Indol-3-yl-tetramethylcyclopropyl Ketones: Effects of Indole Ring Substitution on CB_2 Cannabinoid Receptor Activity

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A series of potent indol-3-yl-tetramethylcyclopropyl ketones have been prepared as CB_2 cannabinoid receptor ligands. Two unsubstituted indoles (**5**, **32**) were the starting points for an investigation of the effect of indole ring substitutions on CB_2 and CB_1 binding affinities and activity in a CB_2 in vitro functional assay. Indole ring substitutions had varying effects on CB_2 and CB_1 binding, but were generally detrimental to agonist activity. Substitution on the indole ring did lead to improved CB_2/CB_1 binding selectivity in some cases (i.e., **7**−**9**, **15**−**20**). All indoles with the morpholino-ethyl side chain (**32**−**43**) exhibited weaker binding affinity and less agonist activity relative to that of their tetrahydropyranyl-methyl analogs (**5**−**31**). Several agonists were active in the complete Freund's adjuvant model of chronic inflammatory thermal hyperalgesia (**32**, **15**).

Introduction

The cannabinoid receptors are members of the G-protein coupled receptor (GPCR)[a] family of receptors. The cannabinoid 1 receptor (CB_1) is found in the central nervous system as well as the periphery,[1] whereas the cannabinoid 2 receptor (CB_2) is found mainly in the periphery, particularly in the immune system.[2] Evidence of CB_2 receptor expression in the CNS has recently emerged.[3] Activation of the CB_1 receptor is thought to mediate the psychotropic effects associated with nonselective agonists such as Δ^9-tetrahydrocannabinol (Δ^9-THC), the principle active component of marijuana. In animal studies, activation of either the CB_1 or CB_2 receptor will result in analgesic activity,[4] and several studies have established that CB_2-selective agonists (relative to CB_1) exhibit efficacy in many rodent pain models and lack the CB_1-mediated CNS effects at analgesic doses.[5] Specifically, CB_2-selective agonists have shown efficacy in preclinical models of neuropathic and inflammatory pain.[5,6] Cannabinoid receptor agonists are also being investigated for use in cancer,[7] multiple sclerosis,[8] osteoporosis,[9] Alzheimer's disease,[10] liver disease,[11] and diabetes.[12]

Aminoalkylindoles (AAIs) and indoles lacking the amine functionality are well-established structural motifs in cannabinoid research.[13] Initial work by Sterling Winthrop led to pravadoline[14] and later to the potent but nonselective cannabinoid ligand **1** (Figure 1).[15] Huffman and co-workers have subsequently published on numerous indole cannabinoid ligands[13] with their work focusing on varying the indole nitrogen substitution and the 3-aryl acyl substituents. Importantly, the work of Huffman and co-workers led to the realization that the amino alkyl side chain, which was previously thought to be necessary for interaction with the cannabinoid receptors,[16] could be replaced with groups lacking the amino functionality. One of the most well-known examples from the Huffman laboratories is **2**, in which the amino alkyl group is replaced by a propyl side chain.[17] Makriyannis and co-workers have also disclosed many aminoalkylindoles, including the CB_2 inverse agonist AM630, as well as the CB_2-selective ligand **3**.[18] Compound **3**, although demonstrating efficacy in a range of preclinical pain models,[18,19] has also exhibited some unique characteristics including an opioid receptor dependency[20] not shared by other CB_2-selective ligands[21] and varied activities in in vitro functional assays.[22,23]

Merck has disclosed a selective CB_2 ligand, **4**, which has the acyl group attached to the indole nitrogen and a morpholino-ethyl side chain in the 3-indole position.[24] Researchers at Bristol-Myers Squibb have reported numerous 3-amide indoles[25] and related indolopyridones.[26] There are several other reported indole-related cannabinoid ligands, including those described by patent applications from Organon,[27] Hoffman-La Roche,[28]GlaxoSmithKline,[29] Sanofi-Synthelabo,[30] and Schering-Plough.[31]

Several structure–activity studies have explored indole nucleus substitutions, both with respect to activity at the CB_1[16] and CB_2[25a] receptors. In a study of the CB_1 receptor structure-activity relationship (SAR), Eissenstat and co-workers[16] reported that only small groups (hydrogen, methyl) were tolerated in the 2-indole position. This conclusion is supported by the work of Huffman and co-workers.[13] The Eissenstat group also reported that substitution in the 5-indole position (methyl, methoxy, fluoro, bromo) was detrimental to activity in both their binding and functional assays. Substitution at the 6-indole position (methyl, methoxy, bromo) resulted in ligands with binding affinity but no functional activity. Substitution at the 7-indole position (methyl, methoxy, fluoro) gave compounds with modest improvements in binding and functional activities relative to the unsubstituted analogs.

Hynes and co-workers have reported on the effects of indole ring substitution on CB_2 activity in their 3-amide indole series.[25a] A C-7 methoxy group increased CB_2 binding affinity, and a C-2 substituent other than hydrogen resulted in decreased CB_2 binding affinity. The 7-methoxy derivatives were used to

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[a] Abbreviations: FLIPR, fluorescence imaging plate reader; CFA, complete Freund's adjuvant; PEG, poly(ethylene glycol); GPCR, G-protein coupled receptor; CB_1, cannabinoid 1 receptor; CB_2, cannabinoid 2 receptor; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; BSA, bovine serum albumin; PWL, paw withdrawal latency.

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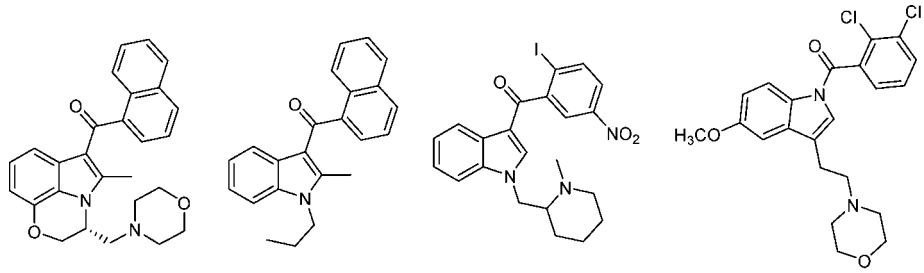


Figure 1. Literature compounds.

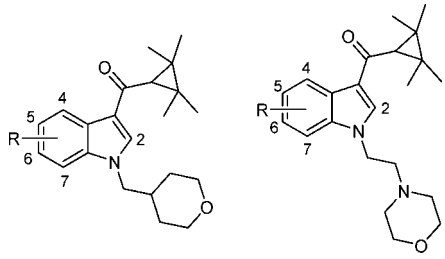


Figure 2. Indol-3-yl-tetramethylcyclopropyl ketones.

investigate other indole substituents. Substitutions at the C-4 and C-6 positions were reportedly not tolerated. Only chloro and fluoro substitutions were described for C-5, with the 5-chloro substitution leading to significantly decreased CB_2 binding affinity relative to that of the 5-hydrogen analog.

