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A Pyridone Analogue of Traditional Cannabinoids. A New Class of Selective Ligands for the CB_2 Receptor

John W. Huffman,[a,*] Jianzhong Lu,[a] George Hynd,[a] Jenny L. Wiley[b] and Billy R. Martin[b]

[a]*Howard L. Hunter Laboratory, Clemson University, Clemson, SC 29634-1905, USA*
[b]*Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613, USA*

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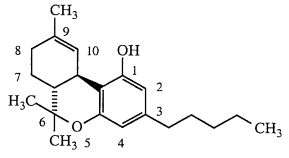
Abstract—A pyridone analogue (**5**) of the potent bicyclic cannabinoid CP 47,497 (**6**) has been synthesized as a model for one conformational isomer of anandamide and to test the hypothesis that an amide carbonyl may serve as a hydrogen bond acceptor in interactions with the CB_1 cannabinoid receptor. Pyridone **5** was synthesized from 6-bromo-2-methoxypyridine (**10**) by palladium catalyzed coupling with 1-pentyne to provide **11**. Catalytic hydrogenation of **11** and hydrolysis to pyridone **13** followed by *N*-alkylation gave 1-propyl-6-pentyl-2-pyridone (**15**). Bromination of **15** gave dibromide **18**, which underwent Heck coupling with cyclohex-2-en-1-one to give enone **19**. Catalytic hydrogenation of **19** gave ketone **20** which was reduced using $NaBH_4$ to alcohol **5**. Reduction of **20** with K-Selectride gave the axial epimer of **5** (**21**). Neither alcohol **5** nor **21** have significant affinity for the CB_1 receptor ($K_i > 970$ nM), but both have moderately high affinity for the CB_2 receptor ($K_i < 60$ nM).

Introduction

At the present time, several structurally diverse classes of compounds have been identified which bind to the cannabinoid CB_1 and CB_2 receptors and which elicit the behavioral effects characteristic of cannabinoids.[1,2] These compounds include inter alia classical cannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC, 1), the principal psychoactive constituent of marijuana, cannabimimetic indoles, pyrroles and indenes, of which WIN-55,212-2 (**2**) is the prototypical example[3] and cannabimimetic fatty acid derivatives, of which the endogenous cannabinoid anandamide (**3**) is typical.[4] These various types of cannabinoids bind to both receptors, and structure–activity relationships (SAR) for each class of compound have been devised.[1–5]



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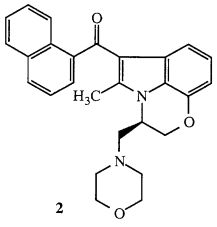

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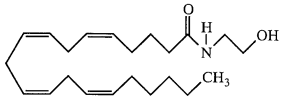

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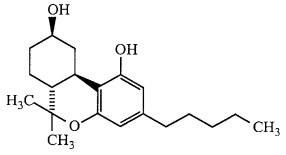

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A number of attempts have been made to develop a pharmacophore to accommodate both the cannabimimetic fatty acid derivatives such as anandamide (**3**) and classical cannabinoids related to Δ^9-THC (**1**).[6–8] Thomas et al. described a model in which the amide carbonyl oxygen of anandamide is superimposed on the pyran oxygen of Δ^9-THC (**1**), the terminal hydroxyl of **3** is aligned with the C-1 hydroxyl of the traditional cannabinoid and the saturated aliphatic chain of anandamide is aligned with the cannabinoid C-3 side chain.[6]

*Corresponding author. Tel.: +1-864-656-3133; fax: +1-864-656-3133; e-mail: huffman@clemson.edu

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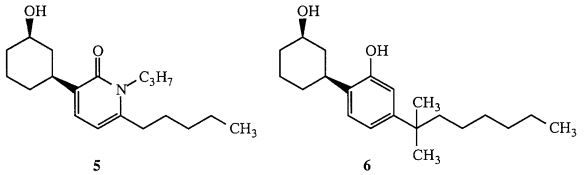
An alternative alignment was described by Tong et al., who employed constrained conformational searching and a CoMFA study which aligned anandamide with a traditional cannabinoid, 9-nor-9β-hydroxyhexahydrocannabinol (HHC, **4**).[7] In this alignment, the five terminal carbons of anandamide correspond to the aliphatic side chain of HHC, the ethanol hydroxyl of anandamide was superimposed on the 9-hydroxyl of **4**, and the oxygen of the amide carbonyl was aligned with the C-1 hydroxyl of HHC. Very recently, Fischera et al. carried out a 3-D-QSAR study to develop a pharmacophore which would accommodate traditional cannabinoids, cannabimimetic indoles and anandamide.[8] The alignment of anandamide and its analogues with Δ^9-THC (**1**) was that suggested by Thomas[6] and the alignment of the indole based cannabinoids with traditional cannnabinoids was similar to that suggested by Huffman and coworkers.[9] All three of these approaches provided results which were consistent with the pharmacology of the compounds included in the studies. In an alternative approach to the interaction of the anandamide and its analogues with the CB_1 receptor, Barnett-Norris et al. employed the conformational memories technique to investigate biologically relevant conformations of anandamide and then carried out docking studies with a computer generated model of the receptor.[10]

