conditions: (a) TFA, rt, 38%.

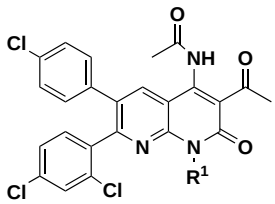
agonists. High potency (IC_{50} < 10 nM) for CB1 binding could be obtained for a variety of functional groups at R^1 other than hydrogen. Typically as the bulk of the group at this position increased so did the CB1 potency. Alkyl and aryl groups had similar potencies as was the case for either isobutyl **22** or benzyl **23** (both 2.3 nM CB1). While ethers such as methoxy **24** or tetrahydropyranyl **25** were well tolerated, a sixfold decrease in potency was observed when R^1 contained a hydroxyl group **26**. When R^1 was a methyl group, the best CB1 potency was observed when R^2 was *N*-acetyl **14** at 7.5 nM CB1, however potency decreased 15-fold (120 nM) for the free amino derivative **15**. The potency remained similarly uninspiring (96 nM) when the amino at R^2 was dialkylated **17**. Finally, when the polar amine function at R^2 was replaced by methyl **19**, we saw a modest improvement in potency to 55 nM. When R^1 is methyl, an acetyl group at R^3 **14** was optimal.

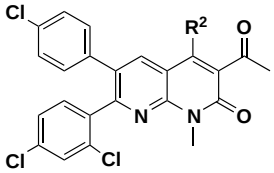
Several compounds (**14** and **22**–**25**) that had been tested in the CB1 assay in vitro were shown to be quite interesting as they had high potency (CB1 IC_{50} = 2.3–7.5 nM) and good selectivity for CB1 over CB2 (between 270- and 2700-fold). In addition to its good CB1 potency, **14** also had the lowest lipophilicity[10] and molecular weight which we viewed as distinct advantages in compounds that must be brain penetrant. Compound **14** was therefore selected for an evaluation of its effects on food intake and body weight changes in diet-induced obese (DIO) rats fed ad libitum on a moderate high fat, high sucrose diet (Table 2).

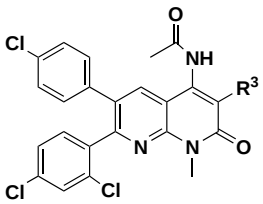
The CB1 inverse agonist **14** was tested at 0.3, 1, or 3 mg/kg, PO.[11] Compound **14** inhibited feeding in a dose-dependent manner. At 3 mg/kg it decreased cumulative food intake from 2 h post-dosing, with an overnight food intake of 6.9 g (67.9% suppression; $p = 0.0009$ vs vehicle treated controls). Compound **14** significantly decreased overnight body weight gain compared to vehicle treatment at all dosing levels with an MED for body weight effects of 0.3 mg/kg (6 g) relative to vehicle treated controls (12 g).

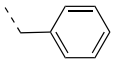
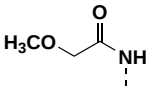
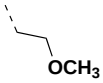
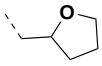
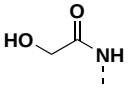
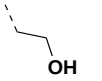
In order to verify that the observed anorexigenic effects were mediated through actions at CB1, an overnight food intake study[12] was conducted with CB1-deficient (*Cnr1*−/−) mice. Based upon a dose–response study conducted in C57BL/6 wild-type mice,[13] ad libitum fed 14–16-week-old male mice (*Cnr1*+/+, $n = 10$; *Cnr1*−/−, $n = 9$) were dosed orally with **14** at 10 mpk (as per the

Table 1. Structures and binding affinities (CB1; CB2) expressed as IC_{50} (nM)







Compound	R^1	CB1; CB2	Compound	R^2	CB1; CB2	Compound	R^3	CB1; CB2
22		2.3; 6200	**14**		7.5; 4100	**14**		7.5; 4100
23		2.3; 5100	**27**		24; 3200	**29**		15; 5400
24		3.7; 1800				**30**		20; 2500
25		5.2; 1400	**28**		46; 3600	**31**		24; 1100
14	CH_3	7.5; 4100	**19**	CH_3	55; 1400	**32**	CH_3	26; 1500
26		32; 1500	**17**		96; 1400	**33**	CN	35; 720
21	H	77; 1400	**15**	NH_2	120; 1100	**34**	H	110; 380

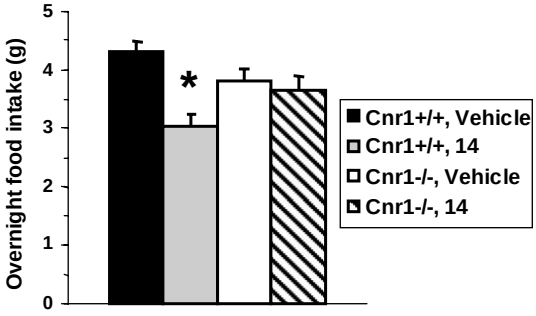


Figure 2. Overnight food intake with wild-type and CB1-deficient mice.