The published literature has been highly biased toward 3-indolyl-acyl aromatic substitutions; however, work in our laboratories led us to re-evaluate the use of nonaromatic rings in this position.[32] Herein we describe the SAR of indole ring substitution on human CB_2 and human CB_1 binding affinity and in vitro CB_2 functional activity for a series of 3-tetramethylcyclopropyl ketone substituted indoles (Figure 2). For the purpose of this discussion, N-1 substitution was limited to the morpholino-ethyl and the methyl-tetrahydropyran side chains (**5** and **32**). The morpholino-ethyl moiety is a well-known structural motif in cannabinoid literature,[13,15,24] and the tetrahydropyranyl methyl group was envisioned to be a truncated version of the morpholino-ethyl moiety. The in vivo activity of selected compounds in the complete Freund's adjuvant (CFA) model of chronic inflammatory thermal hyperalgesia will also be discussed.

Chemistry. All of the indoles discussed in this paper were synthesized by coupling of the appropriately substituted indole with 2,2,3,3-tetramethylcyclopropanecarbonyl chloride (Scheme 1) using EtMgBr and $ZnCl_2$.[33] This resulted in a separable mixture of the C-3-acylated and the *N*-acylated products. The C-3-acylated product then underwent *N*-alkylation with either the mesylate of (tetrahydro-2*H*-pyran-4-yl)methanol or 2-morpholinoethanol.

Biology. The binding affinity of this series of indole ligands was evaluated at recombinant human CB_1 and human CB_2 receptors through competition binding against [^{3}H]-**56** (CP 55,940).[34] In vitro functional activity was assessed in an HEK293 cell line coexpressing the human CB_2 receptor and a chimeric $G_{\alpha q/o5}$ protein to facilitate redirection of the $G_{\alpha i/o}$ signaling to intracellular calcium release responses and enable measurement of calcium mobilization using a fluorescence imaging plate reader (FLIPR) as previously described.[22,35] Maximal efficacy (% max) in the FLIPR assay was determined relative to the response elicited by 10 μM **56**. In vivo activity was assessed using the CFA model of chronic inflammatory thermal hyperalgesia. A solution of CFA in phosphate-buffered saline was injected into the plantar surface of the right hind paw in rats. Thermal hyperalgesia was assessed 48 h post CFA injection.

Results and Discussion

As discussed above, the starting points of our investigation of the substitution of the indole ring were parent compounds **5** and **32**. Indole **5** proved to be one of the most potent compounds in these series with subnanomolar human CB_2 binding affinity ($K_i = 0.21$ nM) and potent ($EC_{50} = 9$ nM), full agonist efficacy in the FLIPR assay. Indole **5** also had relatively high affinity for the human CB_1 receptor ($K_i = 12$ nM) and exhibited only moderate selectivity (57-fold) for the CB_2 receptor. The potency and efficacy of **5** at the CB_2 receptor made it an appealing lead, despite its affinity for the CB_1 receptor. Therefore, the SAR of modifications at the 4–7 positions of the indole ring was investigated (Tables 1 and 3).

Indole **32**, with the morpholinoethyl side chain, was more selective than the tetrahydropyranylmethyl derivative **5** (CB_1/$CB_2 = 192$), and **32** was also a potent ($EC_{50} = 17$ nM) agonist in the FLIPR calcium flux assay. The improved selectivity of **32** as well as its efficacy in in vivo models (vide infra) made it another interesting target for SAR studies with modifications at the 4–7 positions of the indole ring (Table 2).

Looking first to derivatives of **5** (Table 1), analog **6** with electron-withdrawing fluorine substitution in the 4–7 positions exhibited lower CB_2 and CB_1 binding affinities, less potency in the FLIPR functional assay and lower selectivity for CB_2 vs CB_1 (13-fold) than **5**. The 5-fluoro derivative **7** displayed low nanomolar binding affinity for CB_2 and dramatically improved binding selectivity relative to **5** or **6**. Also, there was a marked decrease in functional potency and efficacy in the FLIPR assay as the 5-indole substitution changed from fluoro (**7**) to chloro (**8**) to bromo (**9**) suggesting that increased size of the 5-substituent decreases CB_2 in vitro functional activity. This is supported by a comparison of **16**, **20**, and **24**, which all exhibit potent binding affinity, but where **16** (5-hydroxy) is a potent agonist, **20** (5-methoxy) is a weak agonist, and **24** (5-benzyloxy) lacks agonist efficacy in the FLIPR assay. All 5-halogen substituted derivatives exhibited high levels of CB_2 binding selectivity.

Interrogation of monosubstitution at the 6-indole position also revealed a pronounced effect of substituent size on functional activity. The 6-chloro and 6-hydroxy analogs, **10** and **17**, respectively, were moderately potent and efficacious agonists, whereas the 6-bromo (**11**), 6-methyl (**12**), 6-trifluoromethyl (**13**), 6-methylsulfonyl (**14**), and 6-methoxy (**21**) analogs all lacked agonist activity in the FLIPR functional assay. Interestingly, the 6-benzyloxy analog (**25**) exhibited weak partial agonist

Scheme 1. General Synthesis of Indol-3-yl-tetramethylcyclopropyl Ketones

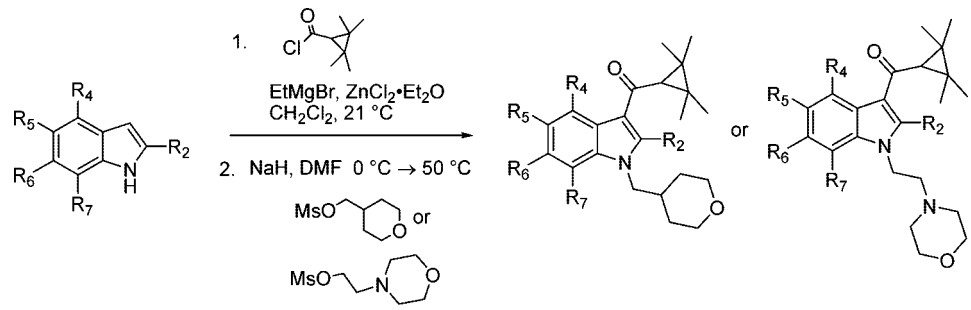
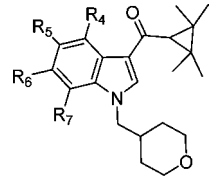