By employing a combination of Dreiding molecular models and the program PCModel, an alternative alignment of anandamide and Δ^9-THC (**1**) has been developed.[11] In this alignment, which is similar to that suggested by Tong et al.,[7] the amide carbonyl of anandamide is superimposed upon the phenolic hydroxyl of Δ^9-THC, and the terminal carbons of anandamide are aligned with the alkyl side chain of Δ^9-THC. The amide nitrogen of anandamide is aligned with C-2 of Δ^9-THC (**1**) and the *N*-substituent of anandamide is considered to correspond to a C-2 substituent on the cannabinoid nucleus. A C-2 substituent should have a relatively minor impact on cannabinoid activity based upon observations made some years ago by Edery,[12] and the more recent observation that 2-iodo-Δ^8-THC has moderate affinity for the CB_1 receptor ($K_i = 89$ nM) and shows typical cannabinoid pharmacology in vivo.[13] Also, a conformationally constrained analogue of Δ^8-THC which has an alkyl substituent at C-2 has good affinity for the CB_1 receptor ($K_i = 22.3$ nM).[14]

On the basis of the alignment described above, a pyridone based cannabinoid (**5**) was designed to test this model. Pyridone **5** replaces the aromatic benzenoid ring of Δ^9-THC with a heterocylic ring containing an amide carbonyl and a bicyclic structure designed in analogy to the potent non-traditional cannabinoid CP 47,497 (**6**).[15] The *n*-pentyl side-chain characteristic of Δ^9-THC is appended to the 6-position of the pyridone. Based on the observation that the analogue of anandamide in which the nitrogen substituent was propyl rather than hydroxyethyl had greater affinity for the CB_1 receptor, and greater hydrolytic stability than anandamide,[16] a propyl substituent was employed as the nitrogen substituent. Comparison, employing PCModel, of a minimized structure of pyridone **5** with that of CP 47,497 (**6**) indicated congruence of the salient structural features of the two molecules. In the alignment of traditional cannabinoids and cannabimimetic indoles suggested by Huffman et al. it was considered that the ketonic carbonyl of the indoles corresponded to the C-1 hydroxyl of traditional cannabinoids.[3,9] In this model, the carbonyl group of the indole serves as a hydrogen bond acceptor, and the amide carbonyl of pyridone **5** provides a possible model for the feasibility of this hypothetical alignment of traditional cannabinoids and cannabimimetic indoles.



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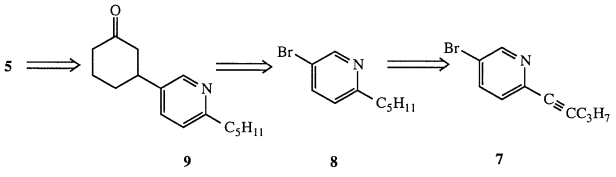
RESULTS

Results

The initial retrosynthetic analysis of pyridone **5** is outlined in Scheme 1. This relatively simple and direct approach employed commercially available 2,5-dibromopyridine as starting material and took advantage of the chemoselectivity of the 2-bromo substituent toward palladium catalyzed coupling with terminal alkynes.[17] Reaction of 2,5-dibromopyridine with 1-pentyne would afford 2-(1-pentynyl)-5-bromopyridine (**7**), selective hydrogenation of which would provide 2-(1-pentyl)-5-bromopyridine (**8**). Halogen metal interconversion of **8**, followed by reaction with 3-ethoxycyclohex-2-en-1-one and mild acid hydrolysis would afford ketone **9**. Conversion of **9** to the appropriate quaternary salt followed by oxidation with potassium ferricyanide would give the target pyridone.[18]

In practice, the palladium catalyzed coupling of 2,5-dibromopyridine with 1-pentyne proceeded smoothly and in good yield to provide 2-(1-pentynyl)-5-bromopyridine (**7**), however the selective hydrogenation of the alkyne in the presence of the aromatic halogen failed.[17,19] Repeated attempts gave only 2-pentylpyridine. In order to circumvent this problem, 2-(1-pentynyl)-5-bromopyridine (**7**) was subjected to halogen metal interconversion and coupling with 3-ethoxycyclohex-3-en-1-one. Catalytic hydrogenation provided ketone **9** and a variety of methods were explored to convert ketone **9** to the corresponding *N*-alkyl 2-pyridone (**5**). However, the result of these efforts were either recovered starting material or products of gross decomposition. A number of alternative approaches



9 8 7

Scheme 1.

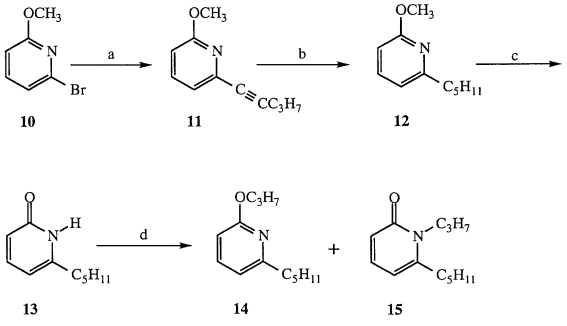
similar in concept to those described above, based upon 2-(1-pentynyl)-5-bromopyridine (**7**) were also unsuccessful.[19]

It seemed probable that the difficulties encountered in converting ketone **9** to the corresponding pyridone were due to steric crowding. An alternative approach was devised in which the oxygen of the pyridone would be introduced early in the synthesis and the alicyclic ring would be introduced subsequently. Accordingly, 2-methoxy-6-bromopyridine (**10**, Scheme 2)[20] was subjected to palladium catalyzed coupling with 1-pentyne to give 2-methoxy-6-(1-pentynyl)pyridine (**11**) in 90% yield. Catalytic hydrogenation of **11** afforded 2-methoxy-6-pentylpyridine (**12**) in excellent (95%) yield. However, attempted directed metalation adjacent to the methoxyl employing *n*-butyllithium was unsuccessful due to the fact that the α-proton of the pentyl group was removed in preference to the aromatic hydrogen.[19]