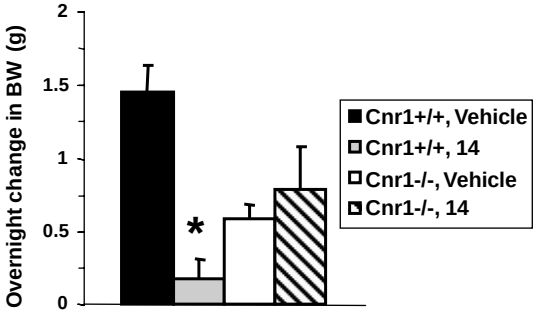


Figure 3. Overnight body weight change with wild-type and CB1-deficient mice.

wild-type mice study). Compound **14** significantly ($p < 0.001$) inhibited 2 h food intake (~49% reduction), overnight (18 h) gains in body weight (~88% reduction; Fig. 3) in wild-type (*Cnr1*+/+) mice, and had no significant effect on any of these parameters in *Cnr1*−/− mice. These data demonstrate that the anorectic actions of **14** are CB1 mechanism-based (Figs. 2 and 3).

With these encouraging results the pharmacokinetic profile of **14** was evaluated in Sprague–Dawley rats, C57BL/6 mice, beagles, and rhesus macaques. Select parameters are summarized in Table 3.

Table 2. Rat food intake/body weight change overnight (18 h) for **14**

Dose (mg/kg)	Δ Body weight (g)	% FI suppression
Vehicle	+12 ± 1	—
0.3	+6 ± 2 ($p = 0.049$)	22 ± 9 ($p = 0.077$)
1.0	+1 ± 2 ($p = 0.002$)	39 ± 13 ($p = 0.077$)
3.0	−4 ± 2 ($p = 0.0003$)	68 ± 14 ($p = 0.0009$)

Table 3. Pharmacokinetic profile of **14**

Animal	*F* (%)	Cl*p* (mL/min/kg)	$t_{1/2}$ (h)
Rat	93	12	>8
Mouse	70	13.9	>8
Dog	100	4.4	>24
Monkey	96	1.4	22

As expected from the strong body weight effects observed in rats, **14** had high bioavailability in this species (93%). Moreover, the rat brain to plasma ratio at 0.25 h was 0.9 and 2.7 at 4 h post IV dosing, indicating that this compound was highly brain penetrant. High bioavailability across all four species was observed. Drug clearance was low in all species resulting in robust half-lives.

In summary, compound **14** is a novel, highly potent, and specific inverse agonist of the CB1 receptor. Oral administration of compound **14** potently suppressed food intake and overnight body weight increases in a diet-induced obese rat model of food intake, and the anorexigenic activity of compound **14** is consistent with its modulation of the CB1 receptor signaling system. The potency, oral efficacy, and excellent pharmacokinetic properties observed for compound **14** clearly demonstrate its utility as a pharmacological tool to evaluate the potential for inhibition of CB1 receptors for the treatment of obesity. Further SAR studies and pharmacological characterization for CB1 inverse agonists derived from our diaryl pyridine lead will be reported from these laboratories in due course.

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5. The calculated log *D* for this compound is 7.9 (Advanced Chemistry Development Log D software version 6).
6. After this work was completed, Ferrarini et al. described 1,8-naphthyridin-4(1*H*)-one-3-carboxamide derivatives as CB2 selective compounds. Ferrarini, P. L.; Calderone, V.; Cavallini, T.; Manera, C.; Saccomanni, G.; Pani, L.; Ruiu, S.; Gessa, G. L. *Bioorg. Med. Chem.* **2004**, *12*, 1921.
7. For full experimental details, see: Debenham, J. S.; Doss, G. A.; Madsen-Duggan, C. B.; Walsh, T. F. WO Patent 2005047285 A1, 2005.
8. Unambiguous structural confirmation of **14** was achieved by ^{1}H–^{13}C correlation HSQC, HMBC, and NOE NMR experiments.
9. The reported binding results were an average of 2–8 independent determinations (each done in replicate), which were normally within 50% of the average value. For both the binding and functional assay conditions, see: Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A. L.; Mitchell, R. L. *Mol. Pharmacol.* **1995**, *48*, 443.
10. The calculated log *D* for this compound is 3.1 (Advanced Chemistry Development Log D software version 6).
11. Food intake assay protocol: compounds were solubilized in 10% Tween 80 in water. DIO Sprague–Dawley rats were dosed PO (orally) 30–60 min before dark onset. There were six rats in each treatment group. Food cups for each cage were weighed every 5 min to measure food consumption (in an automated food intake system) during the 18 h period following dosing with test compounds. Overnight body weight changes were also measured.
12. C57BL/6 mice were purchased from Taconic Farms. *Cnr1* knockout mice were generated by the laboratory of Dr. Andreas Zimmer, National Institute of Mental Health, NIH (Zimmer, A.; Zimmer, A. M.; Hohmann, A. G.; Herkenham, M.; Bonner, T. I. *Proc. Natl. Acad. Sci.* **1999**, *96*, 5780) and generously provided by him.
13. In support of the knockout mice study, **14** was first evaluated in C57BL/6 wild-type mice. Ad libitum fed 12-week-old male mice ($n = 12$ per group) were dosed orally with 14 at 1, 3, or 10 mpk in a 0.225% methylcellulose/10% Tween 80 vehicle ~30 min prior to dark phase of the light cycle. At the highest dose, **14** significantly ($P < 0.0001$) inhibited 2 h food intake (~49% reduction), overnight food intake (~40% reduction), and overnight (18 h) gains in body weight (~143% reduction).