Table 1. In Vitro Biological Activity of Tetrahydropyranyl-methyl Series



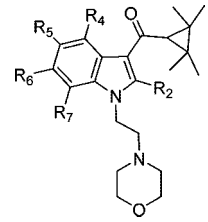
					Human CB_2 Binding		Human CB_1 Binding			CB_2 FLIPR	
Compd	R_4	R_5	R_6	R_7	$pK_i \pm SEM$	K_i (nM)	$pK_i \pm SEM$	K_i (nM)	CB_1/CB_2	EC_{50} (nM) (SEM range)	% max
		1 (WIN 55,212-2)			8.89 ± 0.06	1.3	7.88 ± 0.12	13.3	10	86–163	74 ± 4
		2 (JWH-015)			7.45 ± 0.06	35	5.92 ± 0.10	1204	34	634–961	75 ± 5
		3 (AM1241)			7.94 ± 0.10	11.5	5.90 ± 0.25	1269	110	>10,000	-
5	H	H	H	H	9.67 ± 0.12	0.21	7.91 ± 0.18	12	57	7–12	133 ± 9
6	F	F	F	F	8.31 ± 0.07	5.0	7.18 ± 0.25	66	13	37–43	88 ± 3
7	H	F	H	H	8.80 ± 0.17	1.6	5.92 ± 0.08	1210	756	22–51	88 ± 4
8	H	Cl	H	H	8.52 ± 0.08	3.0	6.30 ± 0.12	512	171	92–172	69 ± 7
9	H	Br	H	H	8.18 ± 0.27	6.6	5.72 ± 0.10	1930	292	280–501	54 ± 10
10	H	H	Cl	H	8.60 ± 0.19	2.5	7.86 ± 0.30	14	5.6	10–32	60 ± 6
11	H	H	Br	H	9.19 ± 0.08	0.65	7.88 ± 0.06	13	20	>10,000	-
12	H	H	CH_2	H	9.83 ± 0.12	0.15	7.56 ± 0.28	28	187	>10,000	-
13	H	H	CF_3	H	8.46 ± 0.08	3.5	7.16 ± 0.18	70	20	>10,000	-
14	H	H	SO_2CH_3	H	8.53 ± 0.10	3.0	5.90 ± .05	1270	423	>10,000	-
15	OH	H	H	H	8.41 ± 0.11	3.9	6.41 ± 0.10	388	99	64–82	94 ± 4
16	H	OH	H	H	8.72 ± 0.11	1.9	6.69 ± 0.15	204	107	7–15	78 ± 5
17	H	H	OH	H	8.07 ± 0.20	8.5	>5.52	>3,000	>353	15–22	107 ± 4
18	H	H	H	OH	8.50 ± 0.05	3.2	6.61 ± 0.09	246	77	>10,000	-
19	OCH_3	H	H	H	8.48 ± 0.09	3.3	5.96 ± 0.20	1090	330	81–101	37 ± 7
20	H	OCH_3	H	H	8.34 ± 0.05	4.6	6.55 ± 0.10	282	61	61–141	35 ± 3
21	H	H	OCH_3	H	9.29 ± 0.27	0.51	7.40 ± 0.20	40	78	>10,000	-
22	H	H	H	OCH_3	9.90 ± 0.10	0.12	7.35 ± 0.11	45	375	>10,000	-
23	OBn	H	H	H	8.03 ± 0.11	9.3	>5	>10000	>1075	383–620	45 ± 6
24	H	OBn	H	H	8.90 ± 0.22	1.3	6.62 ± 0.19	238	183	>10,000	-
25	H	H	OBn	H	9.05 ± 0.06	0.88	7.48 ± 0.02	33	38	135–199	45 ± 4
26	H	H	H	OBn	8.52 ± 0.07	3.1	5.96 ± 0.06	1095	353	>10,000	-
27	H	OBn	OCH_3	H	8.75 ± 0.09	1.8	5.55 ± 0.11	2804	1558	>10,000	-
28	H	OH	OCH_3	H	9.16 ± 0.12	0.7	6.11 ± 0.15	783	1119	>10,000	-
29	H	OH	OH	H	7.67 ± 0.07	21	6.40 ± 0.22	395	19	21–35	106 ± 4
30	H			H	8.99 ± 0.05	1.0	7.55 ± 0.19	28	28	18–41	62 ± 1
31	H	NH_2	H	H	8.03 ± 0.17	9.3	>6	>1000	108	10–21	80 ± 8

activity. All of the 6-substituted analogs exhibited high affinity for the CB_2 receptor. The 6-methylsulfonyl derivative (**14**) and 5-hydroxy (**17**) derivative exhibited low affinity for the CB_1 receptor, and all other 6-substituted analogues investigated had higher binding affinity (<100 nM) for the CB_1 receptor.

Substitutions at the 4 and 7 positions of the indole ring were also investigated. The 4-hydroxy analog (**15**) was a potent, fully efficacious agonist in the FLIPR assay. In contrast, although the 7-hydroxy analog (**18**) demonstrated high affinity for the CB_2 binding site and moderate affinity for the CB_1 receptor, it failed to exhibit agonist activity. Similar trends were obtained with the 4- (**19**) and 7-methoxy (**22**) and the 4- (**23**) and 7-benzyloxy (**26**) analogs. Both 4-substituted analogs exhibited high affinity for the CB_2 receptor, weaker or no affinity for the CB_1 receptor, and weak agonist activity in the FLIPR assay. The 7-substituted analogs (**22**, **26**) exhibited high CB_2 and CB_1 receptor affinity but lacked agonist activity in the FLIPR assay.

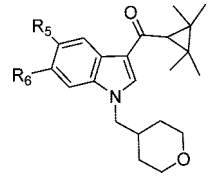
Several 5,6-bis-substituted indole analogues were also investigated (**27**−**30**). Analogues **27** and **28** had good affinity for CB_2 but did not demonstrate agonist activity in the FLIPR assay, which is consistent with the corresponding monosubstituted analogues (**21**, **24**). The diol **29** had lower affinity and was less selective than its monosubstituted analogues (**16**, **17**). Perhaps the most interesting bis-substituted analogue was **30**, which maintained high potency and near full agonist efficacy in the FLIPR assay (18–41 nM, 62%), whereas the similar 5-hydroxy,

Table 2. In Vitro Biological Activity of Morpholino-ethyl Series



						Human CB_2 Binding		Human CB_1 Binding			CB_2 FLIPR	
Compd	R_4	R_5	R_6	R_7	R_2	$pK_i \pm$ SEM	K_i (nM)	$pK_i \pm$ SEM	K_i (nM)	CB_1/CB_2	EC_{50} (nM) (SEM range)	max
32	H	H	H	H	H	8.36 ± 0.17	4.4	6.07 ± 0.14	845	192	14–21	71 ± 4
33	H	OH	H	H	H	7.04 ± 0.11	92	<5	>10,000	>109	>10,000	-
34	H	H	OH	H	H	6.96 ± 0.09	109	<5	>10,000	>92	186–280	62 ± 1
35	H	OCH_3	H	H	H	6.63 ± 0.09	237	>5.46	>3,500	15	>10,000	-
36	H	H	OCH_3	H	H	8.80 ± 0.04	1590	5.89 ± 0.11	1285	0.81	>10,000	-
37	H	OBn	H	H	H	<6	>1000	<5	>10000		>10,000	-
38	H	H	OBn	H	H	8.53 ± 0.07	2.9	6.00 ± 0.04	986	340	>10,000	-
39	H	H	H	H	CH_3	7.57 ± 0.09	27	<5	>10,000	>370	227–497	60 ± 6
40	NO_2	H	H	H	H	6.53 ± 0.11	297	<5	>10,000	>34	>10,000	-
41	NH_2	H	H	H	H	7.49 ± 0.11	32	>5.46	>3,500	>109	50–380	41 ± 5
42	$NHC(O)CH_3$	H	H	H	H	<6	>1000	>5.66	>2,200	-	>10,000	-
43		H	H	H	H	<6	>1000	5.39 ± 0.01	4077	-	>10,000	-