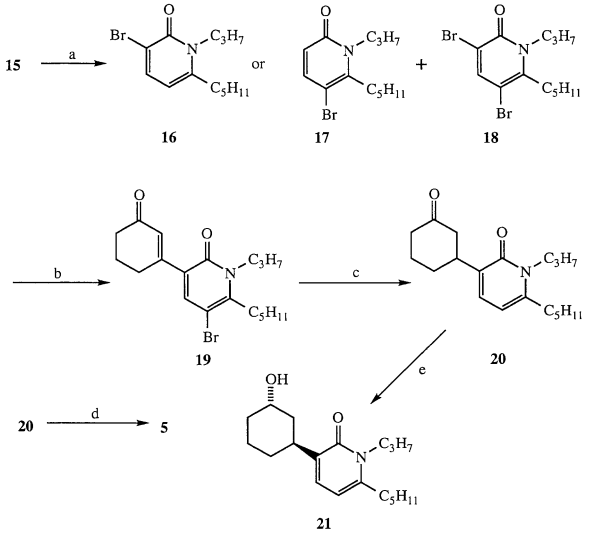
In the successful approach to pyridone **5**, 2-methoxy-6-pentylpyridine (**12**) was hydrolyzed to 1(H)-6-pentyl-2-pyridone (**13**) in 95% yield using hot 48% hydrogen bromide. A number of methods for effecting the *N*-alkylation of pyridone **13** were investigated, all of which afforded *O*-alkylation as the major reaction path. Reaction of **13** with 1-iodopropane in the presence of sodium or potassium carbonate in DMF gave 2-propoxy-6-pentylpyridine (**14**) as the major product, with less than 10% of the desired product, 1-propyl-6-pentyl-2-pyridone (**15**). Similar results were obtained with LDA in THF. A number of years ago, Adams and Schrecker reported that 6-methyl-2-pyridone underwent *N*-alkylation in the presence of sodium ethoxide in benzene.[21] Similar conditions applied to the reaction of **13** with 1-iodopropane afforded **14** and **15** in a ratio of 84 to 13. However, when sodium hydroxide and lithium bromide were used in a one to one ratio in DMF, the ratio of **14** to **15** improved to 73 to 27. It was eventually found that **13** and 1-iodopropane with sodium hydroxide and lithium bromide in a ratio of four to one in DMF gave 1-propyl-6-pentyl-2-pyridone (**15**) in a modest, but acceptable yield of 52% accompanied by 32% of 2-propoxy-6-pentylpyridine (**14**).

It was envisioned that electrophilic bromination of pyridone **15** would provide a mixture of the desired 3-bromopyridone (**16**) and the undesired regioisomer (**17**, Scheme 3). However, reaction of **15** with one equivalent of bromine in acetic acid gave a mixture from which only 16% yield of a monobromo compound could be isolated, in addition to recovered starting material (**15**) and 38% of dibromopyridone **18**. The structure of the monobromo compound was established on the basis of the ^{1}H NMR spectrum in which the aromatic protons appeared as a doublet, $J = 9.6$ Hz, indicating that they were on adjacent carbons. The conclusion that this compound was the undesired 5-bromo isomer (**17**) was based on the observation that there was no NOE enhancement of either aromatic proton when the α-proton of the side chain was irradiated. Acting on the assumption that it would be possible to selectively carry out reaction at one of the two bromine substituents of dibromopyridone **18**, reaction of **15** with excess bromine in acetic acid was carried out to give **18** in 86% yield.

Reaction of dibromopyridone **18** with one equivalent of *n*-butyllithium, at −78 °C followed by quenching with water gave a 16% yield of 1-propyl-6-pentyl-2-pyridone (**15**) and a 32% yield of 5-bromo-1-propyl-6-pentyl-2-pyridone (**17**). Since it was apparent that the 3-bromo substituent was more reactive than the 5-bromo group, the reaction was repeated, however the intermediate organolithium was treated with 3-ethoxycyclohex-2-en-1-one to give a product in 13% yield (Scheme 3), the spectroscopic properties (see Experimental) of which were consistent with those of the desired enone (**19**), however an alternative structure resulting from halogen–metal interconversion at C-5 could not be excluded. The assigned structure (**19**) was confirmed by X-ray crystallography. No attempt was made to optimize the yield for this reaction since it was found nearly simultaneously that palladium catalyzed reaction of dibromopyridone **18** with cyclohex-2-en-1-one under Heck reaction conditions also gave enone **19**, although in only 11% yield.



Scheme 2. (a) 1-Pentyne, $(PPh_3)_2Cl_2Pd/Et_3N/CuI$, 25 °C; (b) H_2(g), 10% Pd(C)/EtOH/20psig; (c) 48% HBr, reflux; (d) 1-iodopropane, NaOH/LiBr/DMSO, 70 °C.



Scheme 3. (a) Br_2/HOAc, 25 °C; (b) *n*-BuLi/THF, −78° C, then 3-ethoxycyclohex-2-en-1-one followed by NH_4Cl, or cyclohex-2-en-1-one, $Na_2CO_3/Pd(OAc)_2$/tri-*o*-tolylphosphine,/DMF; (c) H_2(g), 10% Pd(C)/EtOH/40psig; (d) $NaBH_4$/EtOH, 25 °C; (e) K(*sec*-butyl)$_3$BH/THF, −78 °C to 25 °C, then $NaOH/H_2O_2$/EtOH/H_2O.

This result was surprising since it is known that the Heck reaction usually proceeds with the *syn* addition of the aromatic substrate, to the alkene, followed by *syn* elimination of hydrogen and palladium(II) as the final step.[22] Although this path is not possible with cyclohexenone as a reactant, there is precedent for intramolecular Heck additions to cyclohexenones similar to the intermolecular reaction of **18** with cyclohexenone.[23] It has been noted that in the addition to a conjugated enone, the palladium adduct is actually an enolate which may equilibrate prior to elimination of palladium (II) and hydrogen. A number of experiments were carried out to investigate the potential utility of this reaction and to optimize the yield. It was ultimately found that the yield of enone **19** could be improved to 50% using palladium acetate, tri-*o*-tolylphosphine and sodium carbonate in DMF at 120 °C.

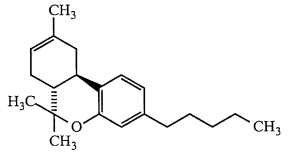
The synthesis of pyridone **5** was completed by hydrogenation of the enone double bond (Scheme 3), which was accompanied by simultaneous hydrogenolysis of the bromine to provide ketone **20**. Sodium borohydride reduction of ketone **20** gave the target pyridone (**5**). The ^{1}H NMR spectrum of this reduction product was consistent with the assigned stereochemistry which showed the carbinol proton as a multiplet at δ 3.73–3.81, consistent with an equatorial hydroxyl group. The axial isomer of alcohol **5** (**21**) was prepared by reduction of ketone **20** with K-Selectride in THF. The ^{1}H NMR spectrum of alcohol **21** showed a carbinol proton as a broadened singlet at δ 4.20, which is consistent with the assigned stereochemistry.

As shown in Table 1, neither alcohol **5** nor **21** have appreciable affinity for the CB_1 receptor, however both alcohols have significant affinity, in the 50 nM range, for the CB_2 receptor. Alcohol **5** was designed as a model compound for one conformation of anandamide (**3**), and also to test the hypothesis that an amide carbonyl group could serve as a surrogate for the phenolic hydroxyl of traditional cannabinoids.[3,9] The distance between the terminal methyl of the pentyl side chain of **5** and the pyridone carbonyl can be brought to 5.74 Å by allowing **5** to assume a minimum energy conformation (although not the global minimum) as determined using PCModel. This distance is somewhat greater than that between the terminal methyl group of anandamide (**3**) and the carbonyl carbon of a conformational isomer of anandamide which has been suggested to be biologically important (4.89 Å).[24] The very weak affinity of alcohols **5** and **21** for the CB_1 receptor suggests that either this conformation of anandamide is not that which is responsible for interaction with the CB_1 receptor or that pyridones **5** and **21** are not appropriate models for the active conformational isomer of anandamide. The observation that alcohol **21** has little affinity for the CB_1 receptor is in itself not surprising in view of the fact that cannabinoids with α-substituents at C-9 are known to have attenuated affinity for the CB_1 receptor.[25]

Table 1. Receptor affinities of pyridones **5** and **21** and Δ^9-THC (**1**)

Compound	K_i (nM)	
	CB_1	CB_2
5	973±105	56±11
21	1054±68	50±4
1	41±2[32]	36±10[33]

Comparison of the structures of CP 47,497 (**6**) and pyridone **5** using PCModel[11] indicate that with the exception of the substitution of an amide carbonyl in **5** for the phenolic hydroxyl of **6** and the presence of a propyl substituent at a position corresponding to C-2 of CP 47,497, there is excellent overlap between the structures of **5** and **6**. However, the affinity of pyridone **5** for the CB_1 receptor (973±105 nM) is many fold less than that of CP 47,497 (**6**, 2.20±0.47 nM),[15] which provides experimental evidence that an amide carbonyl does not substitute for the phenolic hydroxyl of **6** and traditional cannabinoids as a hydrogen bond acceptor. The substitution of a pentyl side chain in **5** for the dimethylheptyl side chain of **6** may also contribute somewhat to the diminished affinity of **5** for the CB_1 receptor. The failure of pyridone **5** to have appreciable affinity for the CB_1 receptor implies that the amide carbonyl of cannabimimetic indoles may also not serve as a hydrogen bond acceptor as suggested previously.[3,9] The fact that several indene derivatives structurally related to the cannabimimetic indoles and which also lack this carbonyl group have high affinity for the CB_1 receptor provides additional evidence that a carbonyl group is not essential for interaction of cannabimimetic indoles and related compounds with the CB_1 receptor.[26]



22

Based on studies employing a mutant CB_1 receptor, Song and Bonner suggested that a hydrogen bonding interaction between the phenolic hydroxyl of traditional cannabinoids such as Δ^9-THC (**1**) and the Pfizer nontraditional cannabinoids such as CP 47,497 (**6**) is important for binding to the CB_1 receptor.[27] This interaction is not important for binding to the CB_2 receptor, inasmuch as methyl ethers derived from analogues of Δ^9-THC (**1**) and several 1-deoxy traditional cannabinoids have high affinity for the CB_2 receptor, but little affinity for the CB_1 receptor.[28] The affinities of pyridones **5** and **21** for the CB_2 receptor (K_i = 56±11 and 50±4 nM, respectively) are similar to that of 1-deoxy-Δ^8-THC (22, K_i = 32±9 nM).[28] As noted above, pyridones **5** and **21** share a general molecular template with CP 47,497 (**6**), which in turn can be related structurally to the traditional cannabinoids, hexahydrocannabinol (HHC, **4**) and Δ^9-THC (**1**). Although pyridones **5** and **21** can be related structurally to cannabinoids **1**, **4** and **6**, their affinities for the CB_1 and CB_2 receptors correlate well with those of 1-deoxy-Δ^8-THC (**22**). Deoxy cannabinoid **22** is closely related structurally to traditional

cannabinoids, such as **1** and **4**, however **22** lacks the phenolic hydroxyl characteristic of traditional cannabinoids with high affinity for the CB_1 receptor. On the basis of these structural relationships and the similarities in affinities for the CB_2 receptor, pyridones **5** and **21** may be considered to be analogues of 1-deoxy-Δ^8-THC (**22**).