Table 3. In Vitro Biological Activity of Additional Tetrahydropyranyl-methyl Analogues



			human CB_2 binding		human CB_1 Binding			CB_2 FLIPR	
compd	R_5	R_6	$pK_i \pm$ SEM	K_i (nM)	$pK_i \pm$ SEM	K_i (nM)	CB_1/CB_2	EC_{50} (nM)(SEM range)	max
44	$O(CH_2)_4OH$	H	6.58 ± 0.11	260	<5	>10,000	>38	>10,000	
45	$O(CH_2)_4Br$	H	7.14 ± 0.11	72	<5	>10,000	>139	>10,000	
46	CN	H	7.52 ± 0.26	30	5.48 ± 0.05	3326	111	226–299	65 ± 3
47	CH_2NH_2	H	<6	>1000	<5	>10,000		>10,000	
48	CH_2OH	H	6.95 ± 0.09	113	<5	>10,000	>88	73–122	53 ± 6
49	CH_2OCH_3	H	7.59 ± 0.012	26	5.77 ± 0.08	1702	65	41–108	50 ± 5
50	$C(O)OCH_3$	H	6.62 ± 0.07	238	<5	>10,000	>42	>10,000	
51	Ph	H	7.23 ± 0.09	58	5.73 ± 0.27	1875	32	>10,000	
52	H	CN	8.30 ± 0.012	5.0	7.50 ± 0.27	31	6.2	45–61	67 ± 7
53	H	CH_2NH_2	7.21 ± 0.26	62	<4	>1,000	>16	>10,000	
54	H	$C(O)OCH_3$	8.86 ± 0.40	1.4	7.60 ± 0.17	25	18	>10,000	
55	H	Ph	9.39 ± 0.13	0.41	6.64 ± 0.16	228	556	>10,000	

6-methoxy analogue, **28**, the 5-methoxy analogue, **20**, and the 6-methoxy analogue, **21**, exhibited little or no agonist activity in FLIPR.

As shown in Table 2, the morpholino-ethyl derivatives were also examined. Analogue **32** is a CB_2-selective compound with high affinity for the CB_2 receptor and potent activity in the FLIPR assay, and it proved to be the most interesting analogue in this group of morpholino-ethyl derivatives. In most cases where direct comparisons can be made between the morpholino-ethyl series and the tetrahydropyranyl-methyl series, the analogues in the latter group demonstrate higher affinity for CB_2, as well as greater potency and efficacy in the FLIPR assay (**33** vs **16**, **34** vs **17**, **35** vs **20**). When comparing the substituted indoles in the morpholino-ethyl series with the parent **32**, it is clear that all indole substitution is detrimental to agonist activity and, in most cases, to binding affinity as well, with the 6-benzyloxy derivative **38** being the one example with binding affinity similar to that of **32**. Finally, as is consistent with the previously reported work,[13,16,25a] substitution at the C-2 indole position resulted in decreased binding affinity at both the CB_2 and CB_1 receptors and decreased functional activity at the CB_2 receptor (**39** vs **32**).

The effects of substitutions on the 5 or 6 positions of the indole ring were further investigated in the tetrahydropyranyl-methyl series as shown in Table 3. As demonstrated in Tables 1 and 2, indole substituents larger than a methyl group generally resulted in loss of agonist activity in the FLIPR assay. Analogues with smaller substituents such as the cyano group (**46**, **52**) did retain agonist activity, as did the 5-hydroxymethyl and 5-methoxymethyl analogues (**48**, **49**); however, these compounds were only partial agonists in the FLIPR assay. With regard to binding, larger groups were tolerated and less polar substitutions displayed better CB_2 activity than more polar substitutions (i.e.,

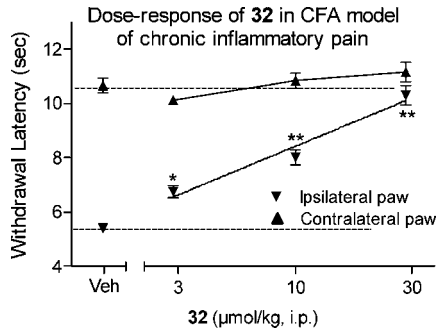


Figure 3. Dose response of **32** in CFA model of chronic inflammatory pain. Thermal hyperalgesia was assessed 48 h post CFA injection; $n = 6$ for each dose; vehicle is 5% DMSO/95% poly(ethylene glycol) (PEG); $*p < 0.05$ vs vehicle control; $**p < 0.01$ vs vehicle control.

45 vs **44** and **48** vs **47**). With respect to binding affinity, the 6 position appears to be more tolerant of larger substitutions than the 5 position (i.e., **54** vs **50** and **55** vs **51**). Polar substitutions also appear to be better tolerated in the 6 position (i.e., **53** vs **47**). However, agonist efficacy was generally lost when large substituents were introduced into any position on the indole nucleus.

In Vitro SAR Summary. Clearly, the effect of indole ring substitution on CB_2 functional activity is sensitive to substituent size, with the effect varying by location of the indole substituent. For example, in the 4- and 5-indole positions the methoxy group is tolerated for partial agonist activity (**19**, **20**), but this substituent is not tolerated in the 6 or 7 positions (**21**, **22**). Binding at both the CB_2 and CB_1 receptors is less sensitive to substituent size with aryl groups (**51**, **55**, **23**−**26**) and relatively large alkyl groups being tolerated (**44**, **45**). The morpholino-ethyl analogues (Table 2) were generally weaker than the tetrahydropyranyl analogues (Tables 1 and 3). All substitution in both series led to decreased CB_2 functional activity in the FLIPR assay but had less of an effect on binding affinities.

Comparing our series with the previously reported work of Eissenstat[16] and Hynes,[25a] several similarities in SAR trends exist. For example, Eissenstat and co-workers demonstrated that C-5 substituents gave weaker CB_1 binding affinity, and this was also true with our compounds (i.e., **7**–**9**, **16**, **24** vs **5**). A C-7 methoxy substituent was reported to enhance both CB_1[16] and CB_2[25a] binding affinity, whereas in our series, C-7 methoxy derivative **22** exhibited weaker CB_1 binding affinity and improved CB_2 binding affinity relative to that of the parent analogue **5**.