Although pyridones **5** and **21** do not constitute viable rigid models for anandamide, they do provide evidence that an amide carbonyl, and by extension the acyl carbonyl of cannabimimetic indoles, does not serve as a surrogate for the phenolic hydroxyl group of traditional cannabinoids in interactions with the CB_1 receptor. However, these pyridones constitute a new class of CB_2 receptor ligands, with affinities similar to that of 1-deoxy-Δ^8-THC (**22**).

Experimental

General

IR spectra were obtained using Nicolet 5DX or Magna spectrometers; ^1H and ^{13}C NMR spectra were recorded on Bruker AC300 or JEOL 500 MHz spectrometers. Mass spectral analyses were performed on a Hewlett-Packard 5890A gas chromatograph with a mass sensitive detector and HRMS data were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. Ether and THF were distilled from Na-benzophenone ketyl immediately before use, and other solvents were purified using standard procedures. Column chromatography was carried out on Universal silica gel (32–63 μ) using the indicated solvents as eluents. All new compounds were homogeneous to TLC and ^{13}C NMR.

2-Methoxy-6-(1-pentynyl)pyridine (11). To a stirred solution of 0.40 g (2.13 mmol) of 2-bromo-6-methoxypyridine in 8 mL of triethylamine at ambient temperature, 0.019 g (0.10 mmol) of CuI was added and the mixture was degassed by passing a stream of argon through the solution for 15 min. After cooling to 0 °C, 0.3 mL (2.24 mmol) of 1-pentyne was added, followed by 0.035 g (0.050 mmol) of dichlorobis(triphenylphosphine)palladium (II). The reaction was stirred for 1 h at 0 °C, allowed to warm to ambient temperature and stirred for 8 h. The mixture was filtered through Celite, and the solvent was removed in vacuo. Chromatography (petroleum ether/ethyl acetate, 30:1) gave 0.34 g (91%) of 2-methoxy-6-(1-pentynyl)pyridine (**11**): ^1H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, $J=7.2$ Hz, 3H), 1.65 (sextet, $J=7.2$ Hz, 2H), 2.42 (t, $J=7.0$ Hz, 2H), 3.94 (s, 3H), 6.65 (d, $J=7.9$ Hz, 1H), 6.98 (d, $J=7.5$ Hz, 1H), 7.47 (t, $J=7.5$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ?13.6, 21.4, 21.9, 53.4, 80.5, 90.2, 110.4, 120.2, 138.3, 140.8, 163.7; MS (EI) *m/z* 174 (100), 146 (35), 130 (12), 117 (17); HRMS calcd for $C_{11}H_{13}NO$: 175.0997, found 175.0998.

2-Methoxy-6-pentylpyridine (12). To a solution of 4.50 g (25.7 mmol) of 2-methoxy-6-(1-pentynyl)pyridine (**11**) in 80 mL of dry EtOH was added 0.5 g of 10% Pd on carbon. The mixture was shaken for 18 h under H_2 at 20 psig. The catalyst was removed by filtration through Celite and the solvent was removed in vacuo to give 4.37 g (95%) of 2-methoxy-6-pentylpyridine (**12**) as a pale yellow oil which was used in the subsequent step without further purification: ^1H NMR (300 MHz, $CDCl_3$) δ 0.82 (t, $J=6.9$ Hz, 3H), 1.20–1.28 (m, 4H), 1.60–1.69 (m, 2H), 2.59 (t, $J=7.8$ Hz, 2H), 3.83 (s, 3H), 6.52 (d, $J=8.2$ Hz, 1H), 6.69 (d, $J=7.2$ Hz, 1H), 7.45 (dd, $J=7.2$, 8.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.5, 29.0, 31.5, 37.8, 53.1, 107.0, 115.0, 138.5, 160.4, 163.6; MS (EI) *m/z* 179 (5), 150 (14), 136 (17), 123 (100).

1(H)-6-Pentyl-2-pyridone (13). A suspension of 0.48 g (2.68 mmol) of 2-methoxy-6-pentylpyridine (**12**) in 12 mL of 48% HBr was heated at reflux for 4 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with ether. The ethereal extracts were washed with water, dried ($MgSO_4$) and the solvent was removed in vacuo to give 0.42 g (95%) of 1(H)-6-pentyl-2-pyridone (**13**) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, $J=6.9$ Hz, 3H), 1.30–1.35 (m, 4H), 1.64–1.72 (m, 2H), 2.61 (t, $J=7.5$ Hz, 2H), 6.06 (d, $J=6.9$ Hz, 1H), 6.41 (d, $J=9.0$ Hz, 1H), 7.37 (dd, $J=6.9$, 9.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.3, 28.1, 31.0, 32.9, 105.0, 116.5, 141.7, 150.4, 165.9; MS (EI) *m/z* 165 (9), 122 (15), 109 (100).

6-Pentyl-2-propoxypyridine (14) and 6-pentyl-1-propyl-2-pyridone (15). To a solution of 0.40 g (2.42 mmol) of 1(H)-6-pentyl-2-pyridone (**13**) in 2 mL of DMSO was added 0.75 g (8.64 mmol) of LiBr, 0.16 g (4.0 mmol) of NaOH and 0.3 mL (3.1 mmol) of 1-iodopropane. The reaction mixture was heated to 70 °C and stirred for 12 h. The mixture was poured into water, extracted with ether, washed with water and brine, dried ($MgSO_4$) and the solvent removed in vacuo. Chromatography (petroleum ether/ethyl acetate, 2.5:1) gave 0.16 g (32%) of 6-pentyl-2-propoxypyridine (**14**): ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, $J=6.4$ Hz, 3H), 1.01 (t, $J=7.2$ Hz, 3H), 1.32–1.35 (m, 4H), 1.70–1.84 (m, 4H), 2.64 (t, $J=7.2$ Hz, 2H), 4.22 (t, $J=7.2$ Hz, 2H), 6.51 (d, $J=8.2$ Hz, 1H), 6.67 (d, $J=7.2$ Hz, 1H), 7.44 (t, $J=7.3$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 10.6, 14.0, 22.4, 22.5, 29.1, 31.5, 37.9, 67.3, 107.1, 114.8, 138.5, 160.4, 163.6; MS (EI) *m/z* 207 (8), 178 (29), 151 (78), 109 (100). Further elution with the same solvent system gave 0.26 g (52%) of 6-pentyl-1-propyl-pyridone (**15**): ^1H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, $J=6.9$ Hz, 3H), 0.99 (t, $J=7.5$ Hz, 3H), 1.36–1.41 (m, 4H), 1.57–1.78 (m, 4H), 2.59 (t, $J=7.8$ Hz, 2H), 3.96 (t, $J=7.8$ Hz, 2H), 5.99 (d, $J=6.9$ Hz, 1H), 6.42 (d, $J=9.0$ Hz, 1H), 7.20 (dd, $J=6.9$, 9.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.3, 13.8, 22.0, 22.3, 28.5, 31.4, 32.9, 45.3, 105.5, 117.6, 138.4, 149.8, 163.6; MS (EI) *m/z* 207 (62), 164 (75), 136 (50), 122 (54), 109 (100); HRMS calcd for $C_{13}H_{21}NO$: 207.1623, found 207.1629.

5-Bromo-6-pentyl-1-propyl-2-pyridone (17) and 3,5-Dibromo-6-pentyl-1-propyl-2-pyridone (18). To a stirred solution of 0.11 g (0.53 mmol) of pyridone **15** in 3.0 mL of glacial acetic acid at ambient temperature was added

dropwise a solution of 0.093 g (0.58 mmol) of bromine in 0.28 mL of acetic acid. The reaction mixture was stirred at ambient temperature for 10 h, poured into saturated aqueous $NaHCO_3$ and extracted with ether. The ether extract was washed successively with water and brine, dried ($MgSO_4$) and the solvent removed in vacuo. The residue was chromatographed (petroleum ether/ethyl acetate, 3:1) to give 0.026 g (17%) of **16** as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J=6.9 Hz, 3H), 1.00 (t, J=7.2 Hz, 3H), 1.37–1.48 (m, 4H), 1.55–1.64 (m, 2H), 1.75–1.86 (m, 2H), 2.79 (t, J=8.1 Hz, 2H), 4.00 (t, J=7.8 Hz, 2H), 6.35 (d, J=9.6 Hz, 1H), 7.35 (d, 9.6 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.2, 13.8, 22.1, 22.4, 27.5, 31.6, 32.8, 47.0, 99.7, 118.8, 142.6, 147.8, 162.2; MS (EI) m/z 287 (33), 285 (31), 244 (10), 242 (10), 189 (100); HRMS calcd for $C_{13}H_{20}BrNO$: 285.0728, found 285.0734. Further elution with petroleum ether/ethyl acetate, 2:1 gave 0.073 g (38%) of dibromopyridone **18** as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J=6.9 Hz, 3H), 1.00 (t, J=7.5 Hz, 3H), 1.35–1.48 (m, 4H), 1.54–1.62 (m, 2H), 1.72 (sextet, J=7.5 Hz, 2H), 2.78 (t, J=7.4 Hz, 2H), 4.04 (t, J=7.8 Hz, 2H), 7.80 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.2, 13.8, 22.1, 22.2, 27.4, 31.6, 32.9, 48.6, 98.5, 113.7, 143.8, 147.6, 158.6; MS (EI) m/z 365 (25), 294 (31), 280 (28), 267 (100); HRMS calcd for $C_{13}H_{19}Br_2NO$: 362.9834, found 362.9826.

3,5-Dibromo-6-pentyl-1-propyl-2-pyridone (18). To a stirred solution of 0.33 g (1.59 mmol) of 6-pentyl-3-propyl-2-pyridone (**15**) in 2.5 mL of glacial acetic acid at ambient temperature was added dropwise 0.59 g (3.5 mmol) of bromine in 3 mL of acetic acid. The reaction mixture was stirred at ambient temperature for 10 h, poured into saturated aqueous $NaHCO_3$ and extracted with ether. The ether extract was washed with successive portions of water and brine, dried ($MgSO_4$) and the solvent removed in vacuo. The residue was chromatographed (petroleum ether/ethyl acetate, 10:1) to give 0.50 g (86%) of **18**, which was identical to the material described above.