In Vivo Characterization. An accumulating body of evidence suggests the potential utility of CB_2-selective agonists in the treatment of pain. To test this hypothesis, several CB_2-selective analogues exhibiting in vitro agonist efficacy were selected for evaluation in the CFA model of chronic inflammatory thermal hyperalgesia. Compound **32**, which exhibited high radioligand binding affinity for the CB_2 receptor ($K_i = 4.4$ nM), very good selectivity ($CB_1/CB_2 = 192$), and high potency and near full efficacy in the FLIPR assay ($EC_{50} = 17$ nM, 71% maximal response), was also efficacious in the CFA model of chronic inflammatory thermal hyperalgesia (Figure 3). The dependence on CB_2 receptor activation was demonstrated by coadministration of **32** with the selective CB_1 and CB_2 receptor antagonists **57** (SR141716A)[36] and **58** (SR144528),[37] respectively (Figure 4). Pretreatment with the CB_1 antagonist **57** resulted in no reduction in efficacy for **32**, whereas pretreatment with the

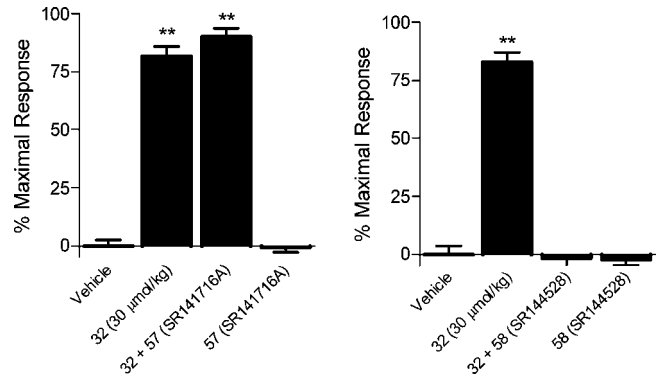


Figure 4. Activity of **32** in CFA model with pretreatment with **57** and pretreatment with **58**. Thermal hyperalgesia was assessed 48 h post CFA injection; $n = 6$ for each dose; vehicle is 5% DMSO/95% PEG; $**p < 0.01$ vs vehicle control.

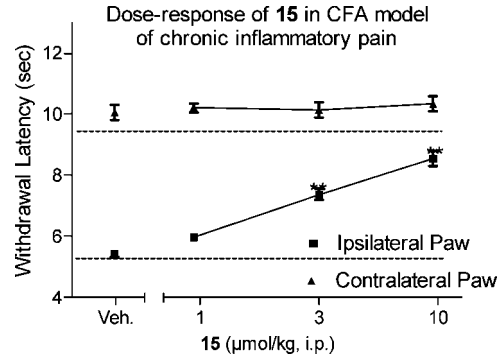


Figure 5. Dose response of **15** in CFA model of chronic inflammatory pain. Thermal hyperalgesia was assessed 48 h post CFA injection; $n = 6$ for each dose; vehicle is 5% DMSO/95% PEG, $**p < 0.01$ vs vehicle control.

CB_2 antagonist **58** completely blocked activity of **32** in this model, providing strong evidence that the effect of **32** is mediated through activation of the CB_2 receptor and not CB_1.

A second analogue (**15**), exhibiting a selectivity and efficacy profile comparable to those of **32**, was selected for evaluation in the CFA model of chronic inflammatory thermal hyperalgesia. Compound **15**, an agonist in the FLIPR assay ($EC_{50} = 73$ nM, 94% response), exhibited high affinity for the CB_2 receptor ($K_i = 3.9$ nM) and 100-fold selectivity vs CB_1, and as shown in Figure 5, it also demonstrated robust dose-dependent efficacy in the CFA model. By contrast, analogue **55**, which demonstrated a comparable radioligand binding affinity and selectivity profile but failed to exhibit agonist efficacy in the FLIPR functional assay, was inactive (data not shown) in the CFA inflammatory pain model.

In summary, two series of indole derivatives were prepared and evaluated for human CB_2 affinity, selectivity against CB_1 affinity, and agonist activity at the human CB_2 receptor. The two lead compounds, **5** and **32**, both demonstrated high affinity for the CB_2 receptor, and both were agonists in the FLIPR functional assay. The tetrahydropyranyl-methyl analogue **5** was more potent than the corresponding morpholino-ethyl derivative **32**, but **5** also exhibited high affinity for the CB_1 receptor ($K_i = 12$ nM). To further investigate the SAR of these compounds, numerous indole-substituted analogues were prepared. In the tetrahydropyranyl-methyl series, several analogues exhibited binding selectivity for CB_2 better than that of **5** (i.e., **7**, **15**–**17**, **29**–**31**, **48**, **52**) while still maintaining high affinity for the CB_2 binding site and good potency and efficacy in the FLIPR assay. In the morpholino-ethyl series, all indole-substituted analogues

exhibited lower affinity and lower potency compared to those of **32**, with only three analogues (**34**, **39**, and **41**) showing any agonist activity in the FLIPR assay. Overall, agonist activity was highly dependent on indole substituent size, particularly in the 6- and 7-indole positions. CB_2 binding affinity proved much less sensitive to substituent size, especially in the tetrahydropyranyl-methyl series where even aryl-substituted indoles retained high binding affinity (i.e., **23**−**26**, **51**, **55**).

Compound **32** is a novel, high affinity ligand for the CB_2 receptor, exhibiting selectivity versus the CB_1 binding site. It displays full agonist efficacy in an in vitro functional assay and is active in a model of chronic inflammatory pain, an effect that is selectively blocked by pretreatment with a CB_2 antagonist and not by a CB_1 antagonist. A more detailed characterization of the in vitro and in vivo properties of this ligand has been published,[38] and a description of the effects of further variations of the indole nitrogen side chain in the 3-cycloalkyl acyl indole series will be forthcoming.

Experimental Section

Radioligand Binding Assays. Membrane samples prepared from HEK cells stably expressing the human CB_2 receptor and the CHO cells stably expressing the human CB_1 receptor were used to perform radioligand binding assays using [^{3}H]**-56** as previously described.[35] Briefly, competition experiments were conducted using 0.5 nM [^{3}H]**-56** in the presence of variable concentrations of test compounds in an assay buffer containing 50 mM Tris-HCl, pH 7.4, 2.5 mM EDTA, 5 mM $MgCl_2$, and 0.05% fatty acid free BSA. After 90 min of incubation at 30 °C, the reactions were terminated by rapid vacuum filtration through UniFilter-96 GF/C filter plates (Perkin-Elmer, Boston, MA) and six washes with cold assay buffer, and the filter plates were air-dried. The bound activity was counted in a TopCount using Microscint-20 (Perkin-Elmer). Nonspecific binding was defined by 10 μM unlabeled **56**. K_i values from competition binding assays were determined with one site binding or one site competition curve fitting using the MDL Assay Explorer software (San Ramon, CA). Data are presented as mean values ± standard error of the mean (SEM) of at least three independent experiments, each of which was performed in duplicate.

Fluorescence Imaging Plate Reader Functional Assays. FLIPR assays were performed using HEK cells stably coexpressing the chimeric $G_{\alpha q/o5}$ protein with the human CB_2 receptor.[35] Briefly, cells were seeded at 75,000 cells per well 1 day prior to the assay, and assays were performed with no-wash dye (FLIPR Calcium Assay Kit, Molecular Device, Sunnyvale, CA) following vendor's instruction. Variable concentrations of test compounds (0.3 nM to 10 μM) and positive control **56** (at 10 μM final concentration) or vehicle negative control were added to cells in the presence of assay buffer (10 mM HEPES, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.05% BSA), and fluorescence responses were measured immediately with a FLIPR machine. Net peak responses were compared with that of 10 μM **56** and expressed as percentages of the **56**-evoked response. EC_{50} values were analyzed with sigmoidal dose response curve fitting using MDL Assay Explorer software. Data are presented as mean values ± standard error of the mean (SEM) of at least three independent experiments, each of which was performed in duplicate.

Chronic Inflammatory Pain Model. Chronic inflammatory thermal hyperalgesia was induced by injection of 150 μL of a 50% solution of CFA in phosphate-buffered saline into the plantar surface of the right hind paw in rats. Thermal hyperalgesia was assessed 48 h post CFA injection.

Thermal hyperalgesia was determined using a commercially available thermal paw stimulator (UARDG, University of California, San Diego, CA) as described by Hargreaves et al.[39] Rats were placed into individual plastic cubicles mounted on a glass surface maintained at 30 °C and allowed a 20 min habituation period. A thermal stimulus, in the form of radiant heat, emitted from a focused projection bulb, was then applied to the plantar surface of each hind paw. The stimulus current was maintained at 4.50 ± 0.05 A, and the maximum time of exposure was set at 20.48 s to limit possible tissue damage. The elapsed time until a brisk withdrawal of the hind paw from the thermal stimulus was recorded automatically using photodiode motion sensors. The right and left hind paw of each rat was tested in 3 sequential trials at approximately 5 min intervals. Paw withdrawal latency (PWL) was calculated as the mean of the two shortest latencies. PWL was measured 30 min post administration in both the CFA-treated (ipsilateral) and uninjected paw (contralateral).

Chemistry. Proton NMR spectra were obtained on a General Electric QE 300 or QZ 300 MHz instrument with chemical shifts (δ) reported relative to tetramethylsilane as an internal standard. Elemental analyses were performed by Robertson Microlit Laboratories or Quantitative Technologies, Inc. Column chromatography was carried out on silica gel 60 (230–400 mesh). Thin-layer chromatography was performed using 250 mm silica gel 60 glass-backed plates with F_{254} as indicator. All materials were commercially available and were obtained from Aldrich unless otherwise specified.

2,2,3,3-Tetramethylcyclopropanecarbonyl Chloride. To a flask containing 2,2,3,3-tetramethylcyclopropane carboxylic acid (13.5 g, 95 mmol) was added 30 mL of thionyl chloride (410 mmol, excess). This solution was warmed to reflux and was stirred for 2 h. The mixture was then cooled to ambient temperature and concentrated under reduced pressure. The residue was diluted three times with 10 mL of benzene and concentrated to remove any remaining thionyl chloride. This was repeated two additional times, and the material was used without further purification or characterization.

Tetrahydro-2*H*-pyran-4-ylmethyl Methanesulfonate. To tetrahydropyran-4-methanol (Combi-Blocks, Inc., 0.15 g, 1.2 mmol) in 10 mL of tetrahydrofuran (THF) at 0 °C was added triethylamine (0.56 mL, 4.1 mmol) followed by methanesulfonyl chloride (0.15 mL, 1.9 mmol). The mixture was stirred at 0 °C for 10 min, the ice-bath was removed, and the reaction mixture was stirred at 23 °C for an additional 1.5 h. The reaction mixture was filtered though Celite with THF and concentrated under reduced pressure. The crude tetrahydro-2*H*-pyran-4-ylmethyl methanesulfonate was used without further purification or characterization.

2-Morpholin-4-ylethyl Methanesulfonate. A solution of 4-(2-hydroxylethyl)-morpholine (5.1 mL, 42 mmol), triethylamine (17 mL, 124 mmol), and methanesulfonyl chloride (4.8 mL, 62 mmol) in 100 mL of THF was processed as described in the procedure for the tetrahydro-2*H*-pyran-4-ylmethyl methanesulfonate to give the crude 2-morpholin-4-ylethyl methanesulfonate, which was used without further purification or characterization.

1*H*-Indol-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone. To a solution of indole (11 g, 95 mmol) in 30 mL of dichloromethane at ambient temperature was added 105 mL of a 1 M solution of ethyl magnesium bromide in THF (105 mmol) dropwise via syringe pump. After the addition was complete, the solution was stirred for 15 min at which time 105 mL of a 1 M solution of $ZnCl_2$ in Et_2O (105 mmol) was added. The mixture was stirred for an additional 30 min, and then 2,2,3,3-tetramethylcyclopropanecarbonyl chloride (95 mmol) in 50 mL of dichloromethane was added via cannula. The mixture was stirred for 6 h at ambient temperature and then was quenched with 50 mL of saturated, aqueous NH_4Cl and diluted with 50 mL dichloromethane. The layers were separated, and the aqueous layer was extracted with 3 × 30 mL dichloromethane. The combined organics were washed with 20 mL of H_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified via column chromatography (SiO_2, 50% ethyl acetate/hexanes) to give 9.7 g of the major regioisomer 1*H*-indol-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone (40 mmol, 42% yield) and 6.1 g of the minor regioisomer of 1-[(2,2,3,3-tetramethylcyclopropyl)carbonyl]-1*H*-indole (25 mmol, 27% yield). ^{1}H NMR (major product) (300 MHz, CD_3OD) δ ppm 1.32 (s, 6 H), 1.33 (s, 6 H), 2.14 (s, 1 H), 7.12–7.24 (m, 2 H), 7.38–7.46 (m, 1 H), 8.02 (s, 1 H), 8.19–8.25 (m, 1 H); ^{1}H NMR (minor product) (300 MHz, CD_3OD) δ ppm 1.29 (s, 6 H), 1.34 (s, 6 H), 1.94 (s, 1 H), 6.66 (dd, J = 3.7, 0.7 Hz, 1 H),

7.16–7.32 (m, 2 H), 7.51–7.58 (m, 1 H), 7.67 (d, $J = 3.7$ Hz, 1 H), 8.32–8.39 (m, 1 H); MS (major and minor regioisomers) (DCI/NH_3) m/z 242 $(M + H)^+$.