5-Bromo-3-(3-oxocyclohex-1-enyl)-6-pentyl-1-propyl-2-pyridone (19), A. To a solution of 0.66 g (1.8 mmol) of dibromopyridone **18** in 4 mL of DMF at ambient temperature was added 0.26 g (2.7 mmol) of cyclohex-2-en-1-one, 0.29 g (2.7 mmol) of anhydrous Na_2CO_3, 0.020 g (0.09 mmol) of $Pd(OAc)_2$ and 0.055 g (0.18 mmol) of tri-*o*-tolylphosphine. The mixture was heated at 120 °C for 12 h under an atmosphere of argon, cooled poured into brine and extracted with ether. The ether extracts were washed with three portions of brine, dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was purified by chromatography (petroleum ether/ethyl acetate 30:1) to give 0.35 g (51%) of **19** as a green solid: mp 83–84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J=6.9 Hz, 3H), 1.02 (t, J=7.2 Hz, 3H), 1.40–1.45 (m, 4H), 1.62–1.76 (m, 4H), 2.05–2.13 (m, 2H), 2.46 (t, J=6.4 Hz, 2H), 2.75 (t, J=5.7 Hz, 2H), 2.82 (t, J=7.8 Hz, 2H), 4.02 (t, J=7.8 Hz, 2H), 6.48 (s, 1H), 7.50 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.3, 13.8, 22.1, 22.3, 22.9, 27.5, 28.2, 31.7, 33.2, 37.5, 47.5, 99.6, 127.8, 128.4, 140.6, 149.2, 157.8, 159.8, 199.9. Anal. calcd for $C_{19}H_{26}BrNO_2$: C, 60.00; H, 6.89; N, 3.68; found: C, 60.00; H, 6.81; N, 3.68.

B. To a solution of 0.73 g (2.0 mmol) of dibromopyridone **18** in 4 mL of dry THF at −78 °C was added dropwise 0.38 mL (0.75 mmol) of 1.98 M *n*-butyllithium. The solution was stirred at −78 °C for 1 h, warmed to ambient temperature and stirred for an additional 2 h. After cooling to −78 °C, a solution of 0.44 g (3.1 mmol) of 3-ethoxycyclohex-2-en-1-one in 1.5 mL of THF was added dropwise and the reaction was stirred at −78 °C for 4 h, quenched with saturated aqueous NH_4Cl and extracted with ether. The ether extract was washed with water, dried ($MgSO_4$) and the solvent was removed in vacuo. Chromatography (petroleum ether/ethyl acetate 5:1) gave 0.10 g (13%) of **19** as a green solid, identical in all respects to the material described in part A, above.

3-(3-Oxocyclohexyl)-6-pentyl-1-propyl-2-pyridone (20). To a solution of 0.10 g (0.26 mmol) of **19** in 30 mL of dry ethanol was added 0.02 g of 10% Pd/C followed by 0.5 mL of triethylamine. The mixture was shaken under an atmosphere of H_2 at 40 psig for 16 h. The solvent was removed in vacuo and the residue was chromatographed (petroleum ether/ethyl acetate 2.5:1) to give 0.035 g (44%) of ketone **20** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J=7.5 Hz, 3H), 0.99 (t, J=7.5 Hz, 3H), 1.36–1.43 (m, 4H), 1.60–1.74 (m, 4H), 1.78–1.84 (m, 2H), 2.03–2.07 (m, 2H), 2.38–2.67 (m, 6H), 3.20–3.25 (m, 1H), 3.96 (t, J=7.8 Hz, 2H), 5.98 (d, J=7.2 Hz, 1H), 7.04 (d, J=7.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.4, 13.9, 22.1, 22.4, 25.1, 28.5, 30.0, 31.4, 32.8, 39.6, 41.3, 45.7, 45.9, 104.9, 131.3, 133.8, 147.7, 162.4, 211.6; MS (EI) m/z 303 (76), 275 (55), 260 (100), 234 (80); HRMS calcd for $C_{19}H_{29}NO_2$: 303.2198, found 303.2199.

***cis*-3-(3-Hydroxycyclohexyl)-6-pentyl-1-propyl-2-pyridone (5).** To a solution of 0.028 g (0.092 mmol) of ketone **20** in 1 mL of dry ethanol at 0 °C was added 0.03 g (0.79 mmol) of $NaBH_4$, the reaction mixture was allowed to warm to ambient temperature and stirred for 18 h. The reaction was quenched with 10% aqueous HCl, extracted with ether, dried ($MgSO_4$) and the solvent was removed in vacuo. Chromatography (petroleum ether/ethyl acetate, 1:1) gave 0.019 g (68%) of **5** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J=7.0 Hz, 3H), 1.01 (t, J=7.2 Hz, 3H), 1.22–1.30 (m, 4H), 1.36–1.49 (m, 4H), 1.62–1.74 (m, 4H), 1.83–1.87 (m, 2H), 2.00–2.04 (m, 2H), 2.14 (d, J=10.7 Hz, 1H), 2.57 (t, J=7.9 Hz, 2H), 2.91 (t, J=12.1 Hz, 1H), 3.73–3.81 (m, 1H), 3.97 (t, J=7.8 Hz, 2H), 5.99 (d, J=7.2 Hz, 1H), 7.06 (d, J=7.3 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.4, 13.9, 22.1, 22.4, 24.3, 28.5, 31.1, 31.5, 32.8, 35.4, 35.9, 41.2, 45.8, 70.8, 105.1, 132.9, 133.4, 146.7, 162.8; MS (EI) m/z 305 (35), 287 (100), 244 (65), 189 (80); HRMS calcd for $C_{19}H_{31}NO_2$: 305.2355, found 305.2355.