[1-(Tetrahydro-2*H*-pyran-4-ylmethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone (5). To a solution of 1*H*-indol-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone (0.15 g, 0.62 mmol) in 8 mL of DMF at 0 °C was added NaH (60% dispersion in mineral oil, 0.12 g, 3.1 mmol). This mixture was stirred at 0 °C for 10 min, warmed to ambient temperature, and allowed to stir for 30 min. The solution was again cooled to 0 °C and tetrahydro-2*H*-pyran-4-ylmethyl methanesulfonate (2.1 mmol) in 5 mL of DMF was added via cannula. The ice-bath was removed after the addition was complete, and the reaction mixture was warmed to 45 °C and stirred for 2 h. The mixture was cooled to ambient temperature, diluted with 10 mL of ethyl acetate and quenched with 10 mL of saturated, aqueous NH_4Cl and 5 mL of H_2O. The layers were separated, the aqueous layer was extracted with 3 × 5 mL ethyl acetate, and the combined organics were dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified via column chromatography (SiO_2, 50% hexanes in EtOAc) to give 0.19 g of **5** (0.56 mmol, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.31 (s, 6H), 1.35 (s, 6H), 1.46 (m, 4H), 1.94 (s, 1H), 2.16 (m, 1H), 3.33 (dt, $J = 11.5$, 2.4 Hz, 2H), 3.98 (dd, $J = 10.5$, 3.1 Hz, 2H), 4.04 (d, $J = 7.5$ Hz, 2H), 7.27 (m, 2H), 7.33 (m, 1H), 7.61 (s, 1H), 8.40 (m, 1H); MS (DCI/NH_3) m/z 340 $(M + H)^+$; Anal. ($C_{22}H_{29}NO_2$) C, H, N.

[6-Hydroxy-1-(tetrahydro-2*H*-pyran-4-ylmethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone (17). A mixture of **25** (0.64 g, 1.4 mmol) and Pd/C (10 wt % palladium on activated carbon, 100 mg) in 20 mL of EtOH and 10 mL of EtOAc was stirred under 1 atm of H_2 (balloon) for 16 h. The system was purged with an inert nitrogen atmosphere. The mixture was filtered, concentrated under reduced pressure, and purified via column chromatography (SiO_2, 50% hexanes in EtOAc) to provide **17** (0.48 g, 1.35 mmol, 94% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.29 (s, 6 H), 1.34 (s, 6 H), 1.38–1.58 (m, 4 H), 1.89 (s, 1 H), 2.06–2.21 (m, 1 H), 3.33 (dt, $J = 11.8$, 2.2 Hz, 2 H), 3.95 (d, $J = 7.1$ Hz, 2 H), 3.97–4.04 (m, 2 H), 4.67 (s, 1 H), 6.76–6.81 (m, 2 H), 7.50 (s, 1 H), 8.25 (d, $J = 9.2$ Hz, 1 H); MS (DCI/NH_3) m/z 356 $(M + H)^+$; Anal. ($C_{22}H_{29}NO_3$) C, H, N.

[6-Methoxy-1-(tetrahydro-2*H*-pyran-4-ylmethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone (21). To a solution of **17** (0.15 g, 0.42 mmol) in 10 mL of THF was added NaH (60% dispersion in mineral oil, 51 mg, 1.3 mmol) followed by CH_3I (39 μL, 0.63 mmol). The mixture was stirred at ambient temperature for 18 h and then was quenched with 3 mL of saturated aqueous NH_4Cl. The mixture was diluted with 10 mL of EtOAc, the layers were separated, and the aqueous layer was extracted with 3 × 3 mL of EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified via column chromatography (SiO_2, 30% hexanes in EtOAc) to provide **21** (86 mg, 0.23 mmol, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.30 (s, 6 H), 1.34 (s, 6 H), 1.34–1.63 (m, 4 H), 1.90 (s, 1 H), 2.05–2.24 (m, 1 H), 3.34 (dt, $J = 11.7$, 2.4 Hz, 2 H), 3.88 (s, 3 H), 3.94–4.02 (m, 2 H), 3.97 (d, $J = 7.5$ Hz, 2 H), 6.77 (d, $J = 2.4$ Hz, 1 H), 6.92 (dd, $J = 8.8$, 2.0 Hz, 1 H), 7.51 (s, 1 H), 8.28 (d, $J = 8.8$ Hz, 1 H); MS (DCI/NH_3) m/z 370 $(M + H)^+$; Anal. ($C_{23}H_{31}NO_3$) C, H, N.

[6-(Benzyloxy)-1-(tetrahydro-2*H*-pyran-4-ylmethyl)-1*H*-indol-3-yl](2,2,3,3 tetramethylcyclopropyl)methanone (25). A mixture of 6-benzyloxyindole (Lancaster, 2.0 g, 9.0 mmol), ethylmagnesium bromide (1.0 M solution in THF, 11 mL, 11 mmol), zinc chloride (1.0 M solution in Et_2O, 11 mL, 11 mmol), and 2,2,3,3-tetramethylcyclopropanecarbonyl chloride (13.4 mmol) in 30 mL of dichloromethane was processed as described in the procedure for 1*H*-indol-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone to provide (6-benzyloxy-1*H*-indol-3-yl)-(2,2,3,3-tetramethyl-cyclopropyl)-methanone (2.0 g, 5.8 mmol, 64% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.25 (s, 12 H), 2.19 (s, 1 H), 5.13 (s, 2 H), 6.86 (dd, $J = 8.6$, 2.2 Hz, 1 H), 6.99 (d, $J = 2.0$ Hz, 1 H), 7.29–7.43 (m, 3 H), 7.44–7.50 (m, 2 H), 8.06 (d, $J = 8.8$ Hz, 1 H), 8.09 (d, $J = 3.1$ Hz, 1 H); MS (DCI/NH_3) m/z 348 $(M + H)^+$.

The (6-benzyloxy-1*H*-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone (0.90 g, 2.6 mmol), tetrahydro-2*H*-pyran-4-ylmethyl methanesulfonate (4.4 mmol), and NaH (60% dispersion in mineral oil, 0.31 g, 7.8 mmol) in 15 mL of DMF were processed as described in the procedure for **5** to provide **25** (0.87 g, 2.0 mmol, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.29 (s, 6 H), 1.34 (s, 6 H), 1.34–1.51 (m, 4 H), 1.90 (s, 1 H), 1.98–2.12 (m, 1 H), 3.30 (dt, $J = 11.7$, 2.4 Hz, 2 H), 3.91–4.00 (m, 2 H), 3.93 (d, $J = 7.1$ Hz, 2 H), 5.15 (s, 2 H), 6.81 (d, $J = 2.4$ Hz, 1 H), 7.01 (dd, $J = 8.8$, 2.0 Hz, 1 H), 7.29–7.43 (m, 3 H), 7.43–7.49 (m, 2 H), 7.50 (s, 1 H), 8.28 (d, $J = 8.8$ Hz, 1 H); MS (DCI/NH_3) m/z 446 $(M + H)^+$; Anal. ($C_{29}H_{35}NO_3$) C, H, N.