***trans*-3-(3-Hydroxycyclohexyl)-6-pentyl-1-propyl-2-pyridone (21).** To a solution of 0.044 g (0.15 mmol) of ketone **20** in 1 mL of dry THF at −78 °C was added

0.31 mL (0.31 mmol) of 1.0 M potassium tri-*sec*-butylborohydride (K-Selectride) in THF. The reaction mixture was stirred at −78 °C for 2 h, allowed to warm to ambient temperature and stirred for 1 h. The reaction was quenched with 1 mL of water and 5 mL of ethanol followed by 2 mL of 15% aqueous NaOH and 2 mL of 30% H_2O_2. After extraction with three portions of ether, the combined extracts were washed with brine and dried ($MgSO_4$). The solvent was removed in vacuo and the crude material was chromatographed (petroleum ether/ethyl acetate, 1:1) to give 0.027 g (61%) of alcohol **21** as a pale yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, J = 6.9 Hz, 3H), 0.97 (t, J = 7.3 Hz, 3H), 1.21 (t, J = 10.8 Hz, 1H), 1.32–1.51 (m, 7H), 1.56–1.65 (m, 3H), 1.69 (sextet, J = 7.4 Hz, 2H), 1.78–1.95 (m, 3H), 2.04 (br d, J = 13.8 Hz, 1H), 2.56 (t, J = 7.8 Hz, 2H), 3.25 (br t, J = 9.2 Hz, 1H), 3.94 (d, J = 5.5 Hz, 1H), 3.96 (d, J = 5.5 Hz, 1H), 4.20 (s, 1H), 5.99 (d, J = 7.3 Hz, 1H), 7.06 (d, J = 6.9 Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 11.4, 13.9, 20.5, 22.1, 22.4, 28.6, 31.2, 31.7, 32.1, 32.2, 32.8, 38.6, 45.8, 66.7, 105.2, 133.2, 134.1, 146.4, 162.9; MS (EI) m/z 305 (35), 287 (15), 262 (45), 244 (63), 234 (100); HRMS calcd for $C_{19}H_{31}NO_2$: 305.2355, found 305.2354.

Receptor binding assays. CB_1 assay. [^{3}H]CP-55,940 (K_D = 690 nM) binding to P_2 membranes was conducted as described elsewhere,[29] except whole brain (rather than cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]CP-55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments.

CB_2 assay. Human embryonic kidney 293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal clone II (HyClone, Logan, UT, USA) and 5% CO_2 at 37 °C in a Forma incubator. Cell lines were created by transfection of CB_2pcDNA3 into 293 cells by the Lipofectamine reagent (Life Technologies, Gaithersburg, MD, USA). The human CB_2 cDNA was provided by Dr. Sean Munro (MRC, Cambridge, UK). Stable transformants were selected in growth medium containing geneticin (1 mg/mL, reagent, Life Technologies, Gaithersburg, MD, USA). Colonies of about 500 cells were picked (about 2 weeks post-transfection) and allowed to expand, then tested for expression of receptor mRNA by northern blot analysis. Cell lines containing moderate to high levels of receptor mRNA were tested for receptor binding properties. Transfected cell lines were maintained in DMEM with 10% fetal clone II plus 0.3–0.5 mg/mL geneticin and 5% CO_2 at 37 °C in a Forma incubator.

The current assay is a modification of Compton et al.[30] Cells were harvested in phosphate-buffered saline containing 1 mM EDTA and centrifuged at 500g. The cell pellet was homogenized in 10 mL of solution A (50 mM Tris–HCl, 320 mM sucrose, 2 mM EDTA, 5 mM $MgCl_2$, pH 7.4). The homogenate was centrifuged at 1600g (10 min), the supernatant saved, and the pellet washed three times in solution A with subsequent centrifugation. The combined supernatants were centrifuged at 100,000g (60 min). The (P_2 membrane) pellet was resuspended in 3 mL of buffer B (50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, pH 7.4) to yield a protein concentration of approximately 1 mg/mL. The tissue preparation was divided into equal aliquots, frozen on dry ice, and stored at −70 °C. Binding was initiated by the addition of 40–50 μg membrane protein to silanized tubes containing [^{3}H]CP-55,940 (102.9 Ci/mmol) and a sufficient volume of buffer C (50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, and 5 mg/mL fatty acid free BSA, pH 7.4) to bring the total volume to 0.5 mL. The addition of 1 μM unlabelled CP-55,940 was used to assess nonspecific binding. Following incubation (30 °C for 1 h), binding was terminated by the addition of 2 mL of ice cold buffer D (50 mM Tris–HCl, pH 7.4, plus 1 mg/mL BSA) and rapid vacuum filtration through Whatman GF/C filters (pretreated with polyethyleneimine (0.1%) for at least 2 h). Tubes were rinsed with 2 mL of ice cold buffer D, which was also filtered, and the filters subsequently rinsed twice with 4 mL of ice cold buffer D. Before radioactivity was quantitated by liquid scintillation spectrometry, filters were shaken for 1 h in 5 mL of scintillation fluid.

CP-55,940 and all cannabinoid analogues were prepared by suspension in assay buffer from a 1 mg/mL ethanolic stock without evaporation of the ethanol (final concentration of no more than 0.4%). When anandamide was used as a displacing ligand, experiments were performed in the presence of phenylmethylsulfonyl fluoride (50 μM). Competition assays were conducted with 1 nM [^{3}H]CP-55,940 or 1 nM [^{3}H]SR141716A and six concentrations (0.1 nM to 10 μM displacing ligands). Displacement IC_{50} values were originally determined by unweighted least-squares linear regression of log concentration-percent displacement data and then converted to K_i values using the method of Cheng and Prusoff.[31]

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