[1-(2-Morpholin-4-ylethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone *p*-Toluenesulfonic Acid (32). 1*H*-Indol-3-yl(2,2,3,3-tetramethylcyclopropyl)methanone (5.0 g, 21 mmol), 2-morpholin-4-ylethyl methanesulfonate (42 mmol), and NaH (60% dispersal in mineral oil, 4.2 g, 104 mmol) in 40 mL of dimethylformamide were processed as described in the procedure for **5** to provide 6.6 g of [1-(2-morpholin-4-ylethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone 18.6 mmol, 90% yield). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.33 (s, 12 H), 2.13 (s, 1 H), 2.46–2.54 (m, 4 H), 2.79 (t, $J = 6.4$ Hz, 2 H), 3.61–3.71 (m, 4 H), 4.37 (t, $J = 6.4$ Hz, 2 H), 7.16–7.30 (m, 2 H), 7.45–7.53 (m, 1 H), 8.11 (s, 1 H), 8.20–8.30 (m, 1 H), MS (DCI/NH_3) m/z 355 $(M + H)^+$.

To [1-(2-morpholin-4-ylethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone (6.6 g, 19 mmol) in 25 mL of EtOAc and 5 mL of EtOH was added *p*-toluenesulfonic acid monohydrate (3.5 g, 19 mmol). No precipitate formed after 10 min of stirring, so the crude material was concentrated under reduced pressure and dried under reduced pressure to give 9.4 g of the title compound (18 mmol, 96% yield). ^{1}H NMR (MeOH-d_4, 300 MHz) δ 1.33 (s, 6H), 1.34 (s, 6H), 2.15 (s, 1H), 2.36 (s, 3H), 3.40 (m, 4H), 3.68 (dd, $J = 7.1$, 7.1 Hz, 2H), 3.90 (m, 4H), 4.73 (dd, $J = 7.1$, 7.1 Hz, 2H), 7.23 (br d, $J = 7.8$ Hz, 2H), 7.26 (ddd, $J = 8.1$, 8.1, 1.4 Hz, 1H), 7.33 (ddd, $J = 7.1$, 7.1, 1.0 Hz, 1H), 7.56 (br d, $J = 8.1$ Hz, 1H), 7.72 (br d, $J = 8.5$ Hz, 2H), 8.15 (s, 1H), 8.29 (dt, $J = 7.8$, 1.0 Hz, 1H); MS (DCI/NH_3) m/z 355 $(M + H)^+$; Anal. ($C_{22}H_{30}N_2O_2 \cdot C_7H_8O_3S$) C, H, N.

[5-Hydroxy-1-(2-morpholin-4-ylethyl)-1*H*-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone (33). A mixture of **37** (1.2 g, 2.5 mmol) and Pd/C (10 wt % palladium on activated carbon, 120 mg) in 50 mL of EtOH was processed as described in the procedure for **17** to provide **33** (0.85 g, 2.3 mmol, 92% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 1.30 (s, 6 H), 1.34 (s, 6 H), 1.87 (s, 1 H), 2.41–2.58 (m, 4 H), 2.70–2.84 (m, 2 H), 3.66–3.81 (m, 4 H), 4.16–4.28 (m, 2 H), 4.84–4.98 (m, 1 H), 6.87 (dd, $J = 8.8$, 2.4 Hz, 1 H), 7.21 (d, $J = 8.8$ Hz, 1 H), 7.73 (s, 1 H), 7.88 (d, $J = 2.7$ Hz, 1 H); MS (DCI/NH_3) m/z 371 $(M + H)^+$; Anal. ($C_{22}H_{30}N_2O_3$) C, H, N.

(5-Hydroxy-1-(2-morpholinoethyl)-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (35). Compound **33** (0.15 g, 0.41 mmol), Cs_2CO_3 (0.4 g, 1.2 mmol), and CH_3I (51 μL, 0.61 mmol) in 5 mL of DMF were combined and stirred at ambient temperature for 72 h. The mixture was quenched with 3 mL of saturated, aqueous NH_4Cl and diluted with 5 mL of EtOAc. The layers were separated, and the aqueous layer was extracted with 3 × 3 mL of EtOAc. The combined organic extracts were washed with 1 × 5 mL of saturated aqueous NaCl, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and recrystallized with 4:1 hexanes/EtOAc to provide **35** (75 mg, 0.20 mmol, 48% yield). ^{1}H NMR (MeOH-d_4, 300 MHz) δ ppm 1.33 (s, 12 H), 2.10 (s, 1 H), 2.47–2.53 (m, 4 H), 2.77 (t, $J = 6.4$ Hz, 2 H), 3.63–3.69 (m, 4 H), 3.84 (s, 3 H), 4.33 (t, $J = 6.4$ Hz, 2 H), 6.89 (dd, $J = 8.8$, 2.7 Hz, 1 H), 7.38 (d, $J = 8.8$ Hz, 1 H), 7.81 (d, $J = 2.4$ Hz, 1 H), 8.06 (s, 1 H); MS (DCI/NH_3) m/z 385 $(M + H)^+$; Anal. ($C_{23}H_{32}N_2O_3$) C, H, N.

(5-(Benzyloxy)-1-(2-morpholinoethyl)-1*H*-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (37). The (5-benzyloxy-1*H*-indol-3-yl)-(2,2,3,3tetramethyl-cyclopropyl)methanone (from the procedure for **24**) (1.1 g, 3.0 mmol), 2-morpholin-4-ylethyl methanesulfonate (5.1 mmol), and NaH (60% dispersion in mineral oil, 0.36 g, 9.1 mmol) in 25 mL of DMF were processed as described in the procedure for **5** to provide **37** (1.2 g, 2.6 mmol, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.31 (s, 6 H), 1.36 (s, 6 H), 1.90 (s, 1 H), 2.39–2.59 (m, 4 H), 2.72–2.87 (m, 2 H), 3.63–3.81 (m, 4 H), 4.13–4.31 (m, 2 H), 5.14 (s, 2 H), 7.01 (dd, $J = 9.0$, 2.5 Hz, 1 H), 7.22–7.28 (m, 1 H), 7.29–7.44 (m, 3 H), 7.45–7.52 (m, 2 H), 7.75 (s, 1 H), 8.07 (d, $J = 2.4$ Hz, 1 H); MS (DCI/NH_3) *m/z* 461 $(M + H)^+$; Anal. ($C_{23}H_{31}NO_2$) C, H, N.

Supporting Information Available: Elemental analysis for all final compounds, experimental information and data for compounds **6–16**, **18–20**, **22–24**, **26–31**, **34**, **36**, and **38–55** and ^{1}H NMR spectra for representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(36) **57** (SR141716A) is *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride and was synthesized at Abbott Laboratories.[22]

(37) **58** (SR144528) is *N*-[(1*S*)-*endo*-1,3,3-Trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide and was synthesized at Abbott Laboratories.[22]

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